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THE CYTOARCHITONICS, THE INTRINSIC ORGANIZATION AND THE AFFERENT TERMINATION PATTERNS OF THE GLOBUS PALLIDUS
A LIGHT AND ELECTRON MICROSCOPIC STUDY

by

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THESIS

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PART I

A. General Introduction

The globus pallidus appears to be a focal structure upon which converge most pathways concerned with non-pyramidal motor function. According to Truex and Carpenter (1964), the globus pallidus may serve as one of the principal subcortical sites involved in the integration of non-pyramidal motor functions. Support for this suggestion may be derived from stimulation experiments. Jinnai (1964) has reported that in man stimulation of the globus pallidus produced rigidity and an increase of the stretch reflexes during and immediately after stimulation.

The globus pallidus may also contain elements involved in fundamental viscero-metabolic activities as have been proposed independently by Heath, Freeman and Mettler (1947) and Mettler (1955). The recent physiological findings of Morgane (1961) suggest that the pallidum may participate in the regulation of the feeding centre in the lateral part of the hypothalamus. Lewin et al (1965) have also noted that inhibition of spontaneous bladder activity may result from stimulation of the pallidum.

All these findings suggest that the functions of the globus pallidus may be generalized and multiple in character, and emphasize the necessity for studying and integrating the cytoarchitectonics, the intrinsic organization

and the synaptology of this nucleus. It is hoped that this investigation will provide some correlation between the anatomy and function of this apparently complex structure.

B. The Structure of the Globus Pallidus

According to Grunstein (1924) there seems to be no embryological, physiological or anatomical grounds for uniting the globus pallidus and putamen into the nucleus lentiformis.

The pallidum is characterized by its strongly myelinated lamina, by the myelinated neuropil and fibers which permeate its interior. It is separated from the putamen by the external medullary lamina, from the caudate nucleus and thalamus by the limbs of the internal capsule.

The pallidum of higher mammals has two segments or divisions, the outer is the larger of the two and extends more anteriorly than the inner one, which is smaller. In lower mammals the two segments are separated by a considerable interval; the medial segment is known as the entopeduncular nucleus. In primates and some of the carnivores the segments are drawn together to form a composite mass.

C. Review of the Literature

1. Cytoarchitectonics and Intrinsic Organization

Nissl preparations of the globus pallidus of mammals, including man, were described by Ramon y Cajal in 1909. In his description of this structure of smaller mammals, namely the cat, rat and rabbit, the lenticular nucleus was divided into three parts. The first or external

part - shaped somewhat like a comma and intimately united to the superior temporal lobe, corresponds to the putamen of higher species. The second part - a rounded infero-internal mass of small cells is situated at the border of the internal capsule. The third part - consists of two or three small cellular masses located at the base, not far from the amygdala is connected with the preceding mass. Cajal identified these two latter cellular masses as the globus pallidus of higher species.

According to Cajal's description, the cells of the globus pallidus are large and fusiform or triangular in shape with very long dendrites. Their axons are very thick and have the pecularity of splitting into two during their course through the nucleus. He also noted that numerous collaterals of these axons, together with a tremendous number of fibers of passage through the nucleus, made it extremely difficult to study the course and terminations of these axons within the pallidum.

In 1911, Bielschowsky using his silver impregnation technique partially clarified the intrinsic ramifications of these axons. He stated that the dendrites of the pallidal cells are surrounded by a sheath of neuroglial cells and that the incoming fibers follow and ensheath the dendrites in their course throughout the nucleus. These findings were confirmed by Vogt (1920).

In 1925, Foix and Nicolesco presented the first pictoral details of these two nuclei. Nissl preparations of human material were studied by these two authors in the course of their investigations on Parkinson's disease. They have succinctly described the cellular structure of the globus pallidus in their statement "Il ne presents, en effet, qu'on seul type de cellules nerveuses: grandes cellules." Further they also noted that the cells in the internal part were somewhat larger than those in the external part.

In his studies on the diencephalon of carnivores, Rioch (1931) described the globus pallidus proper with the caudate nucleus and putamen as belonging to the striatum. He further stated that the entopedunucular nucleus consists of "cells scattered between fibers of the ansa lenticularis" and that these cells are triangular or fusiform in shape, with two to three long cytoplasmic processes. Johnston (1915) also gave the same cytological picture of the pallidal cells in the turtle. In this case the cells were found in the lateral forebrain bundle, the homologue of the ansa lenticularis of higher forms.

Pilleri (1962) showed that the globus pallidus, of the primitive marsupials and rodents, contains only one type of ganglion cell, whose dendrites resemble a "string of pearls." He attributes this pecularity of the dendrites to the black reaction of the Golgi preparation.

Gruenthal (1932) was the first to perform a comprehensive comparative study on the nucleus basalis and the globus pallidus. He drew attention to the important relationship between these two nuclei in lower mammals. In monkey, chimpanzee and man, the nucleus basalis has a subpallidal location, but in lower mammals (mole, rabbit and dog) there exists, in addition to the ventral location of the basalis cells, a dorsal extension between pallidum and putamen. In coronal sections from rostral to caudal levels, the dorsal share of the basalis increases and finally prevails over the pallidum.

Concerning the relative differences in cell size of the globus pallidus of primates, Gruenthal confirmed the larger size of the nerve cells in the pallidum internum as compared to the pallidum externum. This difference is much more pronounced in the monkey than in chimpanzee and man. Gruenthal moreover, assumed that the basal ganglion of lower mammals corresponds to the pallidum externum of the primates, and that Meynert's nucleus has become rudimentary in the primates. This was later refuted by Brockhaus (1942) who showed that in some higher primates the basal nucleus is quite extensive, although its relationship to the pallidum varies with the species.

In their studies on the nervous system of vertebrates, Ariens-Kappers et al (1936) agreed that the "exact nuclear delimitations and homologues of the nucleus basalis of Meynert are not as clear as could be wished."

In the sterotaxic atlases of Jasper and Ajmone-Marsan (1954) and of Snider and Niemer (1961) of the cat, the globus pallidus is shown as a histologically uniform and distinct structure throughout its extent; the nucleus basalis is depicted as an insignificant group of cells restricted mainly to the most rostral part of the globus pallidus.

In contrast to the above findings, Gorry (1963) indicated in his thesis on the comparative anatomy of the basal ganglion of Meynert, that in carnivores (dog, raccoon and cat) the cells of the basal nucleus are "scattered in among those of the pallidum in its caudal extremity", but to what extent was not illustrated either with photographs or diagrams.

The intimate morpho-genetic relationship of the basal nucleus to the globus pallidus was already noted by Kodama (1926), who proposed that it should be considered as a third part of the pallidum.

In reviewing the literature on the structure of the globus pallidus it seems that since the classical studies of Bielschowsky in 1919, little attention has been devoted to the actual intrinsic organization of this nucleus. It was not until 1965, that a renewed attempt was made by Fox and his co-workers, to study the organization of the pallidum in the monkey using both light and electron microscopic techniques.

They presented for the first time the ultra structural details of the organization of this nucleus. They

confirmed that the afferents to the pallidum are mainly of the "en passant" type, making both axo-somatic and axo-dendritic synapses in their course through the nucleus. Similar studies carried out on cats and birds (Fox et al 1966) have shown a similar type of organization in the pallidum of these animals.

In 1966, Mori made a corresponding investigation on the corpus striatum of the rat. He showed that the ultra structural organization of the synaptic endings in the pallidum is comparable to those of higher mammals. However, he further observed that in this nuclear body there are various types of endings. He consequently classified these terminals according to the kinds of synaptic vesicles within them. In the globus pallidus, he indicated that there were at least two main types of terminals. The first kind containing large, uneven synaptic vesicles (500-800 Å in diameter). The other type contains small light synaptic vesicles of about 450 Å in diameter. The author however, did not attach any functional significance to these different types of endings, or gave any indication of whether they emanated from a specific region.

2. Afferent Connections of the Globus Pallidus

The origin of all afferents to the globus pallidus are not fully known, but we are already aware of a number of sources according to Knook (1965). For the purposes of the present study however, only the most important and well established connections will be considered, i.e. caudato-pallidal, thalamo-pallidal (VA), putamino-pallidal and nigro-pallidal.

A detailed summary of the course and terminations of these connections have been described by the present author (thesis, 1966). However for clarity a brief review of these connections will be outlined for the monkey and the cat. Emphasis will be placed on the suggested sites of termination of these fibers within the pallidum.

The classical studies of Wilson (1913-1914) on the monkey have shown that after extensive lesions in the head of the caudate nucleus, degenerating fibers can be seen to terminate in both segments of the pallidum. Subsequent studies by Papez (1938) revealed that fibers from the head of the caudate nucleus (monkey) formed a large funnel shaped radiation which after traversing the internal capsule converged to enter the oral segment of the pallidum and pass through it as scattered fiber bundles. He further stated that many of these fibers seemed to end in the internal part of the pallidum. Similar findings were reported by Ranson et al (1941).

Mettler (1942) reported degenerated axons from the head of the caudate nucleus (monkey) through the external medullary lamina. These degenerated fibers continued ventromedially around the external part of the globus pallidus, to join with other fascicles coming from the putamen. He also observed that a group of fibers which separated from the above at the dorsal edge of the pallidum, was seen to enter its medial segment and "was lost to view".

The first indication that a topical distribution exist from the caudate nucleus to the pallidum was reported by Glees (1945), he found that fibers from the dorsal part of the caudate nucleus terminated in the external segment. He also stated that fibers from the ventral part of the caudate nucleus enter the medial division of the pallidum directly from the internal capsule.

Verhaart (1950) on the other hand found that in the normal brain of the gibbon most of the striatal fibers seemed to terminate in the rostral half of the globus pallidus. He also made the interesting observation that 99% of these fibers were one micron or less in diameter with an extremely fine myelin sheath.

All the studies above were made with the Marchi technique, and this probably explains the failure to trace finer caudate fibers entering the globus pallidus.

In more recent times a better insight into the topographical distribution of the caudate fibers to the

pallidum were obtained by the use of the Nauta-Gygax method introduced in 1954.

Voneida (1960) utilized this improved technique to reassess the anatomical relations of the caudate nucleus in the cat and the monkey. He made small, controlled lesions in the head of the caudate nucleus in these animals and reported that fibers from the head of the caudate nucleus after traversing the internal capsule entered the medial segment of the globus pallidus where most of them ended. The remaining fibers passed through the pallidum on their way to the substantia nigra.

Szabo (1962) using the Nauta silver impregnation technique made a detailed study of the topographical distribution of the striatal efferents in the monkey. In this investigation of the caudato-pallidal connections, he mapped out the course and the termination of these fibers by placing controlled lesions in various areas in the head of the caudate nucleus. When lesions were made in the ventro-medial area, the efferents terminated mainly in the internal segment of the pallidum, lesions placed in the lateral areas of the head of the caudate nucleus revealed projections to the external part of the pallidum, whereas lesions made in the middle part caused terminal degeneration in both segments equally.

It should be mentioned that Voneida (1960) could not find any degenerating terminals in the external segment

resulting from lesions placed in the head of the caudate nucleus in either the cat or the monkey. Szabo (1960) pointed out however, that the lesions made by Voneida were restricted to the medio-ventral area of the head of the caudate nucleus and since this area, as mentioned above, specifically projects to the medial segment of the pallidum, it would account for the negative findings reported by Voneida.

Evidence of the caudato-pallidal connections in the cat has also been reported by Kaneko (1941) and Johnson (1959). The latter author maintains that while a few fibers from the head of the caudate nucleus of the cat terminate in the external segment, the majority seems to end in the entopeduncular nucleus. It is interesting to note, that he was unable to follow degenerated fibers to the substantia nigra, a projection described and well documented by Papez (1938), Verhaart (1950), Voneida (1960) and Szabo (1962 and 1967).

Physiological experimentation in support of the above caudato-pallidal connections were reported by Mettler et al (1952) and Spiegal and Szekely (1961).

The most recent physiological experiments on cats carried out by Purpura et al (1967) are particularly interesting. An attempt was made in their studies to specify the major synaptic events observed in lenticular elements by stimulation of various parts of the head of the caudate nucleus. Both excitatory and inhibitory post-synaptic potentials evoked by caudate stimulation were observed in

cells scattered throughout ventral portions of the lenticular complex and particularly in cells of the medial part of the pallidum and in the entopeduncular nucleus.

Putamino-pallidal Connections

The first detailed investigation of putamino-pallidal connections in the monkey was carried out by Wilson (1913-1914). After putamen lesions he described fine degenerating bundles or pencils of fibers coursing mesially within the putamen. The anterior pencils converge as they pass in a posterior direction, while the posterior converge as they travel anteriorly: the most ventral run in a dorsal direction, the dorsal in a ventral direction, and in this fashion they all converge towards the lateral zone of the pallidum. Here some of them spread out, while others pass on to the medial segment, where they in turn break up into smaller fascicles.

This "cone-like" distribution of putamino-pallidal fibers has been reported in the monkey by Papez (1938).

Marchi degeneration studies carried out on monkeys by Ranson et al (1941), Glees (1945) and Mettler (1945) have all confirmed that the putamen also sends fibers to both segments of the globus pallidus.

Subsequent studies by Johnson and Clemente (1959) in the cat and Nauta and Mehler (1961) in the cat and monkey using the Nauta technique have provided further evidence on

the existence of these connections.

It was not until 1962 that the first attempt was made by Szabo to work out the details of the site of terminations of putamen fibers in the pallidum. In this study it was indicated that the origin, course and destination of the fibers entering the pallidum maintain their relative positions throughout in a dorso-ventral and mediolateral direction.

More recent studies on the efferent projections of the putamen in the monkey by Szabo (1967) further clarified this topographical distribution pattern. Particularly pertinent to this study is his observation that while caudate fibers occupy the dorso medial one-third of both pallidal segments, the putamen efferents terminate in their remaining ventro-lateral parts, in addition to sending other fibers to the substantia nigra.

Nigro-pallidal Connections

Ranson et al (1941) have shown in the monkey with the aid of the Marchi method that fine degenerating fibers from the substantia nigra terminated in both segments of the globus pallidus. Similar findings were reported by Fox and Schmitz (1944).

Rosegay (1944) on the contrary was unable to see any significant Marchi degeneration in the pallidum following massive lesions placed in the substantia nigra of the cat.

In an attempt to resolve this problem Carpenter and McMaster (1964) made controlled experimental lesions in the substantia nigra of monkeys and studied the subsequent degeneration using the Nauta-Gygax method. They concluded that nigro-pallidal fibers enter the ipsilateral globus pallidus and terminate mainly in the internal segment. A few fibers however, were also seen in the external segment.

Thalamo-pallidal Connections

The fiber connections from certain nuclei of the thalamus to the pallidum are not well documented, and the few authors who describe them differ as to their final termination.

Nauta and Whitlock (1954) reported that long fibers from the centrum medianum perforate the internal capsule after traversing the reticular complex and enter the lentiform nucleus. Here most of those fibers terminate in the putamen, but a relatively lesser number end in the globus pallidus. They were unable to find any projection to the pallidum from the nucleus medialis dorsalis thalami.

Powell and Cowan (1956) confirmed that the nucleus centrum medianum in the monkey projects to the putamen but denied that any fibers enter the globus pallidus from this nucleus.

Johnson (1961), in agreement with the above authors, reported that fibers from the centrum medianum in the cat also

sends fibers to the pallidum and putamen. He also reported that a few fibers from the nucleus medialis dorsalis thalami terminated in the globus pallidus, but the majority of these fibers ended in the caudate nucleus.

Ranson et al (1941) employing the Marchi technique described afferents in the monkey via the inferior thalamic peduncle to the internal segment of the globus pallidus from the nucleus ventralis anterior of the thalamus. This was later confirmed by Ranson and Clark (1953).

Physiological experiments in support of the connections from the centrum medianum and other intralaminar nuclei to the lentiform nucleus have been reported by Purpura et al (1966 and 1967).

Numerous studies have been done on the afferent connections to the globus pallidus, and yet very little is known about their actual mode of termination. This lack of knowledge may be largely due to limitations of the present light microscopic techniques.

At present one of the most pressing problems of synaptic studies is to determine the interrelationship between particular morphological features and related aspects of synaptic function.

The morphology of the synaptic terminals can provide clues to functional differences (Gray and Gillary (1966)). The possibility exists that "tight junctions" may characterize electrically transmitting synapses or that granular vesicles

may occur in adrenergic endings. Axo-axonic connections, according to Eccles et al (1962) may be responsible for presynaptic "inhibition".

On the other hand, according to Bodian (1966), and Laramendi et al (1967), the type and mode of terminations in certain systems of the central nervous systems determine, to a certain extent, whether they are inhibitory or excitatory in function.

From the above studies it appears that more accurate knowledge on the fine structure of the type of terminals, and also on the exact mode of termination is necessary before any meaningful correlations can be made between morphology and possible physiological functions.

A preliminary investigation on the probable mode of termination of the pallidal afferents has been carried out previously by the present author using light microscopic techniques (thesis, 1966), but further analysis of this important problem is needed.

Three main aspect of the above-mentioned studies require clarification. A more detailed study, on the cyto-architecture of the globus pallidus in the cat and monkey seems to be necessary not only in regard to its cellular relationship to the nucleus basalis, but also to the orientation and dendritic arborizations of its cells.

All investigators who have studied the intrinsic organization of the globus pallidus agree that the main mode

of termination of the afferent fibers to this nucleus is of the "en passant" type. It is difficult to assume that so many different nuclei can send fibers to the pallidum without the existence of a distinctly organized arrangement of terminations within the nucleus.

Lastly, it appears that no attempt has been made to study experimentally the mode of termination of these pallidal afferents by electron microscopy. A study of this kind should clarify to some extent many of the problems mentioned above.

D. Aim of the Present Study

The present investigation is mainly devoted to the study of the afferents of the globus pallidus. In view of the fact that this nucleus receives fibers from the caudate, the putamen, the substantia nigra and possibly from the nucleus ventralis anterior an attempt will be made to study in greater detail the organization and synaptic arrangements of these afferents within the pallidum. Golgi and electron microscopic techniques will be correlated in order to learn whether or not synaptic terminals belonging to these different afferent systems can be identified or whether they show special localization and distribution upon pallidal cells. Since no detailed analysis has previously been made on the pattern of termination of the afferent fibers, special

attention will be paid to this aspect of the problem utilizing the Golgi method in both the cat and the monkey.

The cytoarchitecture of the globus pallidus and its involvement with the nucleus basalis of Meynert in the cat will also be studied in detail and mapped out. The techniques to be used in this latter investigation will consist not only of Nissl preparations, but will also incorporate the Ramon-Moliner modification of the Golgi method. It is hoped that the combination of these two methods will lead to a clearer understanding, of both the morphology of the neurons and the intricate dendritic arborization of these cells.

PART II

A. Materials and Methods

The present work is based on observations made on a total of 46 brains subdivided as follows:

Golgi method (cats - 10; monkeys - 6)

Experimental (cats) electron microscope - 12

Control (normal) (cats) electron microscope - 10

Nissl method (cats - 5; monkeys - 3)

I. The Golgi Method

Three variations of the Golgi method were used in this investigation. The Golgi-Fox and a modification of the Golgi rapid techniques were used in one series of animals, and the Ramon-Moliner method in another.

The Ramon-Moliner technique (1958) was used mainly to study cell clusters, dendritic interrelations and comparative cell sizes.

For cytoarchitectonic studies, Cresylecht violet stained Nissl preparations were used. Of the 8 series used in this study 6 came from the collection of the Department of Anatomy. Three series were loaned to me by Dr. F. Sanides. The brains of cats and monkeys were sectioned in frontal and horizontal planes.

The Golgi-Fox technique (1951) was used with minor variations. The brains were cut into 2-3 mm pieces in the

usual way, and placed in a 6% zinc chromate solution for 48 hours at room temperature. The pieces were then placed on filter paper and with the aid of a small camel hair brush, the surface of the sections were then gently cleaned off with small amounts of distilled water.

The sections were then rinsed two or three times in 0.75% silver nitrate, and finally transferred to a large quantity of fresh 0.75% silver nitrate (100 mls/piece) for 24-48 hours in the dark at room temperature.

The chromate and silver treatments were repeated three times. With the second impregnation one frequently finds many stained cells, but the axons and their collaterals are incompletely stained, as are the terminal ramifications.

The modified Golgi-rapid method applied in this study is one that is used at the Wayne State Medical School in Detroit. The technique is as follows:

(a) Young animals, not older than 6 weeks, are perfused through the heart with 2.5% phosphate buffered glutaraldehyde (pH 7.2 - 7.4).

(b) The brain is then removed and cut in 2-3 mm pieces in the usual way.

(c) The pieces are placed in an equal mixture of 5% glutaraldehyde (buffered) and 5% potassium dichromate with 1 ml 2% osmic acid added per 50 mls solution and left overnight at room temperature in the dark.

(d) The pieces were briefly rinsed in a 5% dichromate

solution, and subsequently placed in a fresh solution of 5% potassium dichromate with 1 ml 2% osmic acid per 50 mls solution, and left for 4-7 days in the dark at room temperature.

(e) After this period the sections were placed into a 0.75% silver nitrate solution for 24-48 hours in the dark at room temperature after first rinsing briefly in this same solution. Again for complete impregnation of the terminal ramifications steps (d) and (e) were repeated three times.

The sections are finally dehydrated and sectioned in the following way:

(f) The pieces were quickly put through two changes of 95% and two changes of absolute alcohol and 10 minutes in xylene, the whole operation taking no more than one hour.

(g) The sections were left in low melting paraffin (48-52°C) for 10 minutes in the oven. Blocks are made using the same paraffin in the usual manner. Sections 100-150u can then be cut with considerable ease.

(h) After the sections were cut, they were put through two changes of 95%, two changes absolute alcohol and cleared in two changes of xylene, mounted; coverslipping is optional, if coverslips are not used large quantities of permount must be employed.

II. Experimental Procedure - Electron Microscope

Healthy adult cats ranging between 2.4 - 2.6 Kg in weight were anaesthetized intraperitoneally with nembutal. Lesions were made in the brain by a D.C. current of 3 ma. The electrode applied for 15 seconds, through an electrode stereotaxically inserted into the desired area of the brain. This electrode consisted of a stainless steel tubing (24 gauge) with an inner core of 0.0063" stainless steel wire. The wire and tubing were dipped in epoxylite and baked for 3/4 hour at 170°F. This procedure was repeated three times, at the end of which a 1 mm bare area was scraped off at the tip.

After coagulation the animals were allowed to survive for 3-7 days. Then, under nembutal (anaesthesia) fixation of the brain was initiated by perfusion through the heart with cold Ringer's solution, followed by cold 2.5% phosphate buffered glutaraldehyde (pH 7.2 - 7.4). The brain was immediately removed, the globus pallidus carefully cut out, and the external and internal sections of this nucleus removed and kept separately.

These pieces were further sectioned into very small cubes 1 mm or less and placed in buffered glutaraldehyde for two hours in the refrigerator at 4°C. After this period the pieces were rinsed twice with the buffer solution and finally placed in buffered 1% osmic acid for 1-1½ hours at 4°C. Dehydration was carried out in a graded series of acetone 50%, 75%, 95% and 100%, then placed in 1:3, 1:2, 1:1 respectively

of acetone/westopal for two hours each on a rotator, then finally in 100% westopal overnight. The pieces were then embedded in fresh 100% westopal in 00 gelation capsules, and placed in an oven at 45°C for 24 hours and then transferred to a 60°C oven for 24 to 48 hours. Thin sections were cut on a Reichert ultramicrotone and placed on Formvar coated 200 mesh grids. The sections were studied on a Phillips 100, and Zeis electron microscopes.

For controls, the unlesioned sides of the brains were used. Normal cats were also utilized as controls and perfused and prepared in the same manner as described above.

The procedure outlined above for processing tissue for electron microscopy, except for the slight variation in the temperature of the different solutions, is in accordance with the methods used by Dr. J. Metuzals of the Department of Histology, University of Ottawa.

List of Abbreviations

The following abbreviations were used for all figures.

AL - Ansa lenticularis

AM - N. anterior medialis

AMy - Amygdaloid complex

AS - Fibrous astrocyte

AV - N. anteroventralis thalami

CA - Commissura anterior

CC - Corpus callosum

Cd - N. caudatus

Ch - Chiasma opticum

CI - Capsula interna

Cl - Claustrum

EML - External medullary lamina

Fx - Fornix

En - Entopeduncular nucleus

GP - Globus pallidus

GP ext - Globus pallidus Externus

GP int - Globus pallidus Internus

HL - Hypothalamus lateralis

IAM - N. inter antero medialis

IML - Internal medullary lamina

LME - Lamina medullaris externa of thalamus

NB - Nucleus basalis of Meynert

NR - Nucleus ruber

Ped - Pedunculus cerebralis

Put - Putamen

R - N. reticularis

RE - N. reuniens

So - N. supraopticus

TO - Tractus opticus

VA - N. Ventralis anterior

PART III

Results

1. Cytoarchitectonics of the Globus Pallidus and the Nucleus Basalis of Meynert

In Nissl preparations, the cells of the globus pallidus can be easily distinguished from those of the nucleus basalis by their pale coloration, in contrast to the darker staining characteristic of the latter cells.

In the cat, the rostral extent of the globus pallidus begins approximately in a frontal plane, passing through the anterior limb of the commissura anterior. At this level, the cells, medium in size, can be seen to be distributed randomly throughout the nucleus.

Here, the nucleus basalis consists of a few small clusters of cells, diffusely situated ventral to the pallidal cells. As can be seen in Pl. 1, Figs. A and B, these cells also extend laterally and slightly dorsally between the pallidum and putamen.

In the next frontal sections, (every tenth section in this series were studied) the cells of the pallidum are still predominant, but at this level only small clusters of basalis cells are seen in the latero-dorsal and latero-ventral borders of the pallidum. Within these two areas smaller accumulations of the basalis cells can be found

Plate No. 1

(A-B) Cat, cross sections of the globus pallidus at the level of the anterior commissure, showing the location of the basalis cells.

(A) small clusters of basalis cells (arrows) are seen along the ventral border of the pallidum.

(B) a cross section taken more laterally than (A). Arrows indicate basalis cells scattered along the latero-ventral border of the pallidum.

Nissl method, 126X.

GP
B
A
GP
C
B

intermingled with those of the pallidum, Pl. 2.

In sections caudal to the optic chiasma, the basalis cells begin to occupy a greater portion of the pallidum and in these sections the pallidal cells can be seen only with certainty in the most medial aspect of the nucleus. In the lateral part of the pallidum, there is still intermingling of basalis and pallidal cells; but the cells of the nucleus basalis are more predominant and mainly occupy the latero-ventral area and to a lesser extent the latero-dorsal part of the nucleus; this is demonstrated in Pl. 3.

In sections caudal to the above level, the cells of the pallidum are considerably reduced in number and consist only of a few scattered cells in the most medial aspect of the nucleus. At this level the nucleus basalis is quite prominent and occupies the greater part of the lateral aspect of the nucleus, as can be seen in Pl. 4.

In the most caudal levels, a few pallidal cells can still be recognized in the dorsal and ventral borders of the nucleus. However, the major part of the nucleus is almost completely filled with basalis cells. (Pl. 6, Fig. e).

The nucleus entopeduncularis on the other hand does not seem to be greatly involved with the cells of the nucleus basalis. This is evident in Pl. 5 which is a horizontal section through the dorsal part of the nucleus. The cells appear to be arranged into irregular groups throughout the extent of the nucleus, but no basalis cells are evident.

Plate No. 2

(A) Cat, cross section of the globus pallidus at the level of the optic chiasma. Note intermingling of pallidal and basalis cells. The basalis cells (long arrows) can be distinguished by their large size and darker staining characteristics. Short double arrows indicate smaller lighter staining pallidal cells. 320X

(B) Cross section caudal to the above, note predominance of basalis cells. Nissl method, 126X.

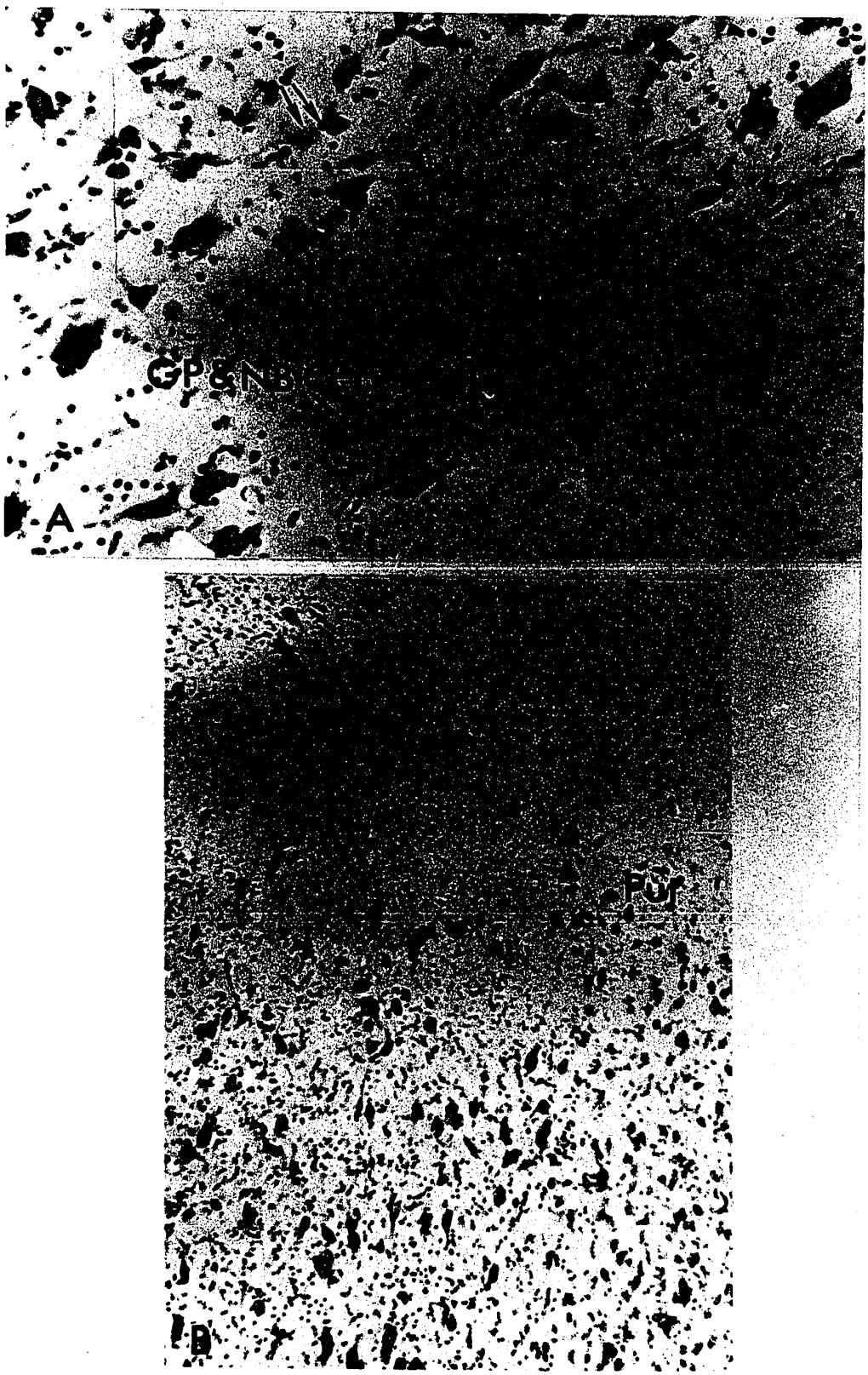
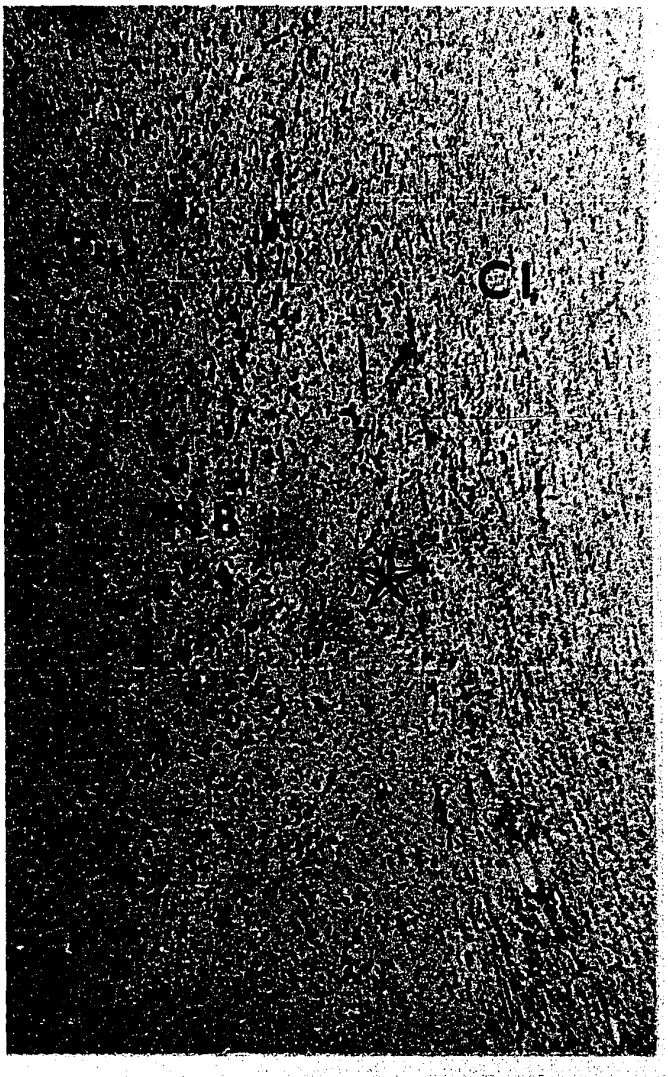

GP & NB
A
Pur
B

Plate No. 3

(A) Cat, cross section of the globus pallidus, slightly caudal to the optic chiasma. The nucleus basalis is prominent here occupying the entire lateral border extending dorsally and ventrally. Nissl method, 50X.

(B) Higher magnification of area (star) shown in (A). Only a few small pallidal cells (GP) can be recognized on the medial border of the nucleus next to the internal capsule. 126X.


Cl
B

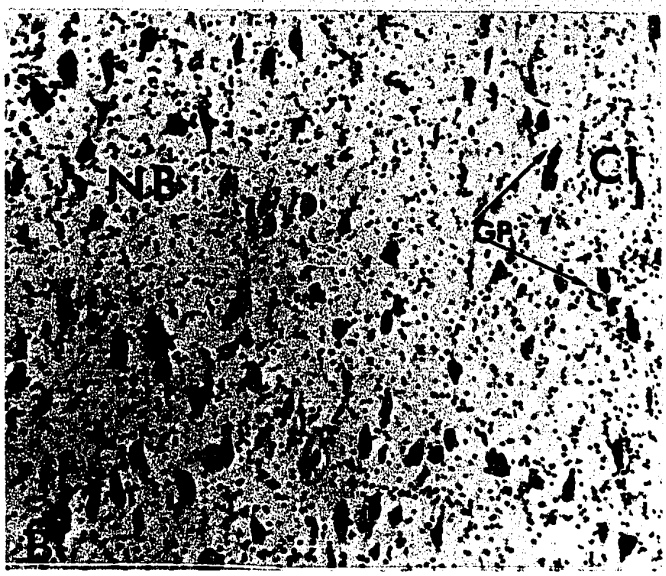
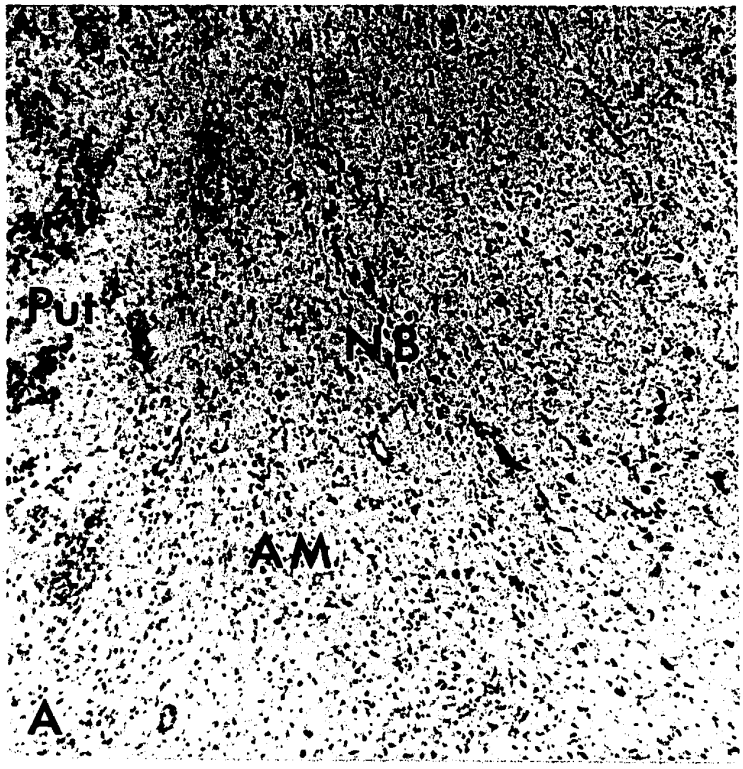

NB
Cl
GP
B

Plate No. 4

(A) Cat, cross section of the pallidum taken at a more caudal level than Plate No. 3. Only basalis cells are apparent here. (B) 50X.

(B) Higher magnification of enclosed area in (A). Note large basalis (NB) cells and the smaller cells of the putamen. Nissl method, 320X.


Put
NB
AM
A

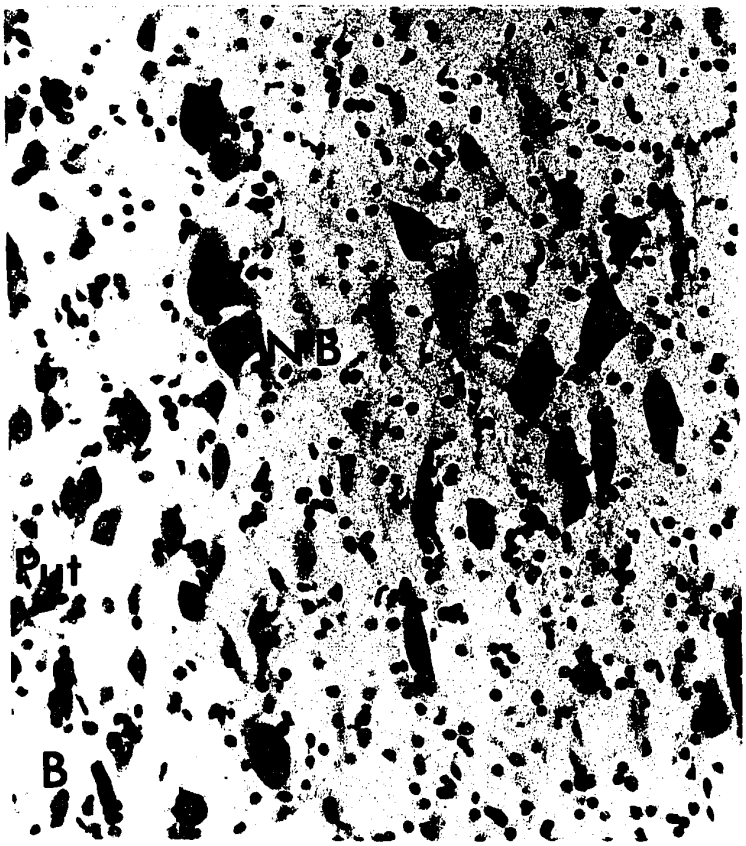
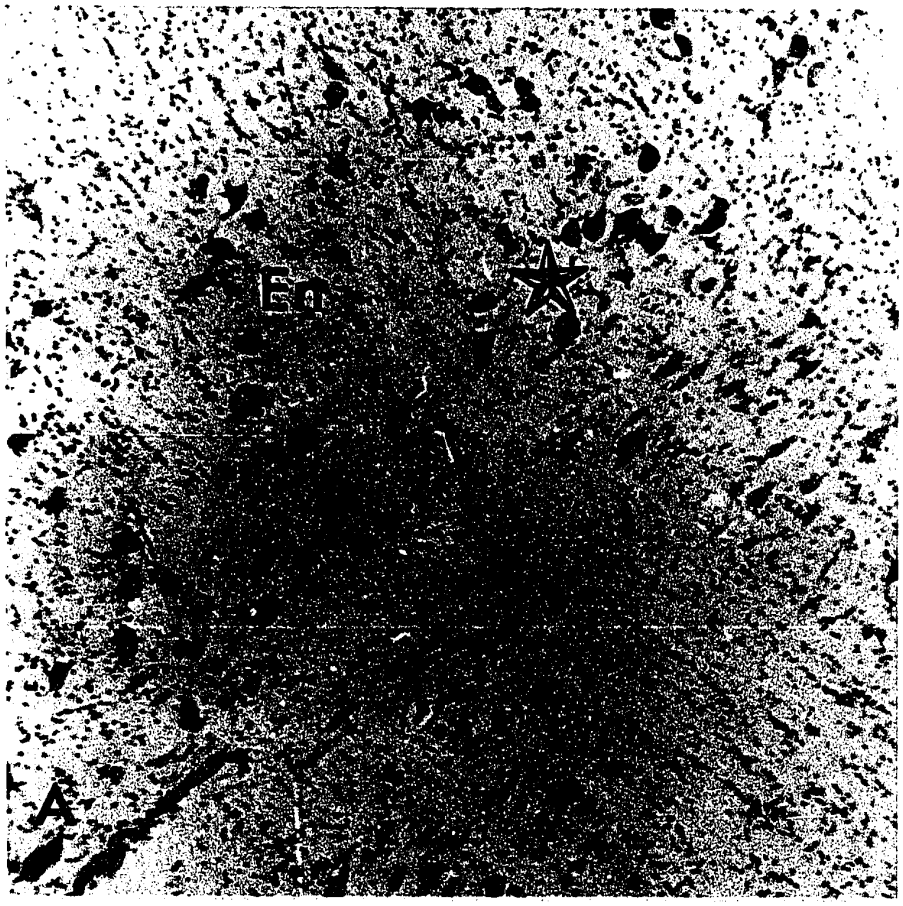
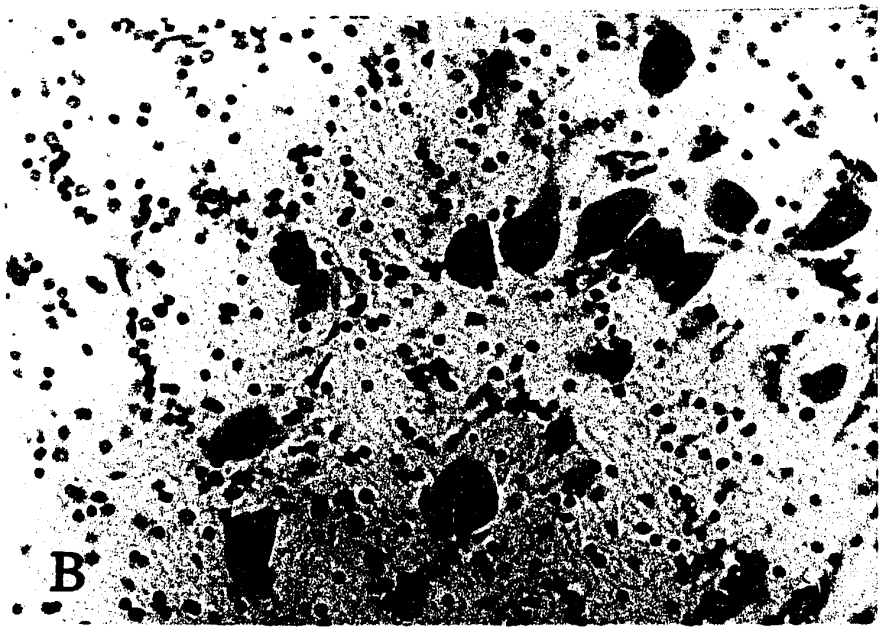

NB
Put
B

Plate No. 5

(A) Cat, longitudinal section of the entopeduncular nucleus. Note irregular arrangement of the cells in small clusters, and also absence of basalis cells. 126X.

(B) Higher magnification of area in (A) (star) showing the typical morphology of the cells. Nissl method, 320X.


Fe
A


B

However, at the level of the supra-optic nucleus, a few scattered basalis cells can be seen mainly at the lateral border of the nucleus entopeduncularis, between the latter and the globus pallidus. These cells were also evident in the Golgi preparations shown in Pl. 11, Fig. B.

The combined results of these cytological studies are summarized in the semi-diagramatic drawings in Pl. 6, Figs. a - f.

The levels depicted in these series of drawings were based on Jasper and Ajmone-Marsan's (1954) atlas of the diencephalon of the cat.

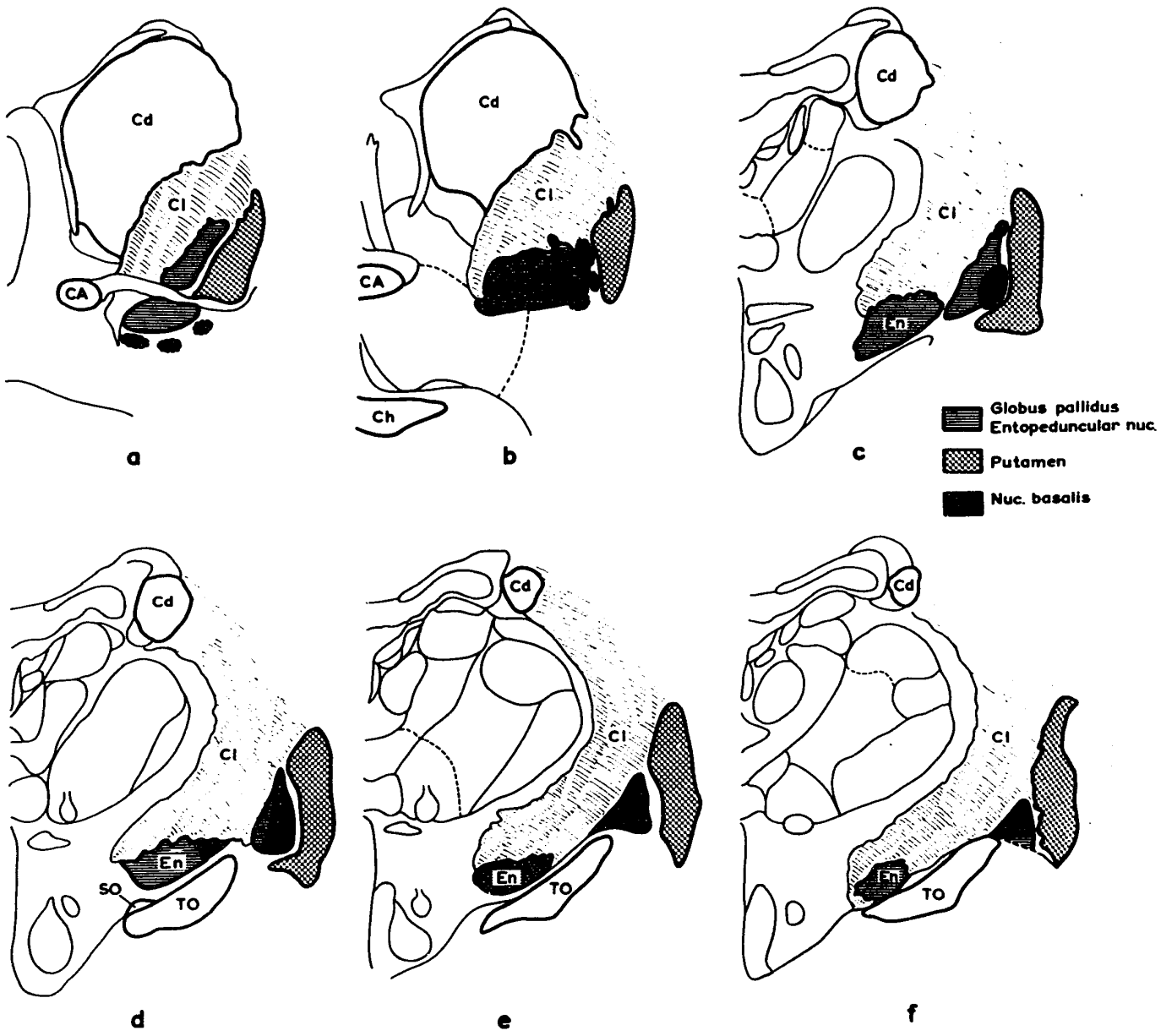
The Nissl series studied in this investigation were controlled by comparing them with their corresponding fiber stain (Heidenhain) sections. These sections in turn were matched as near as can be ascertained with the corresponding sections in the above atlas.

From a cytoarchitectonic point of view, the inter-relationship of the nucleus basalis and the globus pallidus in the monkey has already been studied extensively by Gruenthal (1932), Brockhaus (1942), Olszewski (1952) and Gorry (1963).

It should be stated that whereas the above-mentioned authors disagreed on the extent to which the nucleus basalis intrudes into the external and internal medullary laminae, they nevertheless, are in agreement as to the rostral and caudal extent of this nucleus.

Plate No. 6

(a - f) Diagramatic representation of a series of transverse, Nissl stained sections through the corpus striatum of the cat. (Based largely on Jasper and Ajmone-Marsan's (1954) atlas of the diencephalon of the cat.) The cytoarchitectonics of the globus pallidus and the nucleus basalis of Meynert is outlined in detail. For the meaning of the abbreviations used in this and other figures see page 25.


Cd
Cl
CA
Ch
En
SO
TO
a
b
c
d
e
f
Globus pallidus
Entopeduncular nuc.
Putamen
Nuc. basalis

The studies mentioned above (with the exception of Olszewski) on the cytoarchitectonics of the globus pallidus in the monkey have been mainly concerned with its relation to the nucleus basalis, so that very little attention has been paid to the cytological structure of the pallidum itself. In carnivores, this is quite understandable since the pallidum and the nucleus basalis are so intimately associated. But since this is not the case in higher mammals, a closer look into the cytoarchitectonics of the pallidum itself in the monkey seems worthwhile.

Serial sections of Nissl stained preparations of the monkey brain were studied in a manner similar to studies in the cat, but only a few additional points relevant to this study will be mentioned.

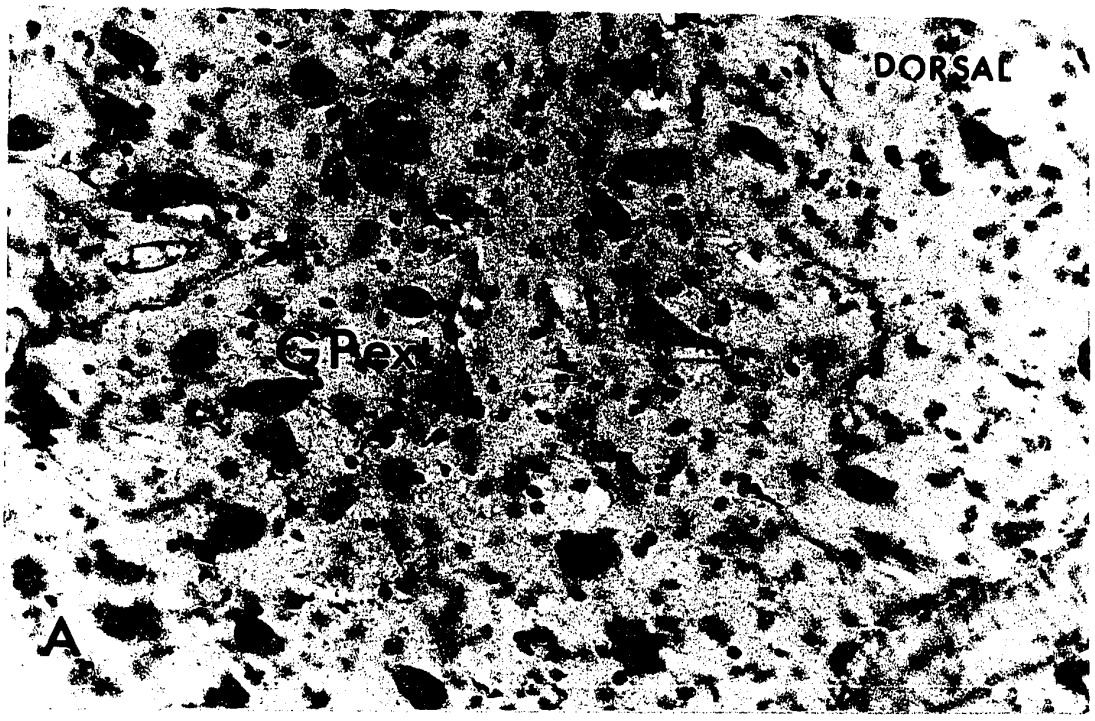
At the level of the anterior commissure, as in the cat, the pallidal cells are, in general, quite uniform in size and appear to be randomly distributed throughout the nucleus. The cells of the basalis nucleus are very sparsely distributed here and are to be found mainly on the basal or ventral border of the pallidum.

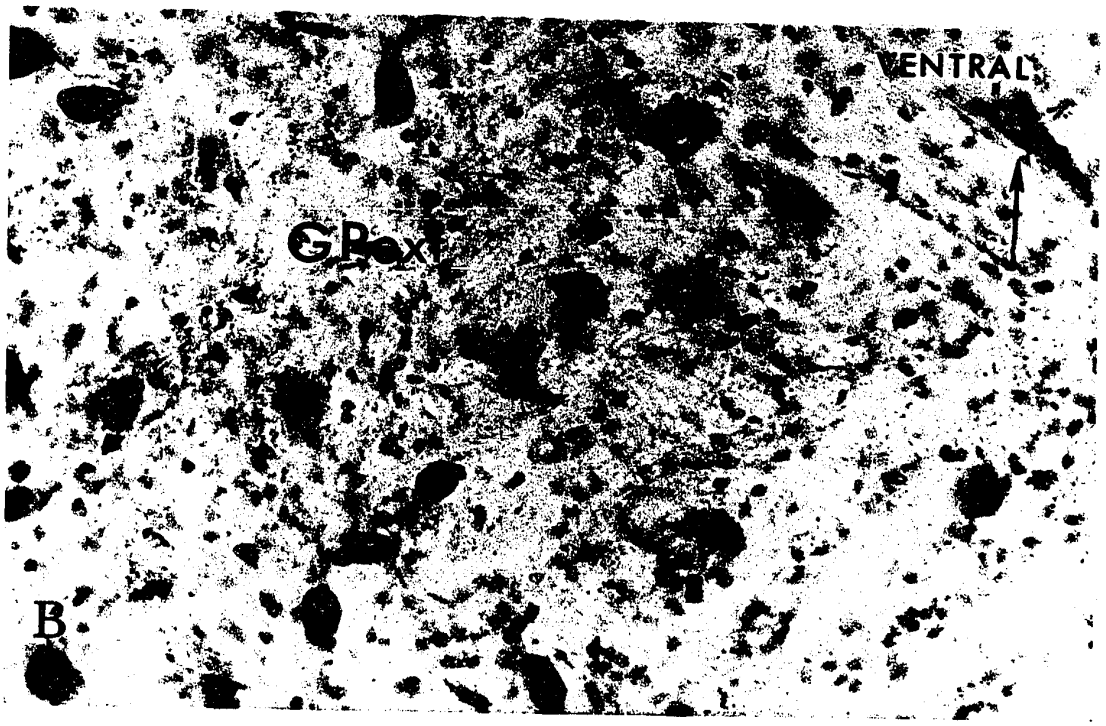
Further caudally, beginning at the level of the optic chiasma, the distribution of the pallidal cells takes on a slightly different appearance as compared to more frontal levels. As seen in Pl. 7, Figs. A and B, the dorsal and dorso-lateral areas of the external part of the pallidum appear to have a higher population of cells than the basal

Plate No. 7

(A) and (B) Monkey, cross-sections of the external part of the globus pallidus to show the differential distribution of cells in dorsal and ventral areas. 320X.

(A) Cross-section of the dorso-lateral area. Note the higher concentration of pallidal cells as compared to the ventro-lateral area (B). Nissl method, 320X.


DORSAL
GPex
A


VENTRAL
GPex
B

areas. The density of the cells seem to decrease progressively in a dorso-ventral direction.

A numerical count on ten randomly chosen slides in these post commissural areas seems to support this observation. On the sections chosen for counting, the appropriate areas were first marked off on the slides and then one microscopic field from each slide was counted. A 16x objective and a 10x ocular were used in each case. The figures for each area were then tabulated and shown in Table 1. These results seem to indicate that the number of cells in the dorsal areas as compared to the ventral regions are in a ratio of approximately 2:1. In the internal part of the pallidum, a some-

Table 1

External Segment - Globus Pallidus

Level	Number and Values of Slides Counted										Average Count
	1	2	3	4	5	6	7	8	9	10	
Dorsal	43	41	39	37	39	34	35	41	33	39	38
Ventral	17	17	15	12	14	17	15	13	18	13	15
								Approx. Ratio			2:1

what similar distribution of cells was evident. Pl. 8, shows a high density of cells in the dorsal part and a somewhat lower concentration in the basal part of the nucleus. Table 2 gives the result of a corresponding cell count on ten randomly chosen slides. The numerical values were obtained in a manner similar to that described above for the pallidum externum.

Table 2

Internal Segment - Globus Pallidus

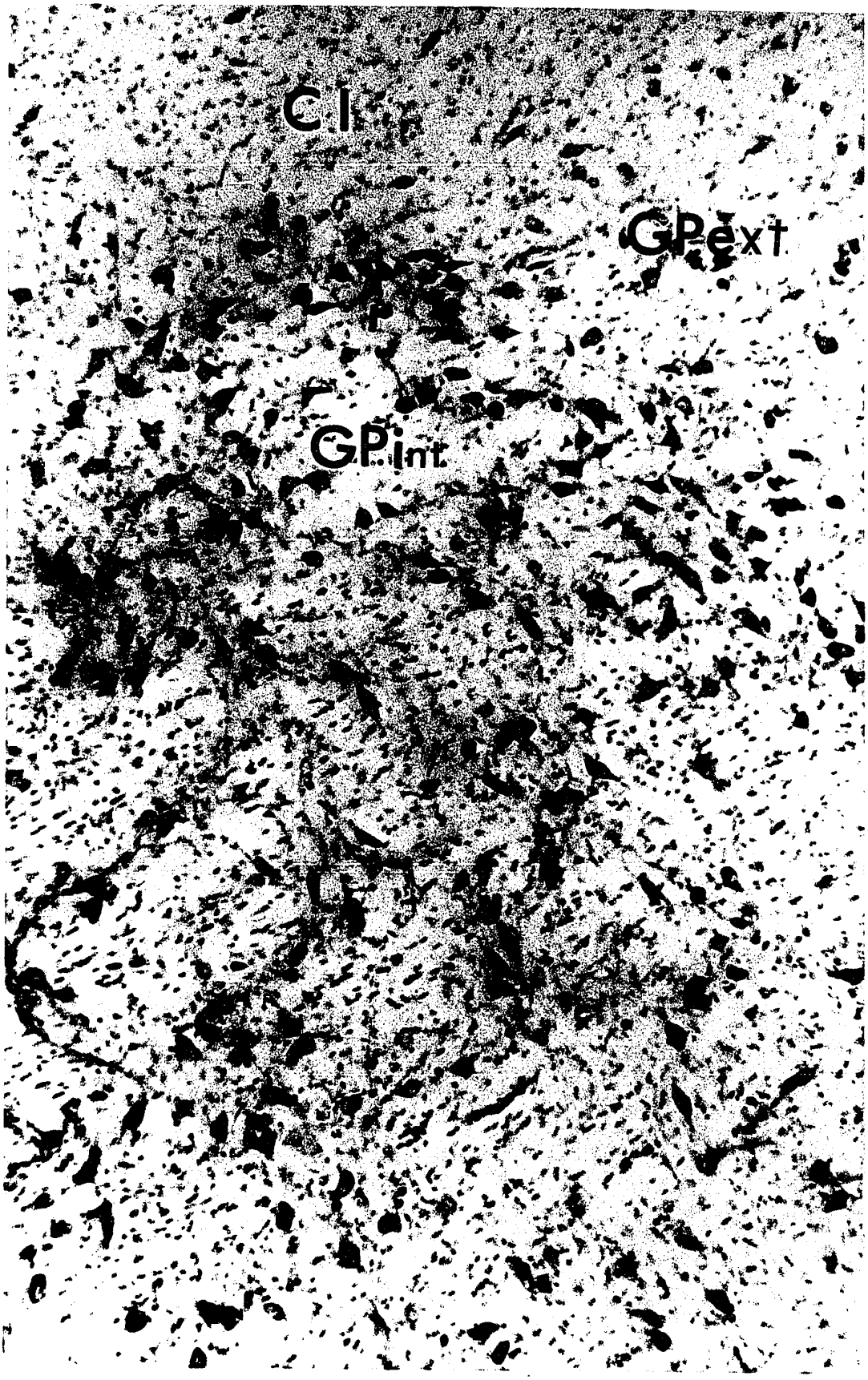
Level	Number and Values of Slides Counted										Average Count
	1	2	3	4	5	6	7	8	9	10	
Dorsal	65	55	51	61	55	63	47	51	66	53	57
Ventral	28	35	33	39	28	36	35	30	27	33	32
									Approx. Ratio		2:1

In the most caudal areas the differences in the density of the cells in the dorsal and ventral parts were not so apparent. The cells within this part of the nucleus which were much reduced in number as compared to more rostral areas were again randomly distributed throughout the nucleus.

The nucleus basalis for the most part occupied a position ventral to the pallidum. As can be seen in

Plate No. 8

Monkey, transverse section of the internal part of the globus pallidus. Note the high concentration of pallidal cells in the dorsal section of the nucleus, as compared to the ventral area at the bottom of photograph. Nissl method, 126X.


Cl
GPext
GPint

Pl. 9, Fig. B, which corresponds to a level approximately through middle 1/3 of the optic tract in a rostro-caudal direction; a few basalis cells can be seen intruding for some distance into the internal medullary stria. A similar intrusion of basalis cells for a short distance into the external medullary stria was also seen between the ventral part of the putamen and the pallidum. (See Pl. 9, Fig. A).

In the most caudal part, where only the external segment of the pallidum remains, a single diffuse band of cells can be seen intermittently scattered throughout the whole length of the external medullary stria. The latter observation is in good agreement with the findings of Olszewski (1952) and Gorry (1963), but is not in accord with the atlas of Snider and Lee (1961).

Cytological Features of the Globus Pallidus

The morphology of the cells in the pallidum as already described by Cajal (1911), Foix et Nicolesco (1925) is characterized by large 'motor' type cells, triangular or fusiform in shape. In Nissl preparation a large pale nucleus and a deeper staining nucleolus are evident in these cells. The nucleus is, as a rule, located centrally and dense accumulations of Nissl substance are evident at each pole of the cell.

As can be seen in Pl. 10, the cells of the internal part are larger than those in the external segment. However,

Plate No. 9

(A and B) Monkey, transverse Nissl sections through the globus pallidus at approximately the middle 1/3 of the optic tract.

(A) Note intrusion of basalis cells only for a short distance into the external medullary lamina.

(B) Shows basalis cells penetrating into the lower part of the internal medullary lamina. 126X.


GPext
Put
NB
A

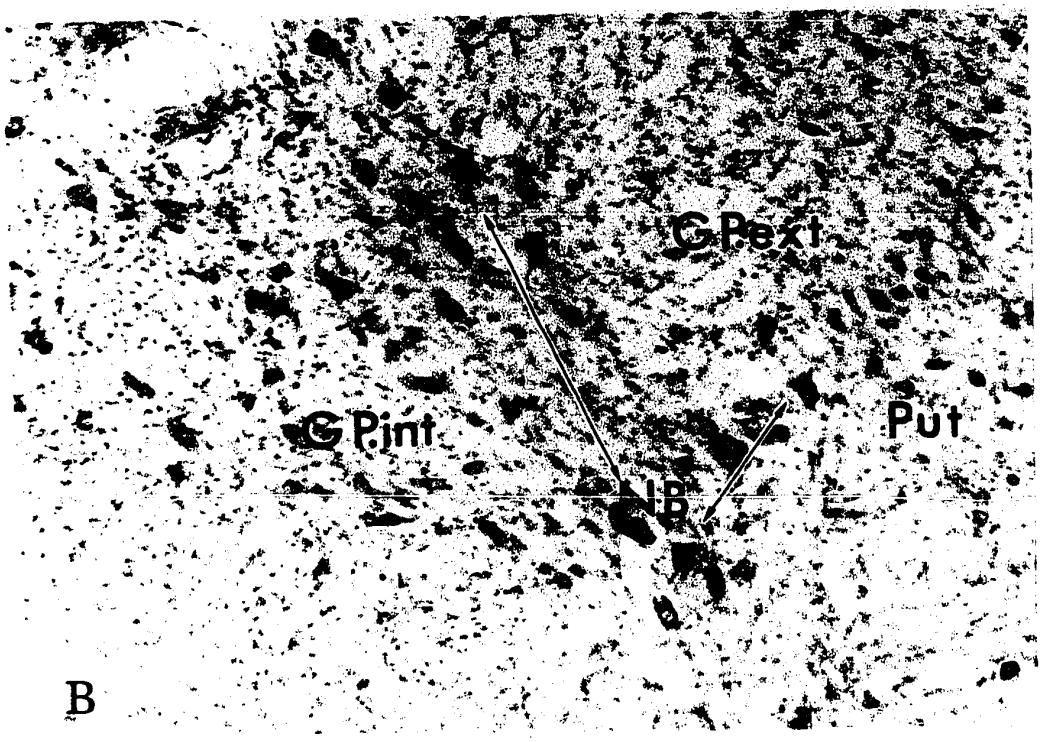
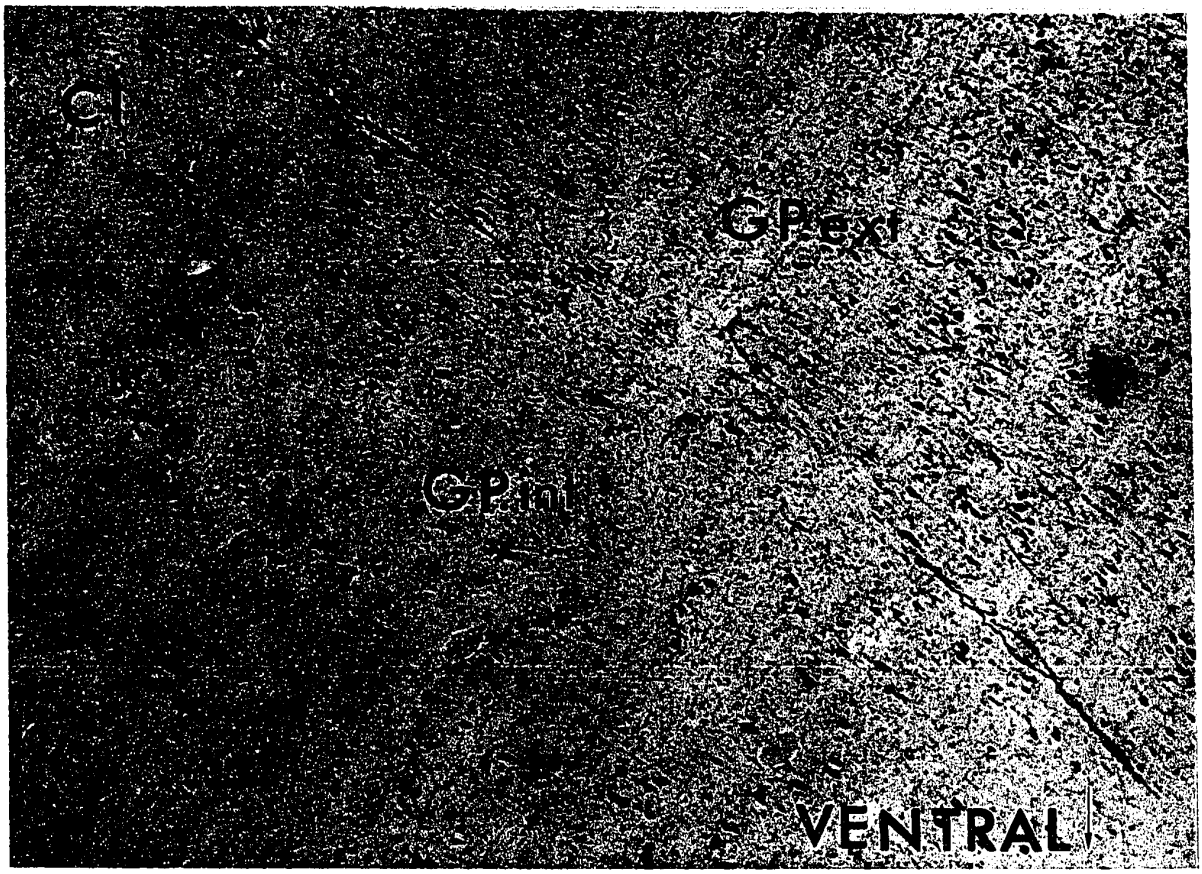

GPext
GPint
Put
NB
B

Plate No. 10

Monkey, cross-section of the pallidum to demonstrate the difference in the cell sizes of the pallidum externum (GP.ext) and pallidum internum (GP.int). Nissl method, 50X.


GPext
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VENTRAL

a few scattered larger type cells can occasionally be seen in the external segment. They can be found either in the central area of the nucleus, or as a single core of cells, in the latero-dorsal and medial aspects of the nucleus. One of these large cells seen in Pl. 7, Fig. B (arrow), is easily distinguished from the deeper staining basalis cells by its pale coloration.

2. Golgi and Electron Microscopic Observation

Cytoarchitectonic studies in general rely mostly on Nissl stained preparations, so that descriptions of the neurons are limited to the cell body. It is not possible, therefore, with this technique to draw any conclusions regarding the form or structure of the axonal and dendritic processes.

In this investigation a series of sections stained by the Ramon-Moliner and Golgi-Fox methods were also processed in the hope of resolving this problem.

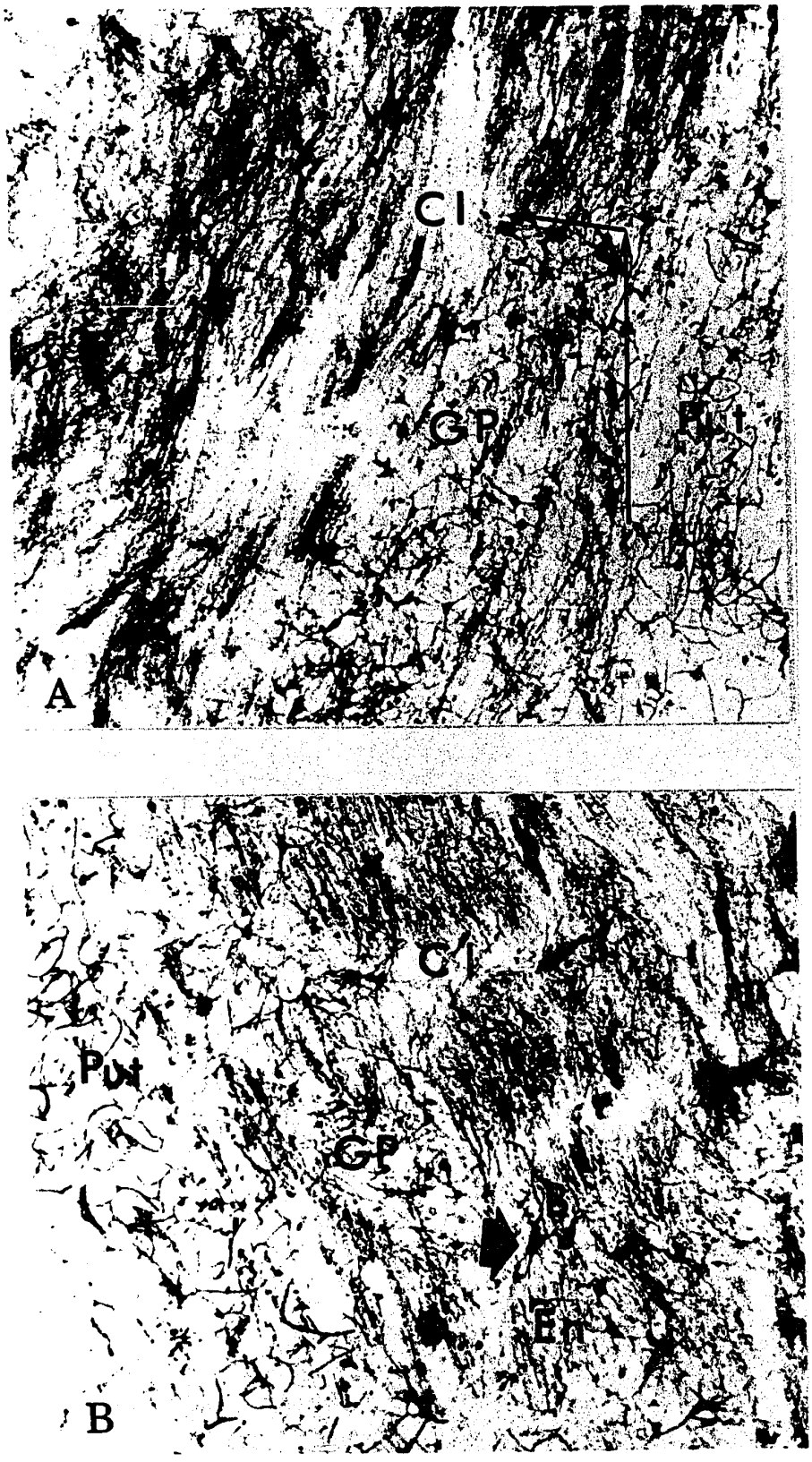
Serial frontal sections of the cat brain by the Ramon-Moliner method were first studied and compared with the Nissl stained materials.

Successful staining was not obtained at the most rostral areas of the pallidum. However, beginning at about the level of the optic chiasma, as shown in Pl. 11, Fig. A, a band of large basalis cells can be seen at the lateral border of the pallidum extending from the base between the

Plate No. 11

(A) Cat, transverse section of the globus pallidus at the level of the optic chiasma. A row of large basalis cells can be seen at the lateral border of the nucleus, within the arrows. Ramon-Moliner method, 70X.

(B) Cat, transverse section at the rostral level of the optic tract, note large basalis cells (B) at the lateral tip of the entopeduncular nucleus (large arrow). Ramon-Moliner method, 70X.


CI
GP
Put
A
CI
Put
GP
En
B

putamen and globus pallidus to the dorso-lateral tip of the latter nucleus, where they appear to form a small cap. The basalis cells are quite distinctive here, due to their large size as compared to the smaller pallidal cells.

At this level the dendrites of the basalis cells penetrate into the pallidum and interdigitate with those of the globus pallidus. Along the length of the lateral border of the pallidum dendritric branches of both pallidal and basalis cells also project deeply into the external medullary lamina. Small bundles of fibers, apparently coming from the putamen, can be seen to enter by streaming along the length of these dendrites (Pl. 12, Fig. B). A more detailed account of the axonal relations to these dendrites will be given when the Golgi-Fox method is considered.

On the dorsal border of the pallidum externum just under the outer border of the internal capsule the cells seem to be orientated in a latero-medial direction. (Pl. 12, Fig.A).

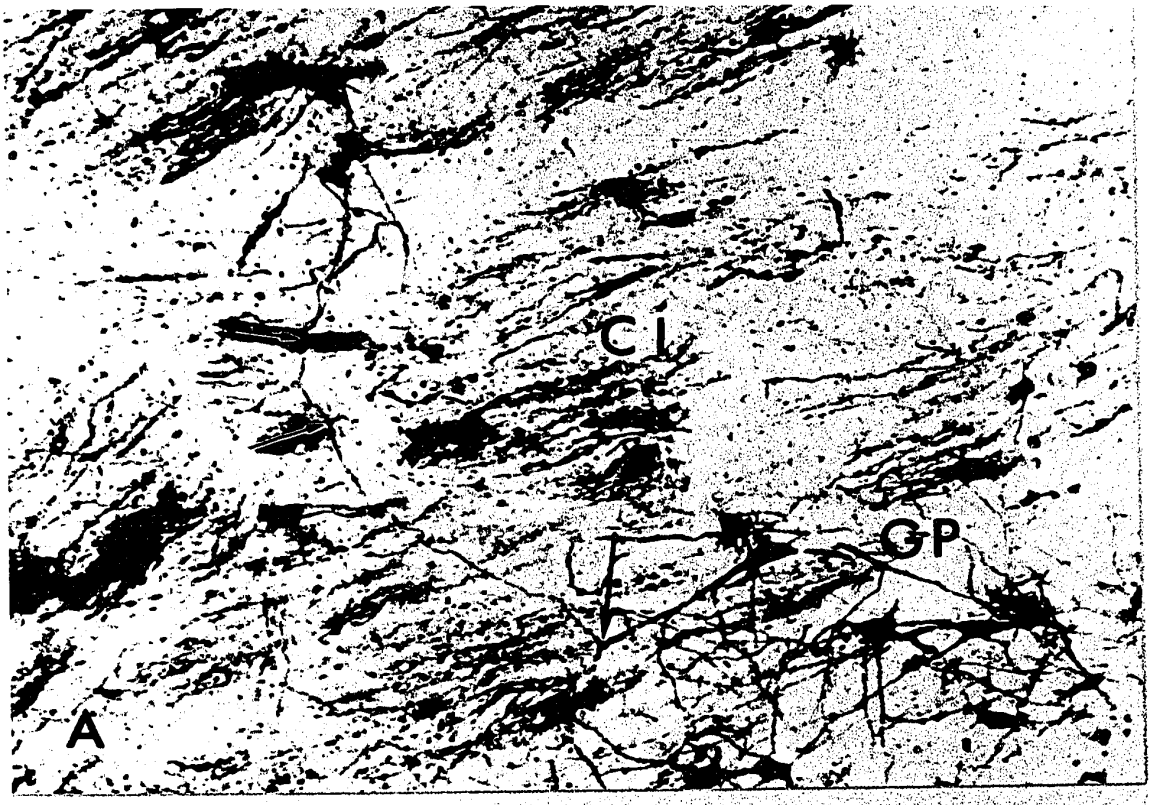
Such an orientation of cells in this area may not be unexpected, since the presence of large bundles of fibers from the internal capsule may cause the cells to be so placed. Nevertheless, a closer look at the dendrites of these cells shows that, while the primary branches of these cells may be orientated in a latero-medial direction, secondary branches extending off at nearly right angles from the main dendrite penetrate the internal capsule and extend to varying distances among its fibers. Pl. 12, Fig. A, shows one of

Plate No. 12

(A and B) Cat, cross-sections of the globus pallidus at the level of the upper 1/3 of the optic tract showing the orientation of the cells and dendritic processes.

(A) Arrows indicate dorsal intrusion of a dendrite along the dorsal border of the pallidum. 176X.

(B) Shows large basalis cells along the lateral border of the nucleus. Note the extension of their dendritic processes into the external medullary lamina and the direction of the fibers within the lamina. Ramon-Moliner method, 176 X.


CI
GP
A


EML
B

these dendrites extending quite deep into the capsule. Pl. 13 (arrow), is another example of a cell at the medial border of the pallidum with one of its branches extending dorsally within the internal capsule. The other branch is extending medially and ventrally.

Within the core of the nucleus at these levels, the dendrites of pallidal cells are not as orderly arranged as along the borders. They appear to ramify in the neuropil in every direction. These dendrites intermingle freely with those of basalis cells, therefore afferent fibers coursing within the nucleus may be assumed to make synaptic contacts with both type of cells.

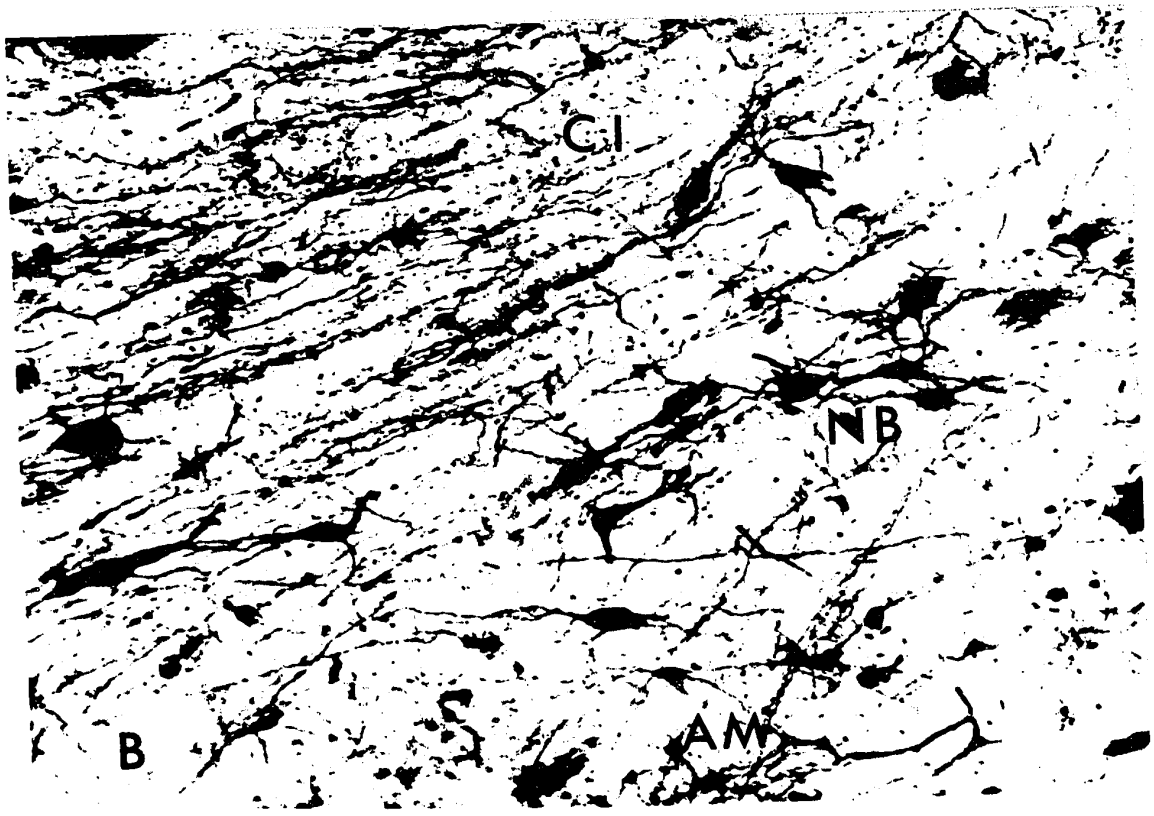
At the medial border of the pallidum in the cat, there is no true internal medullary lamina, and at certain rostral levels, the globus pallidus is seen to be continuous with the entopeduncular nucleus. The dendritic branches of neurons of both nuclei, at this junction, intermingle with each other and small bundles of fibers can be seen coursing from one nucleus to another. Whether these are interneuronal axons or fibers of passage could not be ascertained, since they could not be traced to their cells of origin. At the most caudal limit of the pallidum, only basalis cells persist as was also seen in the Nissl preparation. (Pl. 13, Fig. B).

The cells of the entopeduncular nucleus of the cat are larger than those of the globus pallidus. A similar situation exists in the internal and external segments of

Plate No. 13

(A) Cat, cross section of the pallidum. A large pallidal cell (P) situated at the medial tip of the nucleus is seen to send one of its dendrite (arrow) dorsally into the internal capsule. Note smaller type cells of the pallidum (p) and compare with larger basalis cells (NB) in Fig. B, which is a cross-section at a more caudal level showing mostly basalis cells. Ramon-Moliner method, 176X.


GP
CI
P
P
A


CI
NB
B
AM

the globus pallidus of the monkey. Pl. 14, shows a frontal section of the entopeduncular nucleus, slightly caudal to the optic chiasma. Along the dorsal border of the nucleus just under the internal capsule, the cells can be seen to line up with their main dendrites oriented in a latero-medial direction. A closer examination indicates that the secondary branches of these cells also penetrate the capsule in a similar manner as already described for the globus pallidus. Within the core of the nucleus the cells are again randomly oriented, their dendrites bifurcating in every direction. In Pl. 14, a cell (c) in the centre of the nucleus sends one dendrite in a medio-dorsal direction towards the internal capsule and another branch in a ventral direction, towards the ansa lenticularis, which it reaches and penetrates together with dendrites of other ventrally located cells. At point (A) of the same figure a fiber is seen to enter the nucleus from the ansa lenticularis and make contact with the dendrite of cell (c). Under higher power it is seen that this fiber travels up the dendrite for a short distance. Its subsequent course however, could not be followed due to incomplete impregnation. Nevertheless it can be speculated that since the pallidum contributes to the ansa lenticularis but the putamen does not (Nauta and Mehler, 1966), these fibers entering the entopeduncular nucleus from this tract may be pallidal fibers. On the other hand the nucleus basalis, in the cat, may also send fibers to the entopeduncular

Plate No. 14

Cat, cross section of the entopeduncular nucleus. Note orientation of the cells along the outer border of the internal capsule. Arrow points to fiber from ansa lenticularis (AL) making contact with dendrite of cell (c). Ramon-Moliner method, 176 X.


En
Cr
C
A
AL

nucleus via the ansa lenticularis (Rioch, 1931). It therefore seems that at present, the origin of these fibers remains in doubt.

The morphology of the neurons as seen in the Golgi preparations are in the main either triangular or fusiform in shape but occasionally a few polygonal cells can be seen in both the entopeduncular nucleus and in the pallidum. Pl. 12, Fig. B (arrow) shows one of these cells in the pallidum.

The main dendrites of both pallidal and basalis cells are smooth, thick and very long with few branches and few spines, the latter mainly restricted to the distal parts of the dendrite. In the cat and monkey the general appearance of the dendrites could correspond to the iso-dendritic types according to the classification of Ramon-Moliner (1962). Many secondary or tertiary dendrites, however, are not smooth but take on beadlike appearances. (Pl. 29, Fig. N). Pilleri (1962) also has described this feature of the dendrites in lower forms. He ascribed this phenomena to the "black reaction of the Golgi method". Electron microscopic studies (Pl. 29, Fig. M), indicate that these swellings are real and are not artifacts of the Golgi stain. A close examination of these "swellings" indicate a relatively large accumulation of mitochrondia at this junction. A small spine may also protrude from it. Pl. 36, Fig. 16, shows a cross section at one of these swellings.

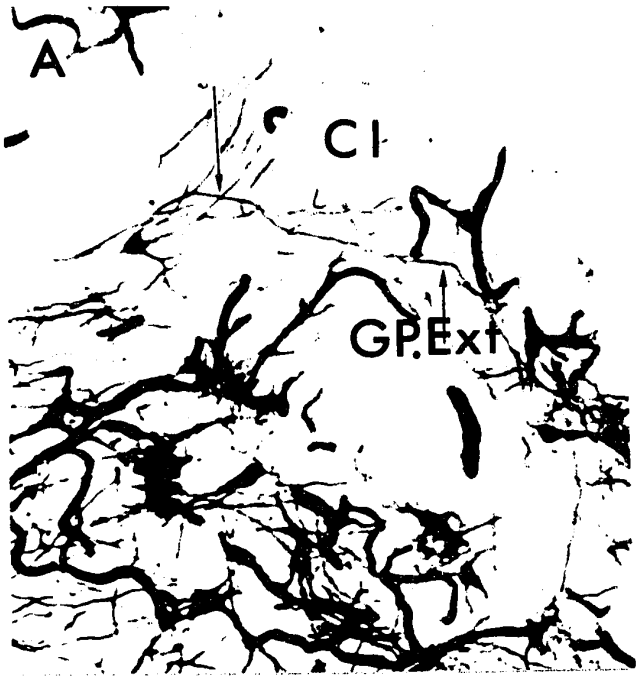
Golgi preparations as a rule do not stain axons, and in many sections, the axonal relationship of incoming fibers to cells of the globus pallidus could not adequately be demonstrated. With a triple impregnation of the standard Golgi-Fox method carried out on another series of monkeys and cats, it was possible to clarify, to some extent, the axonal relationship of these fibers. Using this procedure it was also possible to observe certain additional features not seen in routinely stained Golgi sections.

Pl. 15, Fig. B, is a frontal section which shows two cells located along the outer border of the middle third of the external segment of the globus pallidus of the monkey. The main dendrites are seen descending in a ventral direction in the external medullary lamina. Fibers can be seen to travel along these dendrites in a 'climbing' fashion. On reaching the cell body, these fibers bifurcate freely and are seen to make contact with other cells along their course. While the main dendrites along this border may extend ventralward in the external medullary lamina, secondary branches of these dendrites may be given off at nearly right angles within the lamina; these branches therefore point towards the putamen. (Pl. 15, Fig. B, large arrow). Cells in the upper third of the pallidum externum have primary and secondary branches coursing in dorsal and dorso-lateral directions towards the internal capsule and the putamen respectively, as well as branches coursing in a ventral and ventro-medial direction.

Plate No. 15

(A) Monkey, a pallidal cell is seen at the dorso-lateral tip of the external segment. Note orientation of the main dendrite and extension of secondary dendrite (arrows) into internal capsule. Golgi-Fox. 126X.

(B) Monkey, two typical pallidal cells are seen in the middle third of the lateral border of the pallidum externum. Note orientation of cells and direction of dendrites in the external medullary lamina. Small arrow points to fiber 'climbing' up along dendrite towards cells in the pallidum. Large arrow indicates small dendritic branch extending off at nearly right angles from the main branch. Golgi-Fox. 320X.


A
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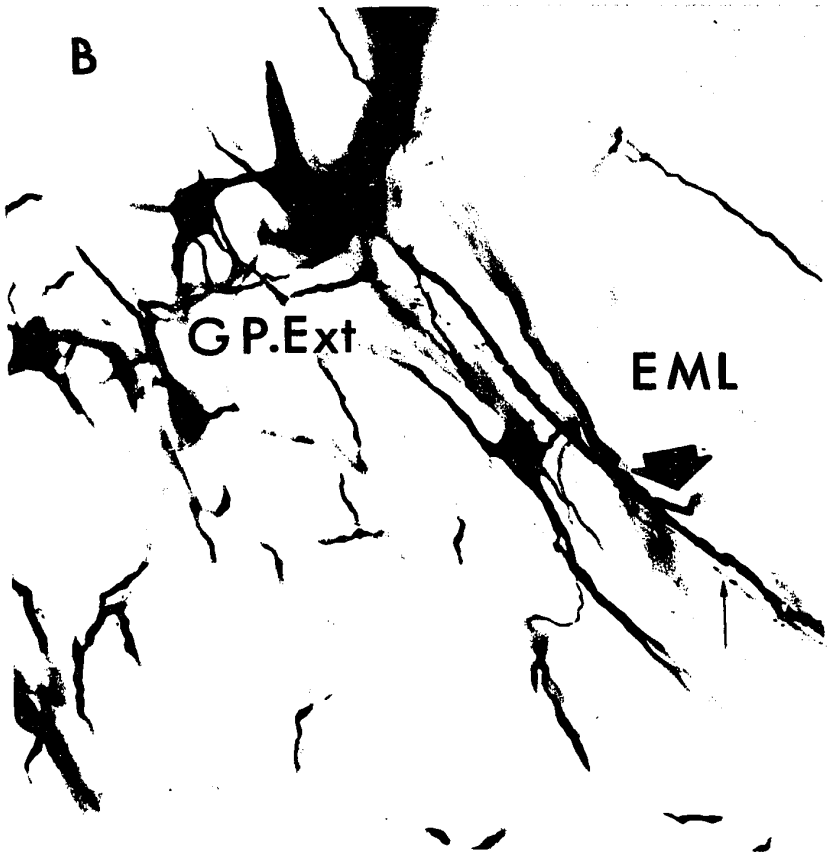
B
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EML

The manner in which these dendrites are orientated conforms in general to the pattern of the incoming fibers. According to Szabo (1967) fibers from the putamen enter the pallidum like the "rays of a fan", the orientation of the dendrites of the pallidal cells therefore may act as a "guide line" for these fibers in their course towards the pallidum. The course of some of these fibers are demonstrated in Pl. 16, Fig. E, which shows two large pallidal dendrites extending horizontally within the external medullary lamina towards the putamen, arrows indicate fibers possibly from the putamen coursing along its length.

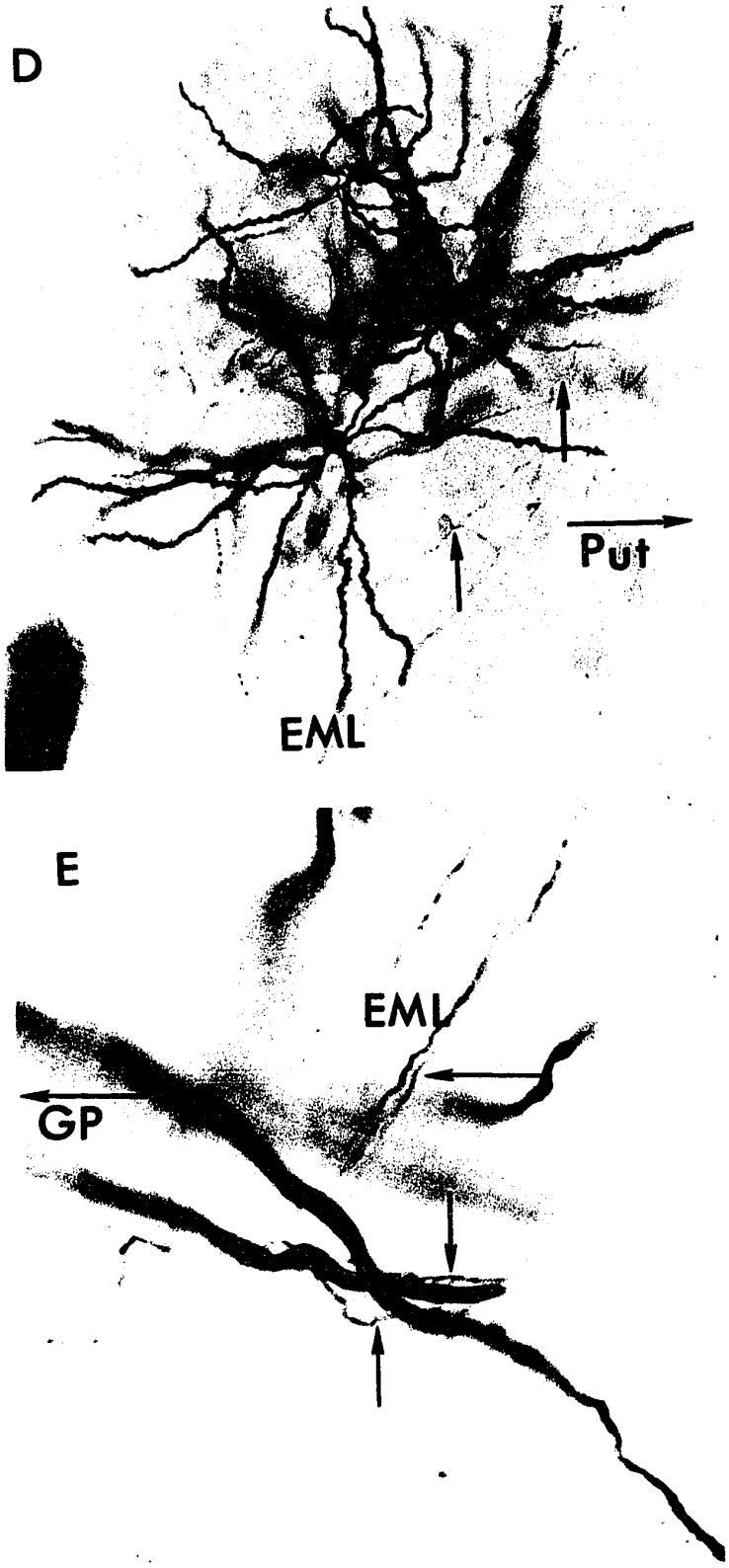
Small groups of cells may also be seen scattered among the fibers of the external medullary lamina, (Pl. 16, Fig. D). These groups of cells appear to be equivalent to the cytoarchitectonic observation of Sanides (1957) of "Insulae terminales". This author described under this term groupings of very small cells along the stria terminalis; the dorsal boundary region of the amygdala and the border of the putamen and pallidum. These cells are smaller than the smallest cells of the putamen and rather densely packed. Fibers within the lamina possibly the "en passant" type can be seen to traverse these 'islands' in their course towards the globus pallidus.

It should be mentioned nevertheless that the cells shown in Pl. 16, Fig. D, resemble, at least morphologically, the smallest type of putamen cells. On the dorsal surface,

Plate No. 16

(D) Photomicrograph of a cluster of small cells found in the dorsal half of the external medullary lamina. These small cells resemble the smallest cells of the putamen. They are situated in a region midway between the putamen and the globus pallidus. Note direction of fibers (arrows). Golgi-Fox. 320X.

(E) Two large dendrites their smooth surface indicative of pallidal origin are seen deep within the external medullary lamina. Arrows indicate fibers of passage coursing along these branches. Golgi-Fox. 800X.


D
Put
EML
E
EML
GP

the dendrites of cells along this border penetrate the internal capsule in a similar manner.

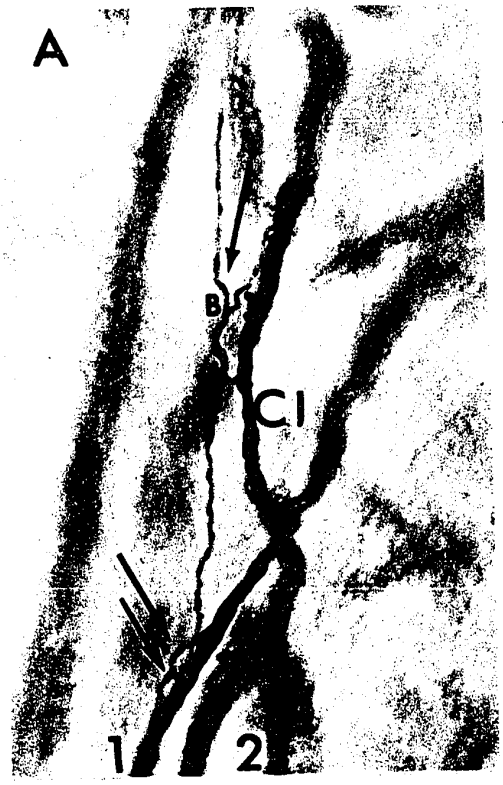
Pl. 17, Fig. A, shows the dendrites (Nos. 1 and 2) of two separate neurons crisscrossing inside the internal capsule. An arrow points to a fiber descending along these two dendrites from the internal capsule. At point (B) a small collateral is given off to dendrite No. 2, the main axon continuing along dendrite No. 1 and making an "en passant" type of synaptic contact with it.

Pl. 18, Fig. A, is a transverse section through the internal medullary lamina. A large blood vessel is seen to course through its centre. On the left, arrows point to a row of cells on the lateral border of the internal part of the pallidum; the cell bodies and main dendrites of these cells are oriented in a dorso-ventral direction, secondary branches can also be seen extending into the stria and at point (D) a few fibers are seen coursing towards the internal segment from the internal medullary lamina. These fibers break up into many branches (double arrows), within the internal segment. Pl. 18, Fig. B, shows two cells in the lower third of the external segment also oriented in a dorso-ventral direction, the main dendrites of these cells extend into the ansa lenticularis and intermingle with dendrites of basalis cells. Secondary dendrites are also given off at point (E) of the same figure and continue within the internal medullary lamina where they interdigitate with dendrites of

Plate No. 17

(A) Monkey, two secondary dendritic branches of cells from the dorsal area of the pallidum are seen within the internal capsule. Arrow shows a fiber from the capsule making contact with both dendrites, at point (B) the fiber gives off a small collateral to dendrite 2, then continues along dendrite 1, making typical "en passant" contacts (double arrows). Golgi-Fox. 800X.

(B) Main dendrite from cell in the centre of the pallidum externum coursing dorsally and latterally towards the external medullary lamina. Note fibers of passage along its course; at points 2 and 3 apparent contacts are made, but at points 1 and 4 in spite of typical swellings no apparent contacts are made with the dendrite at these points. Golgi-Fox. 800X.

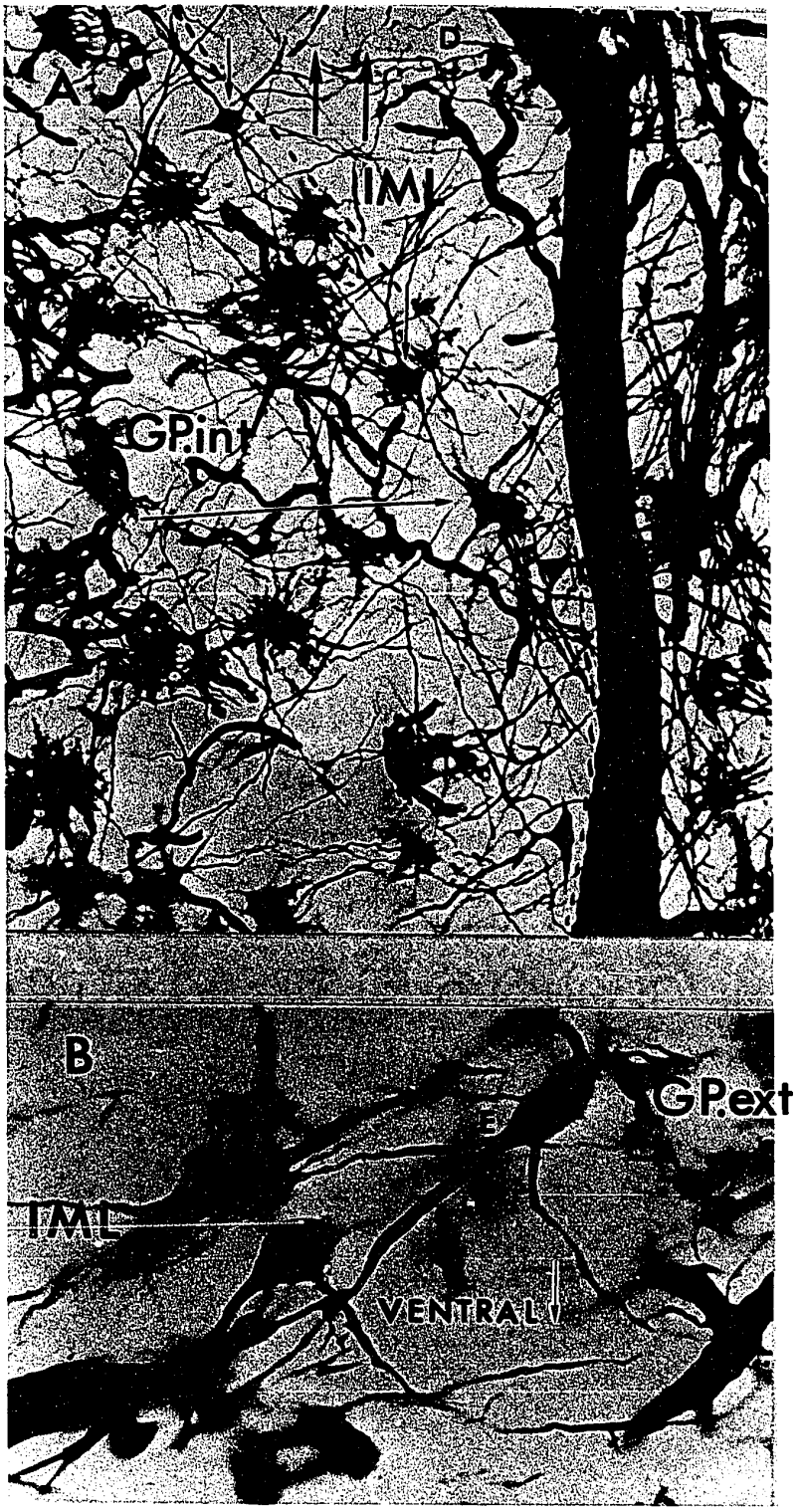

A
B
C1
1
2


B
2
1

Plate No. 18

(A) Monkey, cross section of the pallidum internum - dotted line indicate approximate separation from the internal medullary lamina. Large blood vessel is seen coursing vertically with lamina. Note orientation of cells along the lateral border of the internal segment (arrows). Dendrites are seen to extend off in every direction. Golgi-Fox. 126X.

(B) Two cells are seen on the ventral border of the pallidum externum. Note orientation of cells and dendritic branches. Golgi-Fox. 400X.


A
P
IML
GP.int
B
GP.ext
IML
VENTRAL

the internal segments.

In the central area of the pallidum, a dense neuropil is found in the spaces between the cell bodies and surrounding dendrites. This neuropil contains terminal ramifications which originate from complicated collateral or terminal branches of the many afferent fibers to the pallidum.

Pl. 19, Figs. (C-E), demonstrate some details of the mode and patterns of termination of the afferents to the pallidal cells. Fig. C, shows a small bundle of fibers coursing through the nucleus in a frontal plane. These bundles of fibers which may correspond to Wilson's "pencils" or to Fox's "plexus of nerves" can also be seen in horizontal and saggital planes indicating that they ramify in all directions. Fig. E, indicate the manner in which they travel along the dendrites of the pallidal cells. As shown in this photomicrograph these bundles travel along the course of the dendrites and at certain points (1 and 2) side bundles are given off. These bundles apparently continue along other dendrites. A closer examination of similar bundles indicate that the fibers of these bundles in their course along the dendrites make synaptic contacts, but only at certain intervals. Pl. 17, Fig. B, shows a fiber coursing along a dendrite. At points (2 and 3), there are apparent synaptic contacts but, at points (1 and 4), in spite of the typical swellings in the fiber no apparent contact with the dendrite is seen.

Plate No. 19

(C) Monkey, a large bundle of fibers seen in a cross section of the pallidum internum. Golgi-Fox. 1260X.

(D) Processes of a glial cell to demonstrate differences in the pattern and arrangement as compared to fiber bundles as seen in (C). Golgi-Fox. 800X.

(E) A large dendrite is shown in cross section within the pallidum internum of the monkey. Note manner in which fiber bundles course and branch off (points 1 and 2) along the dendrite. Golgi-Fox. 800X.

C

D

E

Along some of these fibers, groups of bulbous enlargements can be seen which are applied in a "consecutive array" of synaptic contacts around segments of the dendrites, Pl. 19, Fig. E, before branching off within the neuropil.

These patterns of terminals as described above can be classified as the "en passant" type of endings. (See also electronmicrographs Pl. 29, Fig. O and Pl. 36, Fig. 17).

The dendrites of cells in the pallidum are in general almost completely enveloped by synaptic terminals. A somewhat similar involvement of synaptic endings occur on cell bodies. Pl. 20, Fig. D, is a photomicrograph of a cell, (part of which is slightly out of focus) which shows fibers ensheathing the cell body in a "basket like" formation. (See also Pl. 38). Pl. 20, Fig. C, shows a terminal fiber splitting into two before it ends on the cell body.

Higher power photomicrographs of some individual fibers show that in their course through the neuropil, they may emit a single short collateral along its course. In other cases a small "recurrent" type of collateral is given off which seems to travel along, for a short distance, close to the parent fiber, Pl. 21, Fig. E. Pl. 21, Fig. C, shows another type of terminal which ends in a peculiar array of short terminals usually 5 or 6 in a group, but it could not be ascertained with certainty whether this type of ending terminated on cell bodies or dendrites. In Pl. 27, Fig. P and Q, a group of fibers is seen coming together to form a

Plate No. 20

(C and D) Monkey, photomicrographs of two pallidal cells from the pallidum externum. Golgi-Fox. 800X.

(C) Shows the body and dendritic branches of a large pallidal cell. Arrow points to an axon (Ax) which divides into two, just before it makes contact with the cell body.

(D) Another large pallidal cell is seen on which many axons (triple arrows) appear to completely envelope the cell body. A single axon (Ax) is seen to course along the two visible dendrites. A large collateral branch is given off (short arrow).


Ax
C

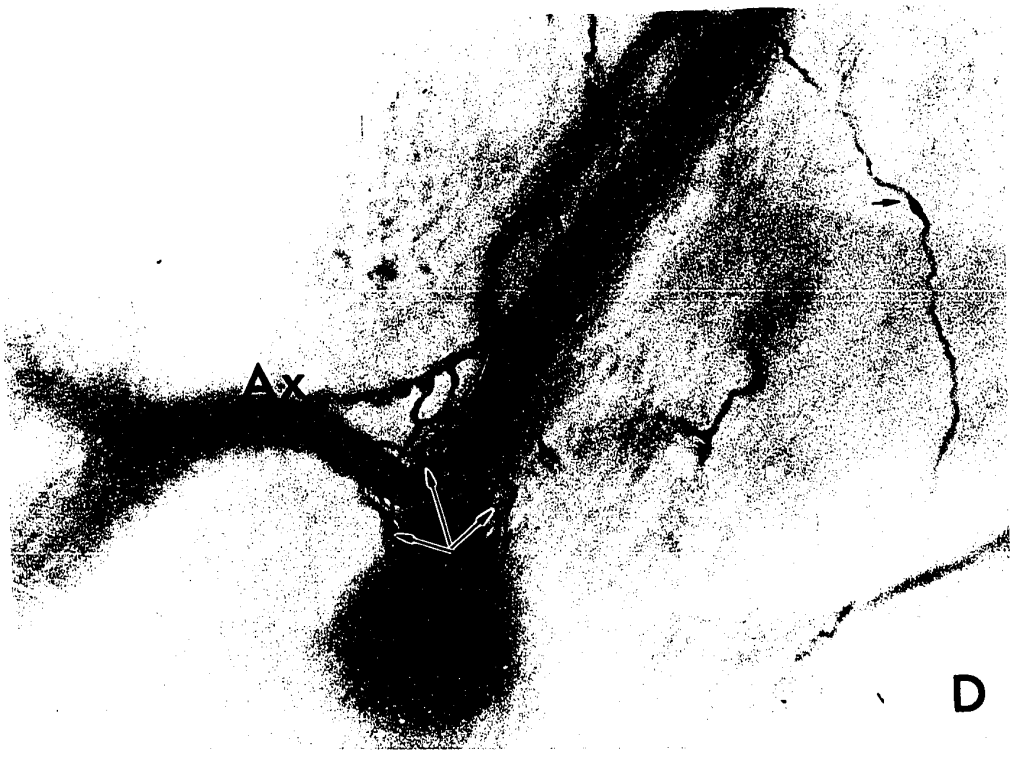

Ax
D

Plate No. 21

(C - G) Patterns of terminal axons within the pallidum externum of the monkey.

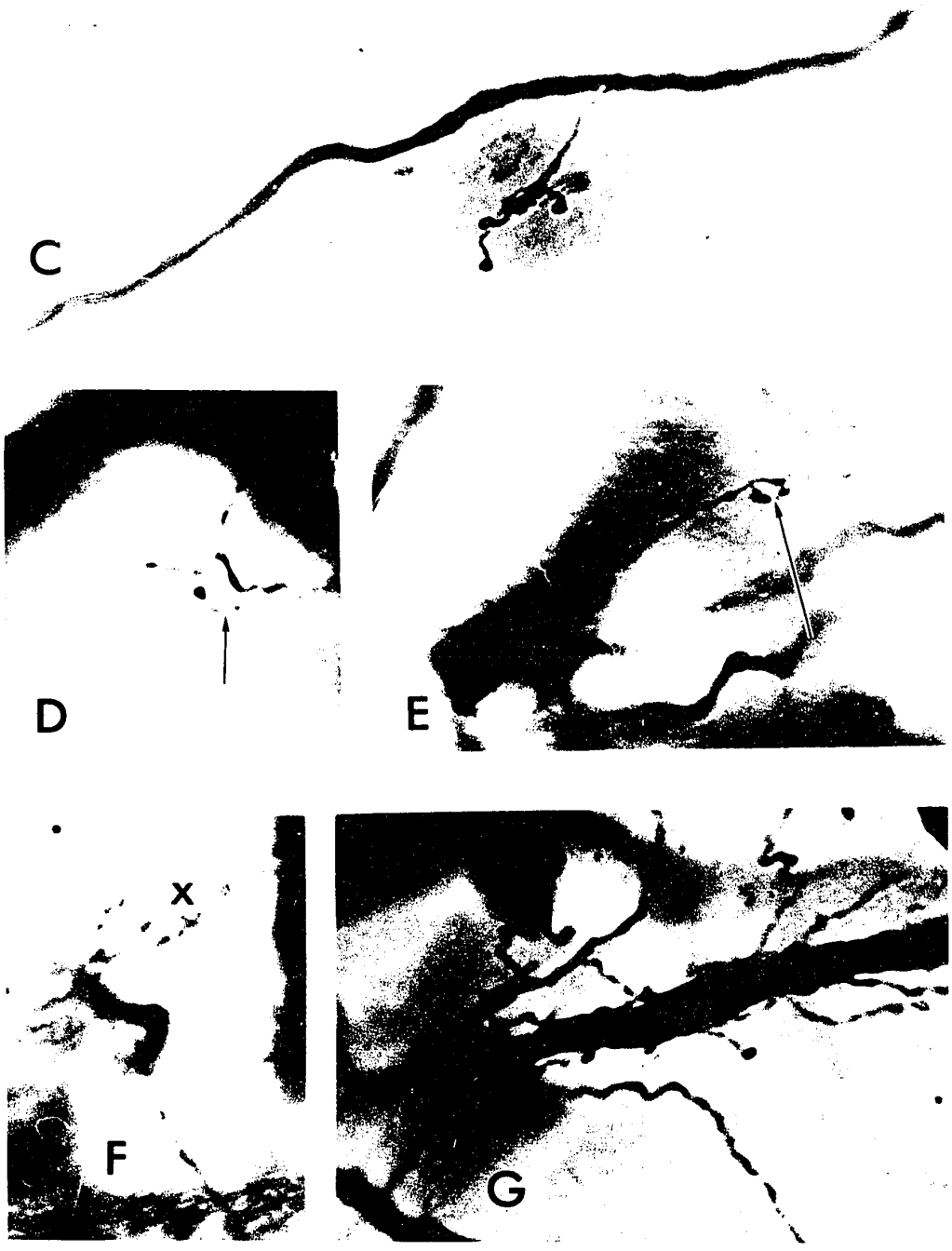
(C) Shows a fiber terminating in a 'grape-like' arrangement. Small collaterals are seen extending off from main axons.

(D) Arrow points to a short collateral given off from a fiber coursing horizontally in the photomicrograph.

(E) Arrow shows another short collateral which appear to course along close to the parent fiber.

(F) Fibers from different directions are seen to meet around point (X). (Compare with E.M. Pl. 28, Fig. P.)

(G) A dendrite is seen coursing horizontally in the photograph. Collateral branches of axons coursing along the dendrite are given off periodically along their course. Golgi-Fox. 800X.


C
D
E
x
F
G

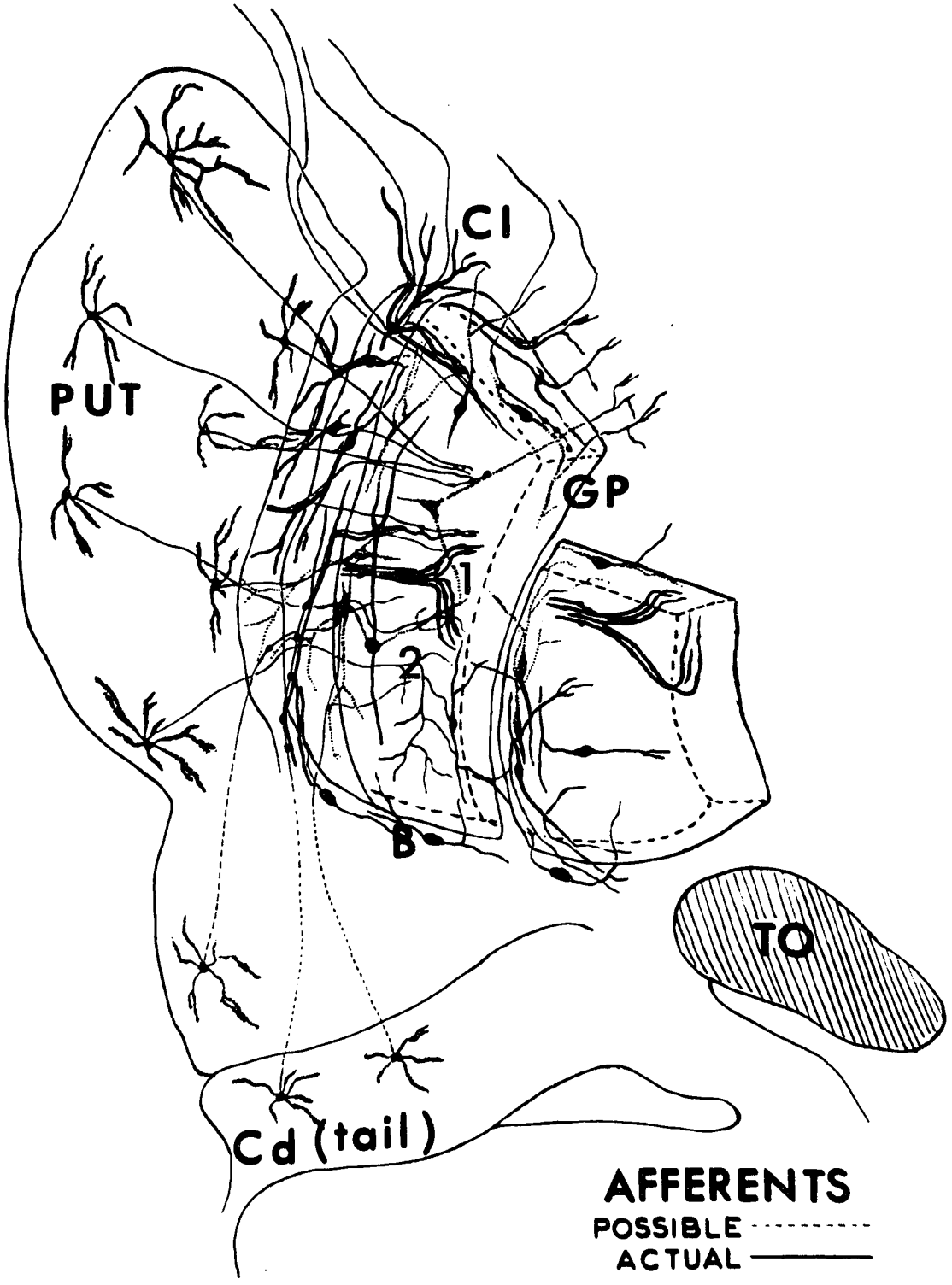
small cluster; the significance of some of these patterns will be discussed in the next section on electron microscopy.

Pl. 22, is a semi-diagramatic tridimensional reconstruction of a typical region of the globus pallidus of the monkey. This reconstruction does not pretend to be accurate in every detail; it is based to a great extent on the observations presented above and represents, therefore a summary of these findings. It should be mentioned that whereas all the photomicrographs of this investigation were made from preparations by the present author, supporting data have been obtained by references to horizontal, frontal and saggital sections, reviewed by the present author, from the many collections of monkey preparations of Dr. C. Fox of the Wayne State University (cited with permission).

The actual course of the fibers within the nucleus was evident on the present Golgi study. However, the origin of these fibers and their course towards the pallidum could not be followed with any degree of certainty by the Golgi method. In this particular aspect, reference was made to the more recent findings of Szabo (1967). An investigation of several Nauta and other fiber stains of the monkey brain was also made from the personal collections of the latter author (cited with permission).

Plate No. 22

Semi-diagramatic tridimensional reconstruction of a cross section approximately at the rostral level of the optic tract. The cells along the lateral border of the pallidum externum, send their dendrites dorsally, ventrally and horizontally into the external medullary lamina. Fibers from the upper and middle parts of the putamen enter the globus pallidus by coursing along the dorsal and horizontal branches. Fibers from the lower part of the putamen and possibly from the tail of the caudate nucleus (dotted lines) ascend into the lower part of the external medullary lamina; course along the ventrally oriented dendritic branches of the pallidal cells to enter the globus pallidus. Fibers from the putamen course through the pallidum as fine fiber bundles both in the external and internal segments (1). They are seen to ramify in every direction. Other fibers seen within the pallidum break up into many branches (2). Stippled neurons represent deeper lying cells. Fibers from the internal capsule enter the pallidum by coursing along the dendrites which protrude within it from cells along the dorsal border of the globus pallidus. Dendrites of basalis cells (B) at the ventral border of the pallidum, intermingle freely with dendrites of pallidal cells.


CI
PUT
GP
1
2
B
TO
Cd (tail)
AFFERENTS
POSSIBLE
ACTUAL

Electron Microscopy of the Normal Structure of the Globus Pallidus

The capricious nature of the Golgi methods may often lead to erroneous interpretation. However, when this method is used in conjunction with electron microscopy a greater degree of reliance in the interpretation of the results may be obtained. But any such comparisons must be made with extreme caution, due largely to the differences in preparations and sectioning of material in these two obviously disimilar techniques. In this study therefore, only the apparent similarity of the structure as revealed by these two methods will be considered.

Large Nerve Cells in the Entopeduncular Nucleus and in the Globus Pallidus of the Cat

Light microscopy has revealed that the cells in the entopeduncular nucleus are mainly of the large type, spindle shaped or triangular. The globus pallidus on the other hand contains cells that are relatively smaller in type than those of the former nucleus. The major part of the globus pallidus however was seen to be taken over by cells of the nucleus basalis of Meynert; at least in the more caudal levels of the nucleus.

Under the light microscope it was quite easy to distinguish basalis cells from pallidal cells. Under the electron microscope however easy distinction between these two types could not be made. The fine structure of most of the cells encountered in both the entopeduncular nucleus and

in the "pallido-basalis" complex (Pl. 23 and 24), appear identical. The nucleoplasm in these cells appear, very coarse and light. The nuclear form is much distorted by many indentations, so that the nuclear membrane has an irregular contour. The Golgi apparatus in these cells is quite prominent and consists of the usual well known structural components of this cell organelle. The granular endoplasmic reticulum appear to be restricted, but not entirely to each pole of the cell. Large vacuoles can also be seen scattered throughout the cytoplasm. Neurotubles and neuro-filaments are fairly numerous especially at the dendritic off shoots (Pl. 38).

All these components inside the cell membrane varied somewhat from cell to cell, but these differences were so slight that it was difficult under the electron microscope to make any definite distinction between the cells, of the globus pallidus, nucleus basalis and the entopeduncular nucleus.

The characteristic feature of cells in these nuclei as pointed out by Mori (1966), is that the greater surface area of their somas in addition to their dendritic processes, is covered with numerous axon terminals of various kinds. In this study synaptic terminals on cell bodies were variable, many sections showed parts of cell bodies that were mainly covered by synaptic terminals, but in other sections cell bodies were seen on which only a few of these terminals were apparent. The areas on the soma devoid of synapses were occupied by glial processes.

Plate No. 23

Electron micrograph of a typical cell in the entopeduncular nucleus of the cat. Nucleus (N) is centrally located and accumulations of granular endoplasmic reticulum (Nissl subs) (ER) appear to be more concentrated at each pole of the cell. Note glial cell (Mg) and one terminal bouton (t) double arrow. 15,000X.

N
ER
Mg

Plate No. 24

Electron micrograph of a cell in the pallidum externum showing the typical morphology of the cytoplasm. G.C.-Golgi complex. E.R.-granular endoplasmic reticulum. Mv-multivesicular body. N-nucleus. 24,000X.

Neuropil in the Entopeduncular Nucleus and Globus Pallidus

Plate 25 is an electron micrograph of a typical cross-section passing through the neuropil of the entopeduncular nucleus of the cat. Many dendritic trunks (Dp) which have been cut transversely appear completely surrounded by a number of terminals (arrows), showing characteristic aggregations of mitochrondia, synaptic vesicles and thickening of the apposed membranes between terminals and dendrites. This axo-dendritic complex appear to be the most common in the entopeduncular nucleus and in the globus pallidus.

A detailed examination of Pl. 25 shows that many axon terminals surround and make contact with dendrites of every calibre, as evidenced by the diameters of these dendrites. These terminals, appear in general to ensheath the entire length of most of these dendrites. Nevertheless, a small number of dendrites can be found on which synaptic terminals are not so numerous. Small areas on these dendrites have no synaptic terminals; these areas being taken over by glial processes. (Pl. 28; Fig. W).

Synaptic Organization

In the subsequent series of micrographs, further attempts will be made to correlate some of the particular features observed in electron micrographs with studies made with the Golgi method.

Pl. 26, Fig. R, is an electron micrograph of a longitudinal section through a bundle of fibers, which can be

Plate No. 25

Electron micrograph of a typical section through the neuropil of the entopeduncular nucleus of the cat. Cross sections of many dendrites (Dp) can be recognized among the numerous myelinated (MYf) and unmyelinated (UNf) fibers. Note also the different calibre of the unmyelinated fibers. Large arrow on right hand side points to some of the larger calibre fibers. These dendrites are in most cases completely surrounded by terminals (small arrows). Surrounding these profiles are sheaths of glial processes (Gf). Large arrow on the left hand side indicate a small cluster of terminals forming a "cap" at the distal end of a dendrite (Dp). Surrounding this small "cluster" is a glial process (seen as a white band) just above star symbol. Small curved arrow, points to the terminal end of the dendrite (Dp). 11,000X.

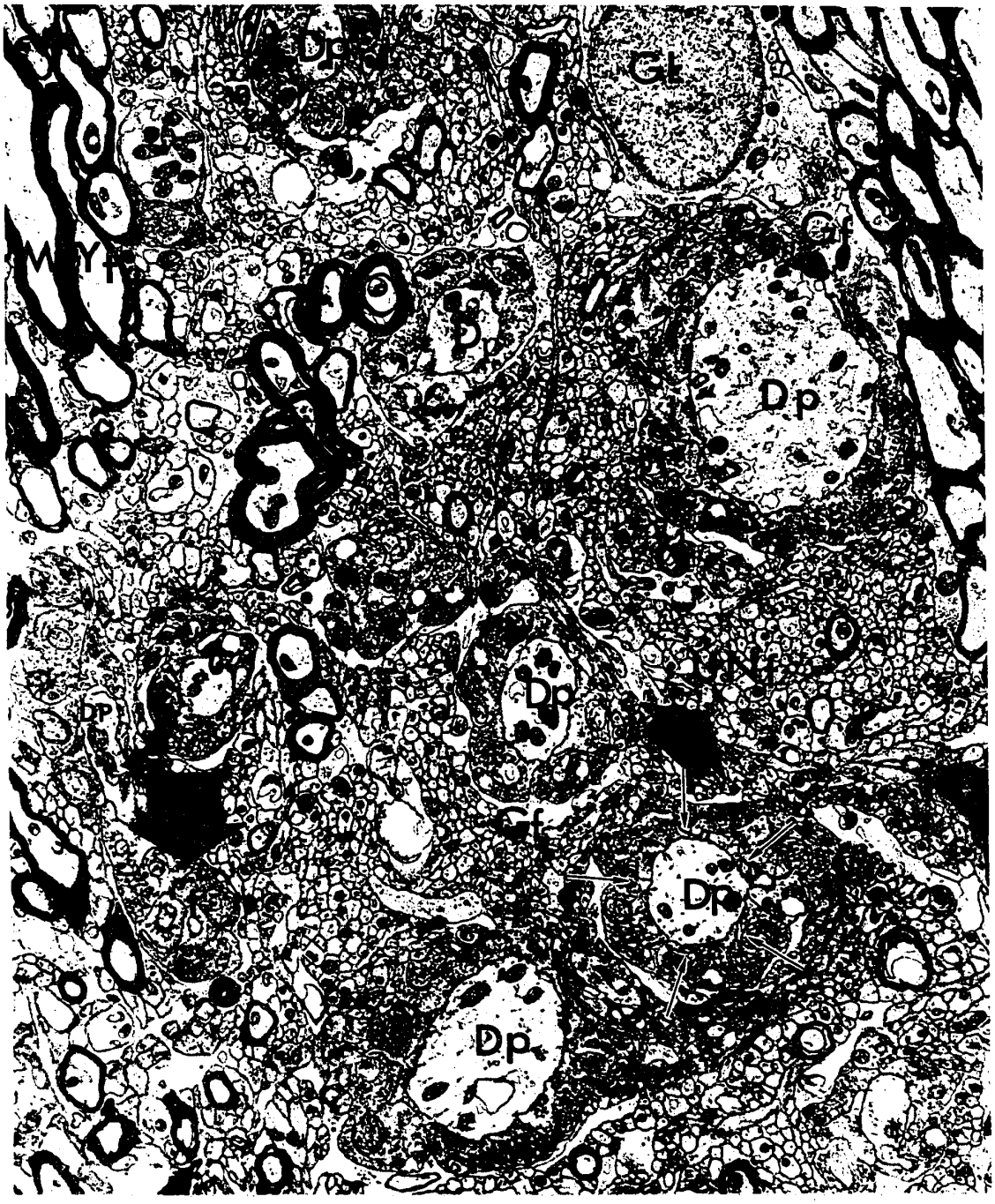
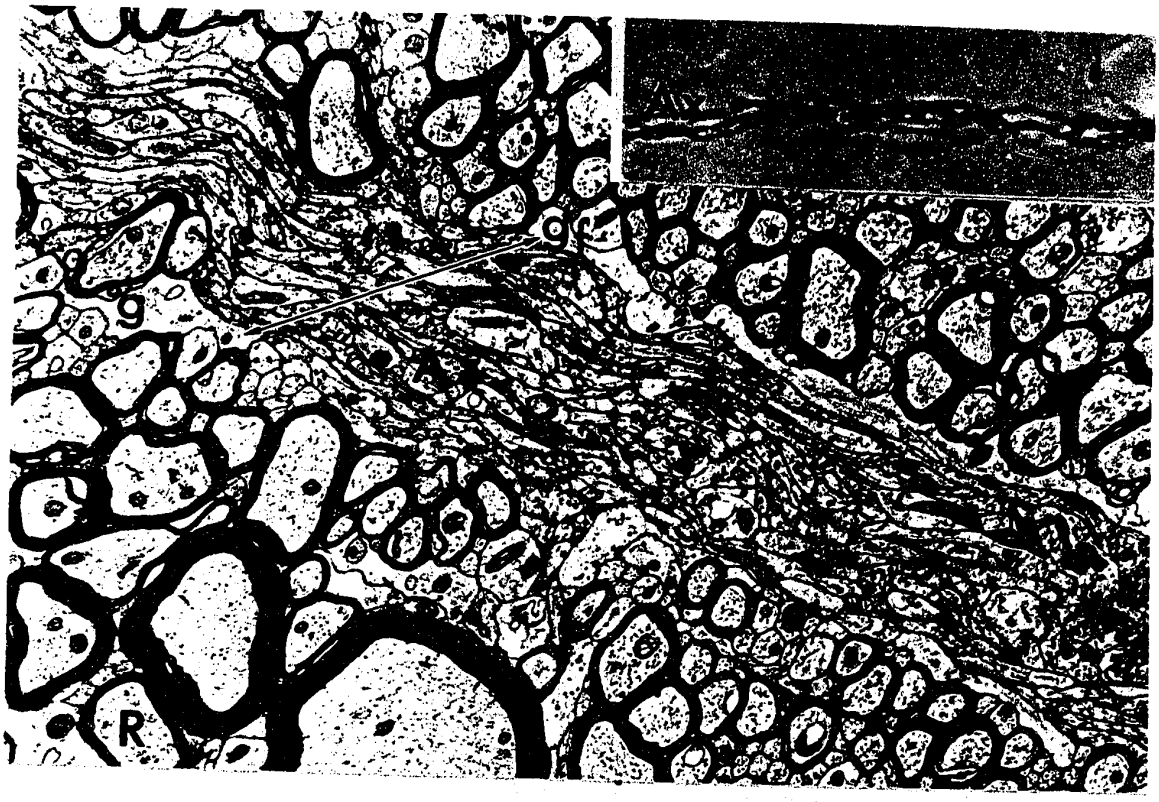

Dp
Gl
Myf
Dp
Dp
Dp
Dp
Gf
Dp
Dp
Dp

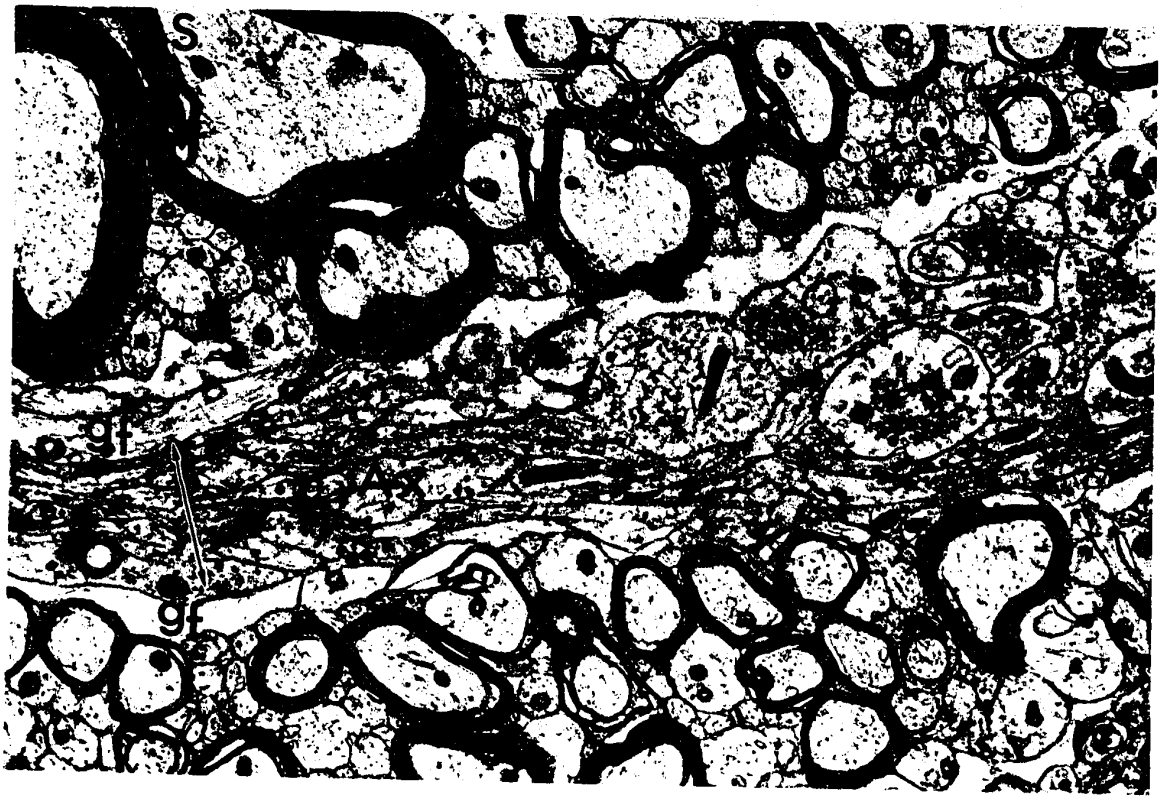
Plate No. 26

(R-S) Cat, electron micrographs of two longitudinally cut bundles of fibers in the entopeduncular nucleus. These bundles of fibers are ensheathed by glial processes (Gf) throughout their entire length, in most cases.

(R) Star indicates synaptic vesicles within the bulbous part of the axons, compare with Golgi section (insert). E.M. 11,300X. Golgi-1260X.

(S) A slightly higher magnification of the terminal part of one of these bundles. The fibers (Ax) are seen to terminate as typical bulbous endings (t) on or near dendrite (D). Synaptic vesicles of various types can be seen in these terminal endings. 16,000X.


g
g
R


S
gf
gf
Ax

recognized by the bulbous thickenings along their course. Within these thickenings numerous synaptic vesicles (star) can be found. Pl. 26, Fig. S, is another section along such a bundle. Many of these fibers are seen to terminate in typical bouton-like endings (t) around the dendrite (D). Surrounding these fiber bundles are glial processes (gf) which seem to encase these bundles like an "insulated cable." This glial ensheathment appear to persist throughout most of the extent of these bundles.

Pl. 27, Fig. P, shows two different fiber bundles (f_1 and f_2) which meet in a "cluster-like" formation (large arrow). This formation appears to represent another view of a bundle of fibers of passage. A somewhat similar arrangement of fibers is seen in the Golgi micrograph (inset), which shows a bundle of axons with their bulbous enlargements forming a cluster in the middle of the fiber bundle. In Pl. 27, Fig. R, a single collateral (stippled) is seen to extend from the main fiber (Ax); typical of many fibers coursing through the neuropil (compare with Golgi preparation inset).

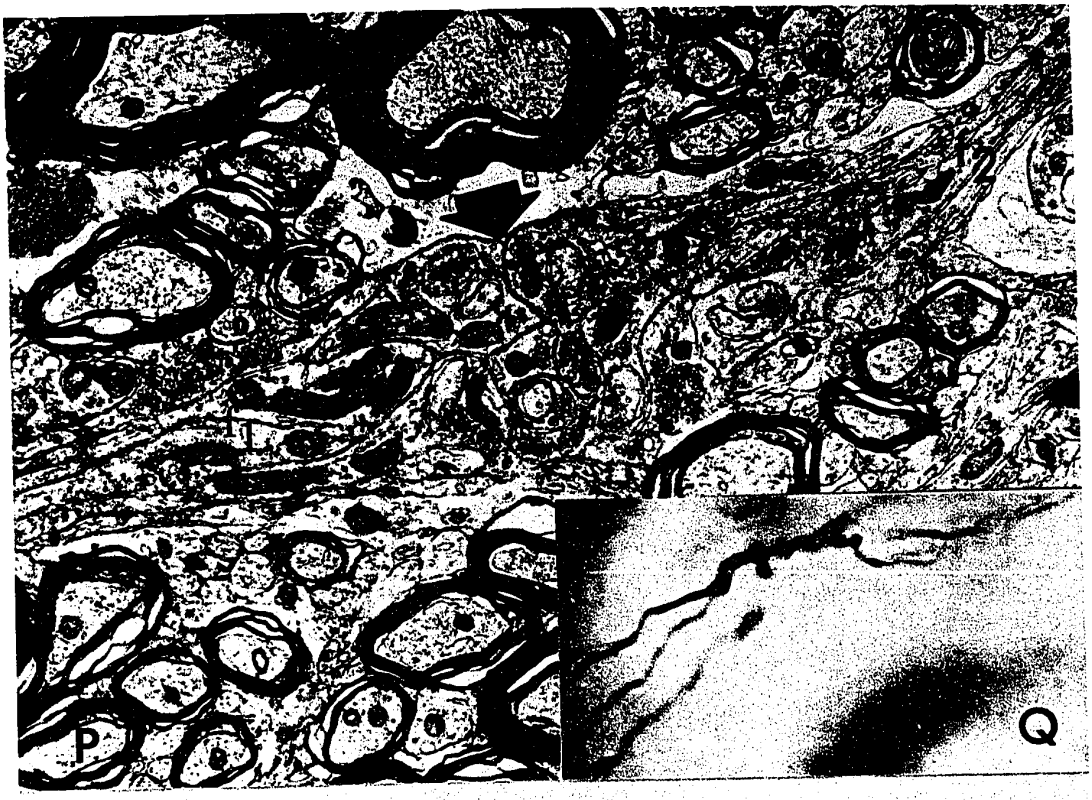
Occasionally in this study axon to axon contacts were encountered (Pl. 28, Figs. X and Y and Pl. 41, Fig. 28). At many of these junctions there were no apparent concentration of synaptic vesicles in contact with the slightly thickened membranes. It appears therefore, that this junction represents simple attachment points between similar adjacent terminals. Valverde (1966), who investigated by serial sections, similar attachment points in the cells of the

Plate No. 27

(P) Electron micrograph of two fiber bundles (f_1 and f_2) in the entopeduncular nucleus which appear to meet at arrow. 15,000X.

(Q) Inset photomicrograph of an apparently similar Golgi preparation. 800X.

(R) Electron micrograph of a section of the entopeduncular nucleus, arrow points to a fiber of passage giving off one small collateral (t). 15,000X.
The Golgi picture (inset) appear to be similar. 900X.


P
Q

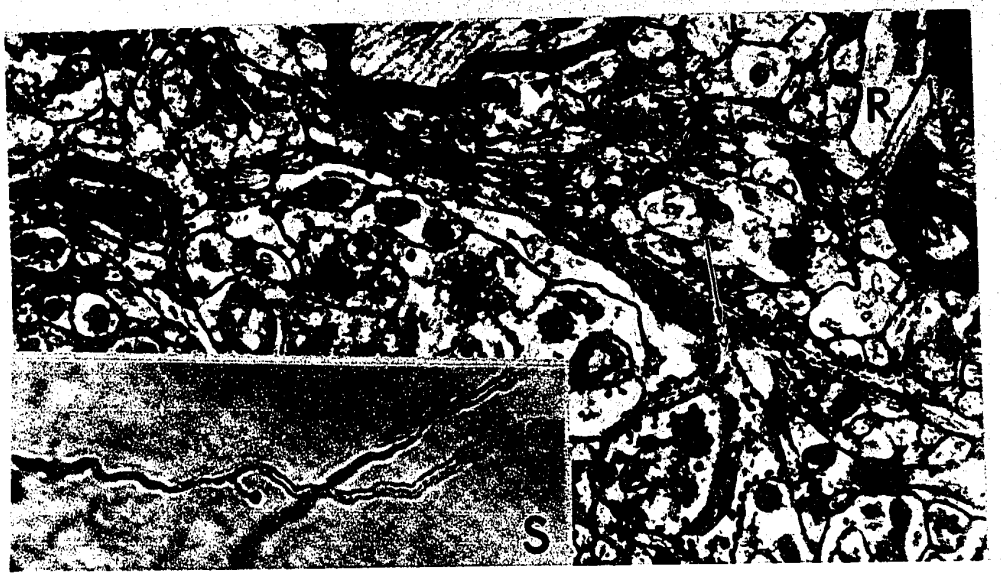

R
S

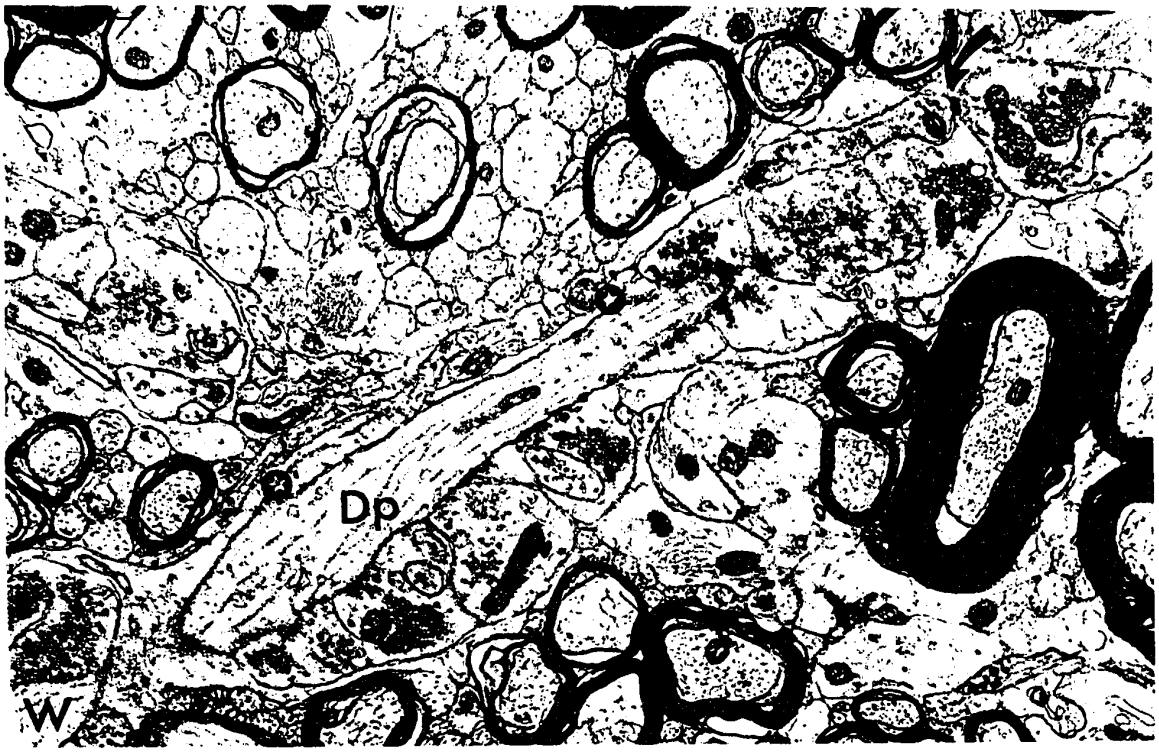
Plate No. 28

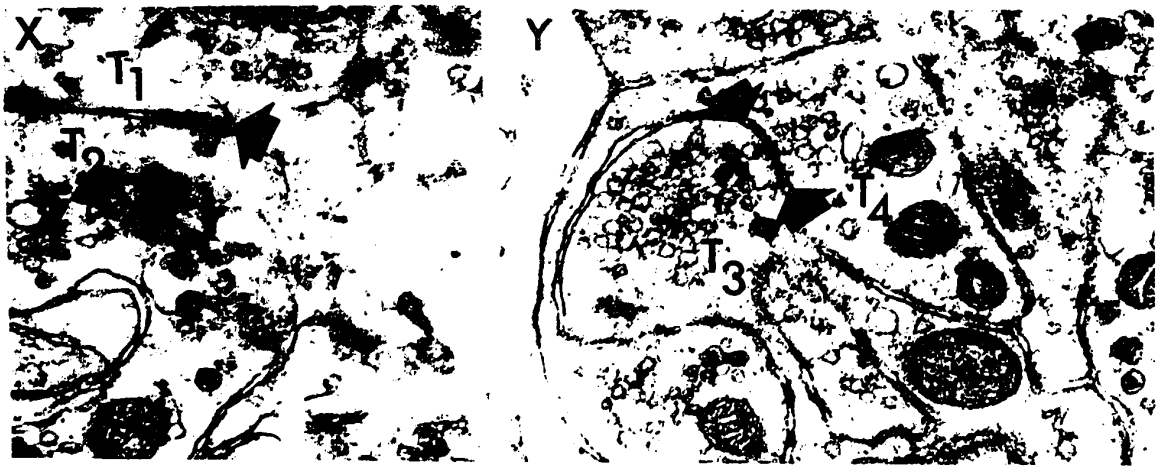
(W,X,Y) Electron micrographs of different types of terminals seen in the entopeduncular nucleus of the cat.

(W) Arrow indicates desmosome-like attachment between a preterminal ending and a terminal bouton. Note also between stars the dendrite (Dp) is free of synaptic contacts. 18,000X.

(X) Shows two terminals (T_1 and T_2), arrow shows the desmosome-like attachment between them, no synaptic vesicles are seen near either membrane thickenings. 45,000X.

(Y) Arrows point to apparent contact between T_3 and T_4. Note closer approximation of synaptic vesicles to the membrane in T_4. 45,000X.


Dp
W


X
T1
T2
Y
T4
T3

posterior column nuclei showed that these axo-axonal junctions proceed from a single fiber, which digitates in the form of clusters of two, three or more boutons having attachment contacts between them. This latter observation of Valverde correlates well with the "grape-like" formation of boutons seen in the present Golgi preparations (Pl. 21, Fig. C).

On the other hand rounded profiles of cell processes containing vesicles can be found in which they share a typical synaptic specialization that may be suggestive of an "axo-axonal" contact (Pl. 28, Fig. Y, and Pl. 41, Fig. 28). In these electron micrographs synaptic vesicles can be seen to accumulate on both sides of the membrane thickenings of these contacts. In Pl. 28, Fig. Y, the synaptic vesicles, appear nearer to the membrane in terminal T_4 than they are in terminal T_3.

Since these types of terminals occur very infrequently, additional study is required to clarify the true nature of this type of contacts in the pallidum; possibly utilizing serial sections.

From the preceding observations, there appears to be a fairly good agreement between the Golgi findings and those of electron microscopy. For example, the typical "en passant" type of synaptic contacts, the beaded appearance of some of the smaller calibre dendritic branches, the short single collateral fibers given off from the larger axons, the "grape-like" clusters of short axon terminals. The dimensions of the majority of fibers measured from the Golgi preparations were

approximately in the range 0.4 -1.0 μ; the dimensions of the finely myelinated and unmyelinated fibers measured from the electron micrographs were in the range 0.8 -1.2 μ and 0.1 - 0.6 μ respectively.

All the above structures observed in Golgi preparations correspond fairly well with profiles of the same order of magnitude in the electron micrographs.

In addition to the above, a careful examination of the electron micrographic material on the pallidum, revealed two additional important facts. Apart from the typical axo-somatic and axo-dendritic synapses, terminal boutons were seen to make synaptic contact with dendritic spines in two definite ways. In the first type the bouton can be seen to surround entirely the spine as seen in Pl. 29, Fig. P. In the second, the bouton may make contact with both the spine and the dendrite simultaneously as illustrated in Pl. 36, Fig. 16. However, the latter observation must be viewed with some caution, since only serial sections can varify this type of relationship. This question requires further study.

Terminal endings in the central nervous system have been classified recently according to the type of synaptic vesicles within them. Bodian (1966) reported three types of synaptic vesicles in the spinal cord of the cat, while Mori (1966) classified at least five types in the corpus striatum of the rat.

In this investigation three distinct types of terminals (Pl. 30, Figs. D and E, T_1 T_2 and T_3) with different kinds of synaptic vesicles within them were found in the

Plate No. 29

(N) Electron micrograph of a dendrite (Dp) in the pallidum of the cat. 15,000X. Note apparent swellings at each pole, with large accumulations of mitochondria, compare with (N) a photomicrograph of a secondary dendrite. Golgi method. 320X.

(O) Longitudinal section of a dendrite (Dp) showing continuity of one bouton en passage (stippled). 15,000X.

(P) Electron micrograph showing typical axo-spinal contact (arrow). 15,000X.


Dp
N
Dp
M
Dp
P

Plate No. 30

(D - E) Electron micrographs of three different types of terminal boutons (T_1, T_2, T_3) seen in the entopeduncular nucleus of the cat. 46,500X.

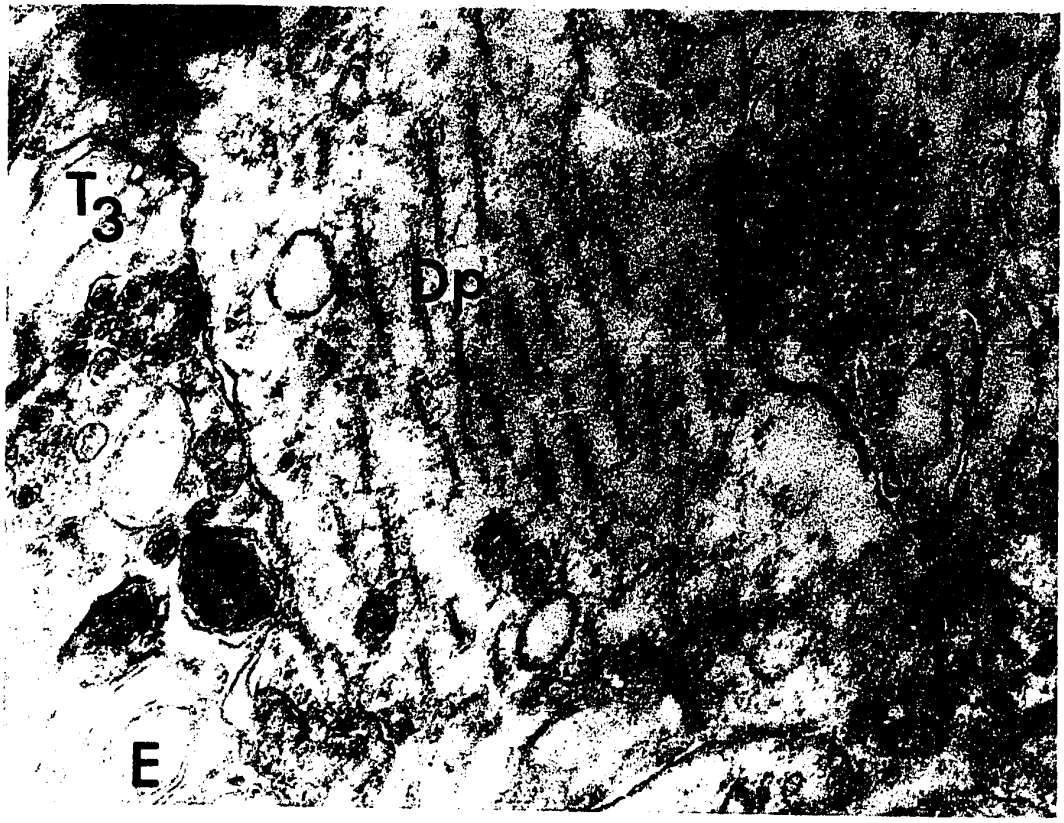
Approximate dimensions of these different types are:

T_1 - 285-450 Å

T_2 - 500-800 Å

T_3 - 800 Å and above


T1
T2
D


T3
Pp
E

pallidum of the cat. The first two types of terminals are very frequently found in other areas of the central nervous system. The type of synaptic vesicles as shown in terminal T_1 corresponds to Mori's (1966) γ-terminals. The vesicles in these terminals are small, light and fairly round of 285-450 Å in diameter. Terminals with larger uneven synaptic vesicles, type T_2 (500-800 Å in diameter) are also found and these should correspond to Mori's (1966) δ-terminals. Both of these types were present on dendrites as well as on somas of neurons. Synaptic endings (T_3, Fig. E, Pl. 30) which mainly contain large dark-core vesicles (800 Å or more in diameter) are exceedingly rare and are mostly found around dendrites. Synaptic terminals incorporating all three types of vesicles were also found with equal frequency.

The functional significance of these different types of terminals remain to be clarified and are presently under active study by Bodian (1966), Laramendi (1967) and Urchizono (1967).

Neuroglial Cells

There were three main types of glia found in the pallidum. Pl. 31 demonstrates the morphology of these cells which can be classified as oligodendroglia and astroglia according to the criteria of Gray (Ref. Kurtz, 1964). Another type of glial cell shown in Pl. 23 and Pl. 31, Fig. P, and labelled (MG) can be easily distinguished from the other two types by the darker staining characteristics of both the cytoplasm and nucleus. These cells were found around the cell bodies of neurons or scattered profusely throughout the neuropil. They are also quite frequently seen in close contact with oligodendroglia cells. Whether these cells are transitional

Plate No. 31

(P-Q) Electron micrograph of three different types of glial cells in the pallidum of the cat. 18,000X. (MG) Microglia? (OL) Oligodendroglia (AS) Astrocyte. 18,000X.

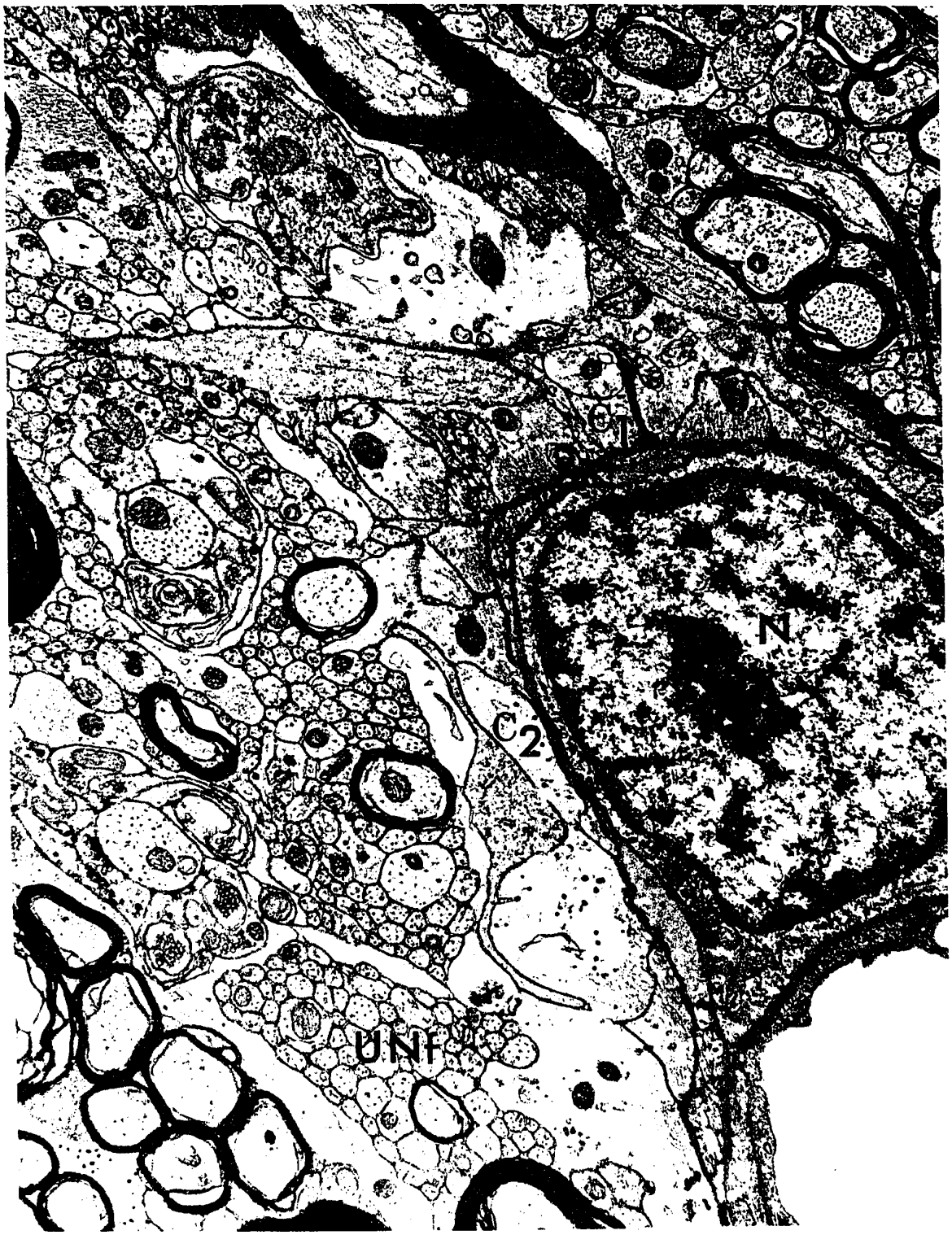
(Q) Arrow points to fibrous process of astrocyte (AS) encircling both the dendritic process (Dp) and the many synaptic terminals surrounding it.

P
MG
BL

Q
AS
DP

Plate No. 32

Electron micrograph showing astrocytic processes surrounding a capillary at point (C_1) and lighter staining processes containing dark granular substance in addition to fibrillar material, surrounding the capillary at point (C_2). Note also its ensheathment of a small bundle of unmyelinated fibers (UNf). (N) nucleus of pericyte. 24,000X.


C1
N
C2
UNI

forms of the former types (Mori, 1966), or microglia cells could not be ascertained. The oligodendroglia cell differed from these unknown types by the lighter and coarser granular appearance of its nucleus, which also contained smaller patches of dark granular material scattered randomly throughout the nucleus. The cytoplasm was also much lighter stained than the latter. Two kinds of astrocytes (AS) were found. A fibrous type and a protoplasmic type. These cells can be recognized by the lighter and finer granular appearance of their nuclei, the cytoplasm contains, in addition to the usual organelles, many bundles of fibrillar materials. The processes of cells of the fibrous type, can be seen to ensheath both dendrites and terminals (Pl. 31, Fig. Q). Cells of the protoplasmic type, send out processes which terminate around capillaries, in the form of "perivascular end feet" (Pl. 32).

Whether any or all of these types of cells perform a phagocytic function could not be ascertained, and this question remains to be clarified by further study.

Although electron microscopy has substantiated to some extent concepts derived from light microscopy of the structure and localization of synaptic contacts, the intrinsic organization of the globus pallidus might attain additional meaning if the mode and patterns of terminations within this nucleus could be related to specific afferent systems. In order to achieve this objective a series of controlled lesions were placed in various areas of the cat brain, and the subsequent degeneration in the globus pallidus was studied by electron microscopy. The results of these experiments are presented in the next section.

Experimental Observation

Before describing the lesions made in different areas of the cat's brain, it appears necessary to comment briefly on the respective photographs of these lesions as seen in Pl. 32, 34, 37 and 40.

Before the blocks for the structures under study were processed, the lesions were grossly verified immediately following the perfusion. This procedure necessitates slicing the brain into 2-3 mm pieces around the approximate area of the lesion. Once the lesion is judged satisfactory, small blocks must be removed in the appropriate areas under study and in many cases the areas to be removed are situated at the same level as the lesion, in which case the small slices are further mutilated, by the removal of these small blocks making it virtually impossible in most cases to cut thin frozen sections from the remaining thin slabs for photography.

In this investigation the following procedure was used to minimize this problem of sectioning. A slab of gelatin (made from a 5% solution) is first positioned on the microtone and frozen in the usual way. The thin slice of tissue is then placed on the frozen gelatin and sectioned as usual. In spite of this precaution it is difficult to prevent some distortion of the tissue once the dry ice is placed around it. In cases in which the block was taken from the same frontal level as the lesion, some fragmentation

of the consequently sectioned brain material was often unavoidable. When the lesion extended into other structures; the degeneration of which might interfere with the results, the brains were discarded.

Lesions in the Substantia Nigra of the Cat

Pl. 32 illustrates three successful lesions placed in the substantia nigra. In each case the coagulating electrode entered the nucleus laterally at an angle to the horizontal plane, after passing through the temporal cortex and the medial geniculate body. In cats SNP 3 and 5 the coagulating current was applied first in the medioventral part of the nucleus, then the electrode was withdrawn 2 mm and the coagulation repeated. In SNP 4 the coagulation was restricted to the dorsolateral part of the nucleus.

In each case, the lesion in the substantia nigra was quite extensive. Concomitant damage to the cerebral peduncule was present but tegmental structures were spared.

The criteria of determing terminal degeneration electron microscopically, is based on the observations of many investigators who have studied degeneration in other areas of the central nervous system. Among these are Colonier and Gray (1962) and Colonier (1964) who studied degeneration in the rat cerebral cortex; Szentagothai (1966), the lateral geniculate body; Smith et al (1966) the cerebellum.

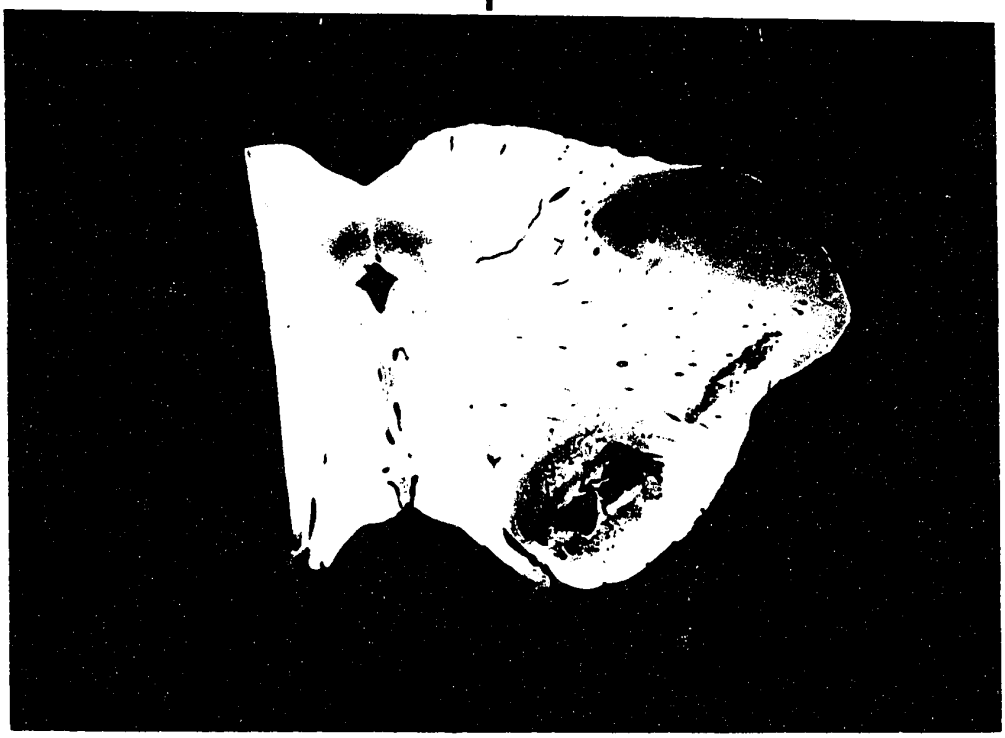
Plate No. 32

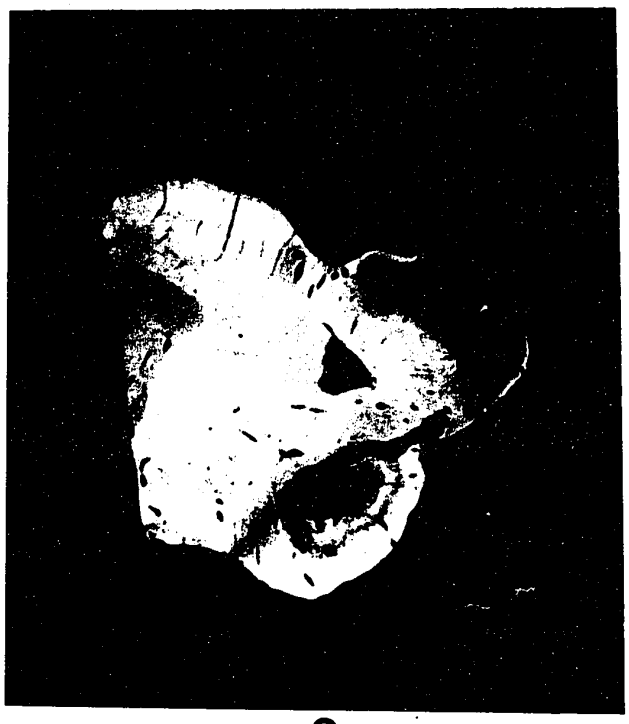
Figs. (1-3) Photomicrographs of lesions in the substantia nigra of the cat. 5X.

(1)(3) Cats SNP 3 and 5, the lesions destroyed most of the medial and lateral areas of the nucleus, some involvement of the cerebral peduncle can also be seen.

(2) Cat SNP 4, the lesion was restricted to the lateral aspect of the nucleus.

1





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Degenerative Synaptic Changes

The most extensive destruction to the substantia nigra is evidenced in cat, SNP 3 (killed after 5 days). In spite of this extensive damage to the nucleus, Pl. 33, shows the typical sparse number of degenerating terminals evidenced throughout the entopeduncular nucleus.

One type of degenerative change seen five days postoperatively is shown in Pl. 33, Figs. 4 and 5 (arrows). The degenerating terminals (Dt) is filled with clumped synaptic vesicles having greater electron density than normal; another degenerating terminal, Fig. 7 (double arrow), shows many electron-dense fragments possibly the end products of mitochondrial disintegration.

In cat SNP 4 (killed after 6½ days) the degenerative changes that occur after this period is shown in Pl. 33, Fig. 6. The synaptic vesicles frequently aggregate in one large clump giving a honeycomb appearance. This clump of vesicles is usually situated in one region of the terminal, leaving the remainder almost empty. A similar observation was made by Smith (1966) in the cerebellum of the cat. Nevertheless, it was not uncommon to observe a few boutons showing the same degenerated appearance as seen after a five-day lesion. In none of these degenerating terminals was there any evidence of neuro-filaments.

Regardless of the appearance of the degenerating boutons, they were seen to synapse mainly on dendrites.

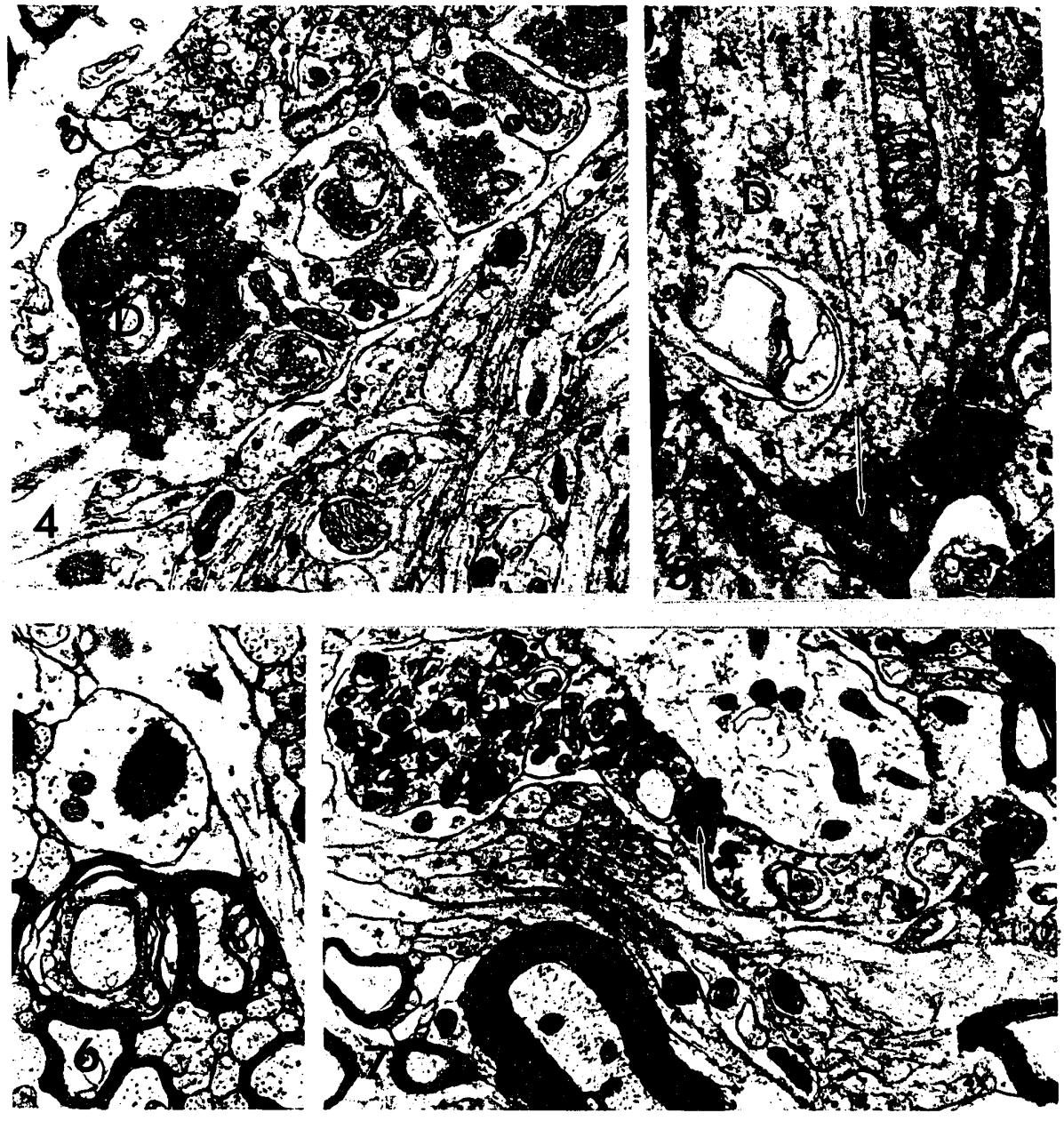
Plate No. 33A

Figs. (4-7) shows various forms of degenerating terminals seen in the entopeduncular nucleus after destruction of the substantia nigra of the cat.

(4 and 5) Degenerating terminals (Dt) from material taken five days after placing a lesion in the substantia nigra, (arrow) points to swollen mitochondria(m) in Fig. 5. Fig. 4-18,000X; Fig.5-35,000X.

(6) Shows a degenerating preterminal bouton 6½ days after a substantia nigra lesion. The synaptic vesicles are clumped in one region of the terminal leaving the other areas bare. 18,000X.

(7) Shows another degenerating terminal (single arrow) synapsing on dendrite (D). Double arrow indicate another degenerating terminal containing many electron-dense fragments. 17,800X.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Axosomatic synapses were not encountered on any of the 100-150 neurons in the brains with substantia nigra lesions.

Degenerative Changes in Axons and their Myelin Sheath

A detailed examination of the degenerated axons indicate that only the finely myelinated and unmyelinated fibers showed any sign of degeneration. There was no clear cut indication of degeneration of the heavily myelinated type of fibers.

Putamen Lesions

Pl. 34, shows three lesions placed in the putamen of the cat. In PUP 1 and 3 the lesions were restricted to the most lateral and basal parts of the putamen. The coagulating electrode was introduced at various angles in the frontal plane. The purpose of this manoeuver was to prevent any extensive damage to the internal capsule. Photographs of these two lesions indicate that the electrode after entering the head of the claustrum descended within the extreme capsule. In addition to a lesion in the lateral part of the putamen, it destroyed a large section of the claustrum. In PUP 2 the electrode was again introduced vertically, but in a slightly dorso-rostral direction, the resulting lesion was restricted to the most dorsal part of the putamen just slightly lateral to the internal capsule.

Plate No. 34

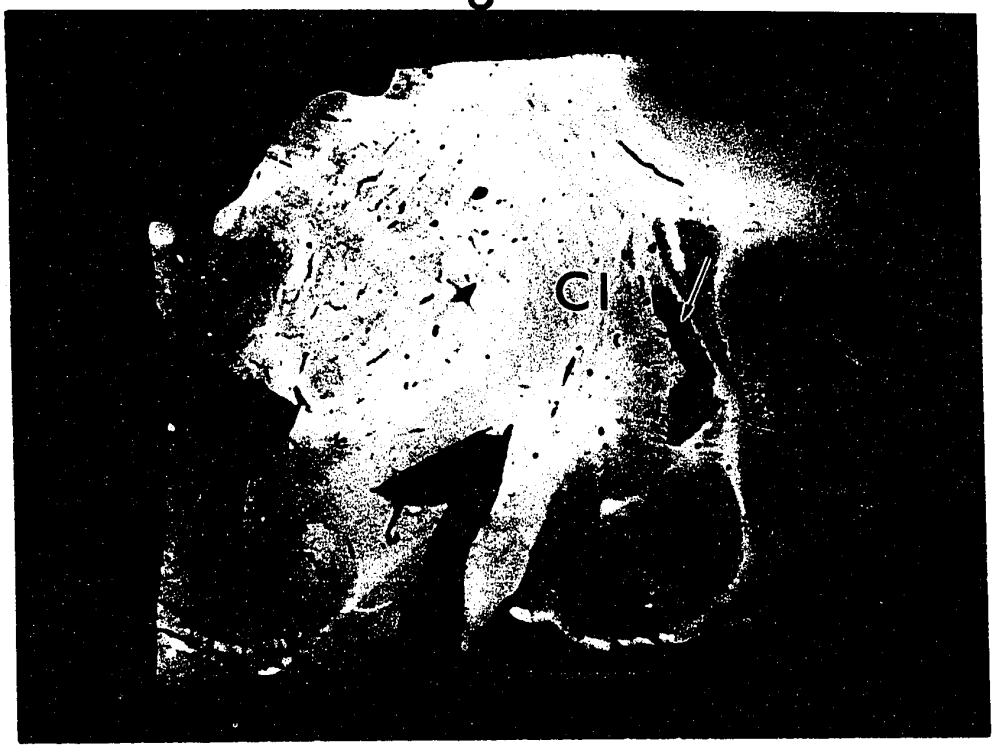
Figs. (8,9,10) Photomicrographs of lesions in the putamen of the cat. 5X.

(8) Cat PUP 1, the lesion (arrow) destroyed mainly the lateral aspect of the nucleus. The electrode also damaged the rostral part of the claustrum and also infringed upon the extreme capsule.

(9) Cat PUP 2, the lesion (arrow) damaged the dorsal tip of the nucleus mainly.

(10) Cat PUP 3, the lesion (arrow) destroyed the lateral part of the putamen, and involved a large area of the claustrum.

8



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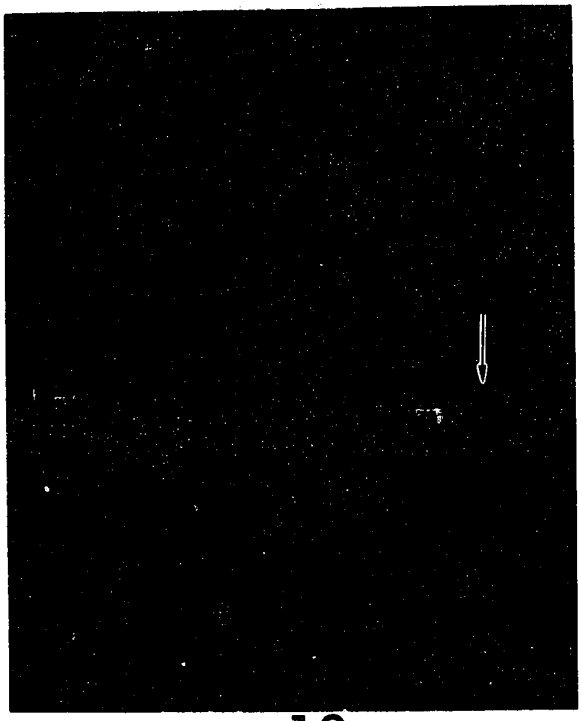

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Degenerative Changes in Synaptic Terminals

Plates 35 and 36 show some of the characteristic changes seen after putamen lesions. Mitochondrial changes, together with the "honey-comb" appearance of the bouton, are in general reliable signs of degenerative changes. Plate 35, Figs. 12 and 13.

Unlike lesions in the substantia nigra, degenerating boutons can be seen both on the dendrites and on the soma of cells within the entopeduncular nucleus and in the globus pallidus. Efforts to determine the type of synaptic vesicles within these degenerating terminals were made extremely difficult by the changes these vesicles undergo during degeneration. However, in the early stages of degeneration it appears that only the types T_1 and T_2 as shown in Pl. 30 and other terminals with mixtures of the two former types are the only kinds to undergo degeneration. No evidence of degenerative changes in type T_3 could be found.

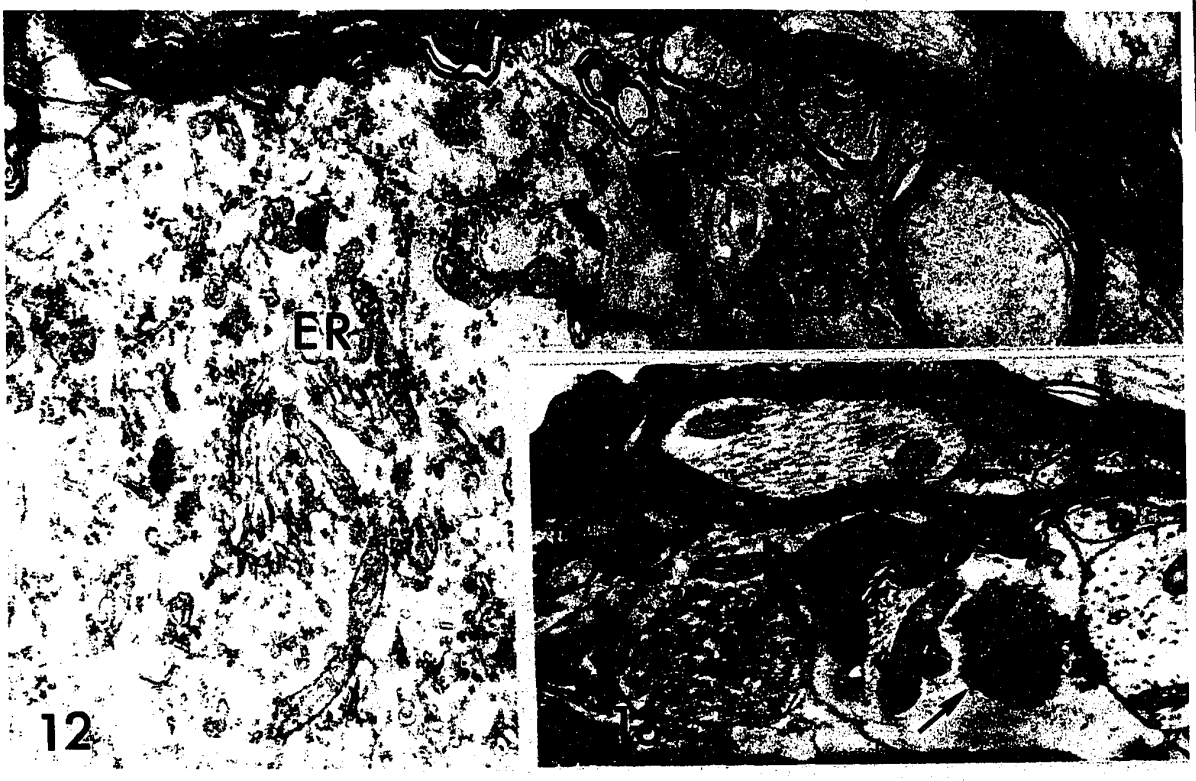
Plate 36, Fig. 17, is an electron micrograph taken from the entopeduncular nucleus 4½ days after a putamen lesion. A typical normal "bouton en passant" (Ep) can be seen coursing towards the bottom of the picture. Two large axon terminals Ax_1 and Ax_2 can also be seen on the top and right hand side of the electron micrograph respectively. Whether these two profiles show some signs of degeneration,

Plate No. 35

Fig. (12) Small degenerating terminal (large arrow) seen on a pallidal cell soma five days after a putamen lesion.

(13) Clumping of synaptic vesicles were also seen in some boutons five days postoperatively. 18,000X.

(14 and 15) Degenerating axons of various calibre, seen as early as three days (Fig. 14) and after five days (Fig. 15) following a putamen lesion in both the entopeduncular nucleus and in the globus pallidus. 18,000X.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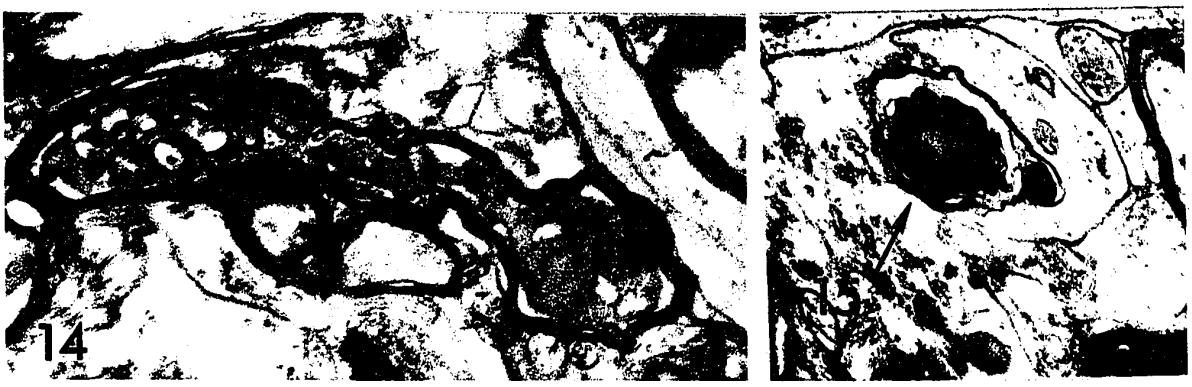
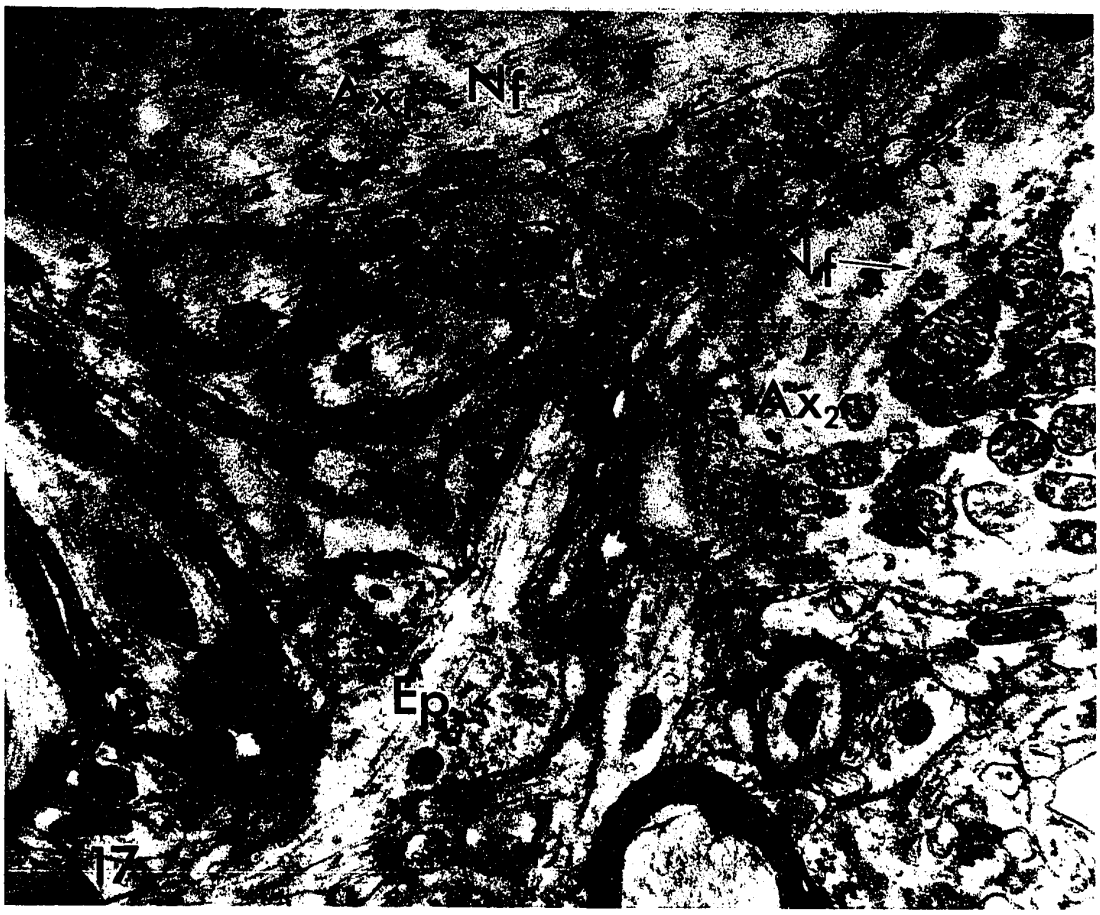

14

Plate No. 36

Fig. (16) Cross section of a dendrite; possibly at a "beaded" area, note large accumulations of mitochondria. At this early stage it is difficult to state positively whether any of the terminals showing some "myelin-like" figures are undergoing degeneration. Three days postoperatively. 11,500X.

(17) Two large axon terminals (Ax_1 and Ax_2) 4½ days after a putamen lesion. Note small strands of neurofilaments (Nf and small arrows) within these terminals. A few synaptic vesicles can be seen at each end of the terminals (large arrows). 18,000X.
A typical bouton en passant (Ep) is shown in bottom centre of Fig. (17). Note that after the axon loses its myelin, the unmyelinated portion continues through the neuropil with swellings containing typical synaptic vesicles within them.

16



17

for example swelling, or whether these profiles should be considered as normal, remains an open question.

Degenerative Changes in Axons and Their Myelin Sheaths

All of the putamen lesions showed degenerative changes mainly in the moderately myelinated and unmyelinated axons (Pl. 35, Figs. 14 and 15). The myelin showed various stages of degeneration in all putamen and caudate lesions, becoming more disordered with time.

Caudate Lesions

Lesions were placed in the head of the caudate nucleus in three cats as shown in Pl. 37. The coagulating electrode was introduced in a vertical direction in each case.

It should be pointed out that ablation of large areas in the frontal and parietal regions of the cortex failed to show any degenerating terminals in either the globus pallidus or in the entopeduncular nucleus of the cat, (thesis, 1966). Similar negative findings from the

Plate No. 37

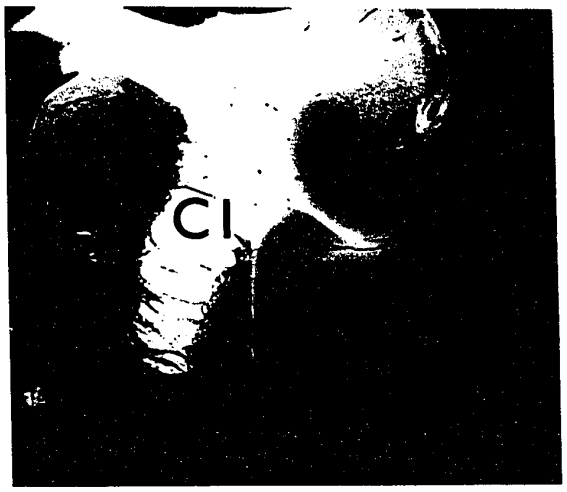
Figs. (18-20) Lesions in the caudate nucleus of the cat. 5X.

(18) Cat CAP 1, the lesion destroyed the medioventral part of the head of the caudate nucleus, and involved some fibers of the internal capsule.

(19) Cat CAP 2, this lesion was very small; it caused damage to only a small area in the mediolateral part of the head of the caudate nucleus.

(20) Cat CAP 3, the lesion was restricted to the dorsal part of the head of the caudate nucleus.

18



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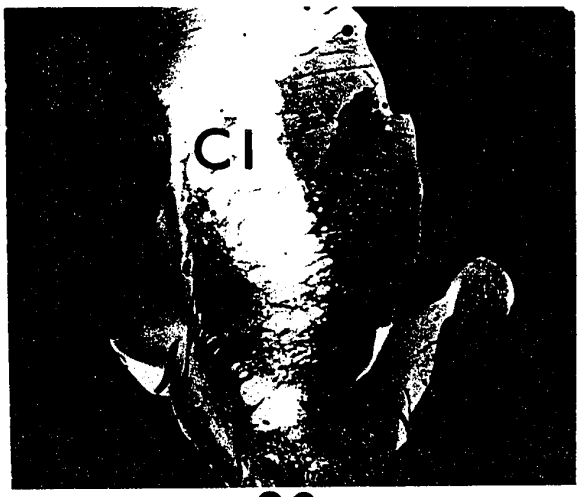

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precentral and postcentral cortex in man were also reported (Petras, 1965). The results of these studies indicated that it was relatively safe to enter the caudate nucleus via the frontal regions of the cortex.

In cat, CAP 1 extensive damage was caused to the medioventral area of the nucleus. The lesion also encroached upon the medial fibers of the internal capsule. In cat CAP 2 the lesion was restricted to the medio-dorsal aspect of the nucleus. The lesion in cat CAP 3 was not very extensive and damaged only a small area of the mediolateral part of the nucleus.

All lesions, however, produced degenerative changes in the entopeduncular nucleus somewhat similar to those of putamen lesions.

Large numbers of degenerating boutons were evidenced on most of the dendrites encountered (Pl. 39, Fig. 23). Degenerating boutons were also observed on the soma of cells, similar to the pattern observed after putamen lesions. As already shown in a previous investigation (thesis, 1966) boutons appear to be grouped in clusters on the cell soma, with the largest aggregates on the proximal ends of the dendrites. Degenerating boutons were more often seen at these locations after caudate lesions (Pl. 38). Degenerating fibers, mainly of the moderately myelinated type, were seen frequently throughout the neuropil (Pl. 39, Figs. 22 and 24).

Plate No. 38

Electron micrograph of part of a cell of the entopeduncular nucleus. Small accumulations of neurofilaments (Nf) are seen at this area of the cell which marks the beginning of a dendritic process. Dt_1 and Dt_2 are two boutons in different stages of degeneration. Dt_1 appear to be at an earlier stage of degeneration than Dt_2. 24,000X.

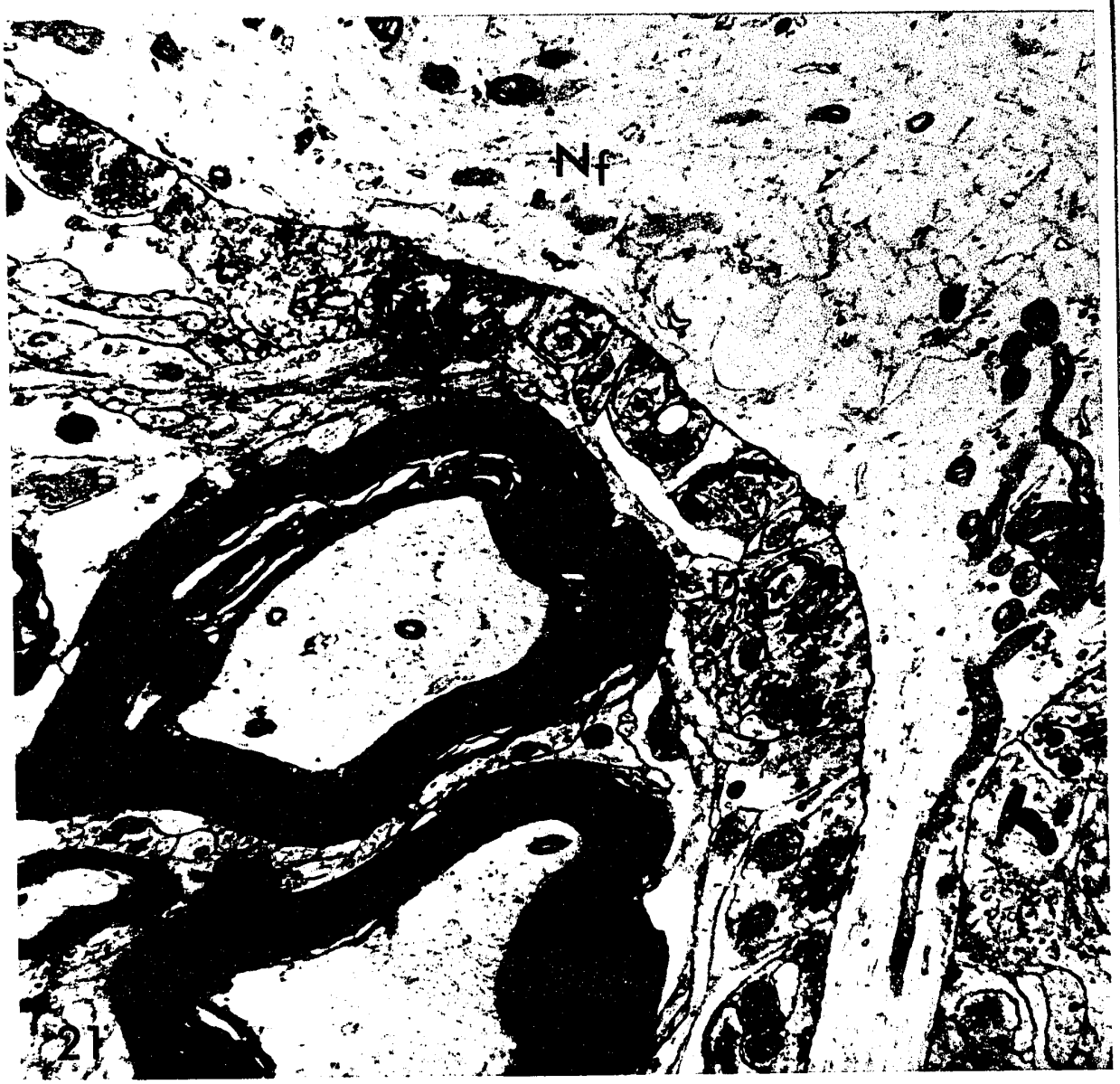

Nf
21

Plate No. 39

Fig. (22) Cross section of a degenerating myelinated fiber (large arrow), showing fragmenting mitochondria and accumulations of dense osmiophilic materials, five days after a caudate lesion. 28,000X.

(23) Longitudinal section of a dendrite (D) displaying many degenerating terminals (arrows) around its periphery. (Star) indicate a "periscopic" myelin figure which is indicative of an early stage in degeneration. (Large arrow) shows degenerating bouton apparently being "pinched" off from main body, five days after caudate lesion. 35,000X. (Inset) degenerating myelinated axon. 24,000X.


22


D
23

Lesions in the Nucleus Ventralis Anterior of the Thalamus

The lesions placed in the nucleus ventralis anterior is shown in Pl. 40. Fig. 24 illustrates the specific area of the thalamus in which the lesions were placed in each case. This section corresponds to Sec. 150, Fr. 12.0 of the stereotaxic atlas of the cat by Jasper and Ajmone-Marsan (1954). Since it was important that no part of the caudate nucleus should be damaged, the electrode in each case was introduced in a vertical plane quite medial to the body of the caudate nucleus. In each of the lesions, the electrode tract, after entering the cortex, was seen to traverse through the corpus callosum, the fornix, and the anterior thalamic nuclei before entering the medioventral one third of the nucleus ventralis anterior. In VAP-1 and 2 the lesions were in the medial tip of the nucleus with some involvement of the rostral cap of the reticular nucleus. In VAP-3 the lesion extended to both the dorsal and ventral areas of the nucleus. Some damage to fibers of the external medullary lamina of the thalamus was also evident.

All three lesions, however, damaged the head of the reticular nucleus anteriorly and encroached upon the nucleus ventralis lateris caudally.

In cat VAP-1 (killed after 7 days) only the globus pallidus was investigated. In this nucleus no sign of any

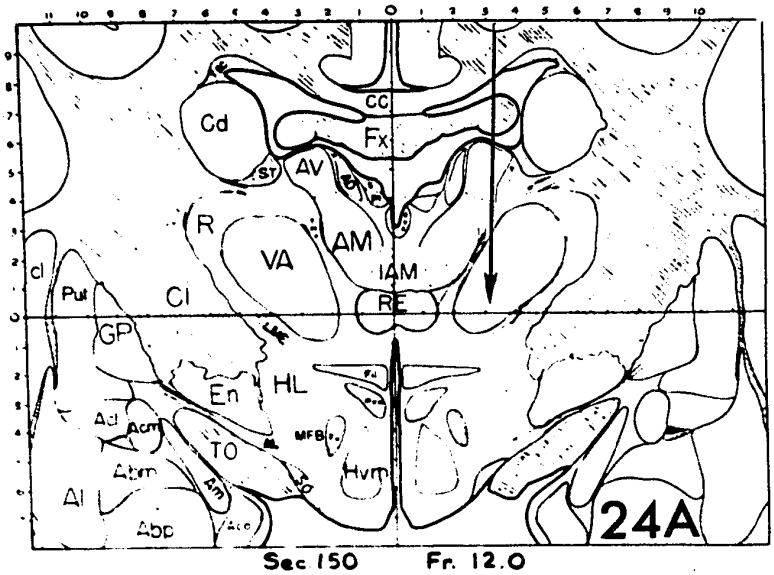
Plate No. 40

Fig. (24) Arrow indicate direction of coagulating electrode and site of destruction of the nucleus ventralis anterior. A more precise location of these lesions is shown in Figs. 25-27. X5.

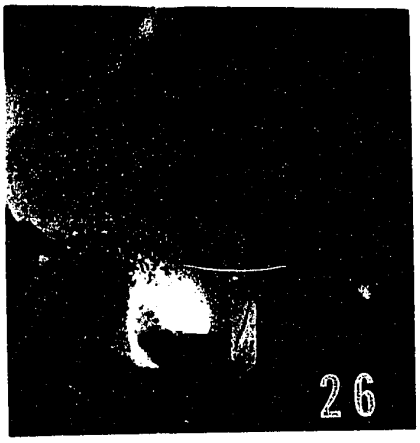
(25 and 26) Cats, VAP-1 and VAP-2. The lesions are confined to the medial tip of the nucleus.

(27) Cat, VAP-3. The lesion caused damage to the dorsal and ventral areas of nucleus.

In each of the above lesions, the reticular nucleus rostrally and the nucleus ventralis lateralis caudally were also damaged to a slight degree.



Sec 150 Fr. 12.0



degeneration in either terminals or in fibers was found.

In cat VAP-2 (killed after 3 days) signs of early degeneration can be observed (Pl. 41, Fig. 28). Formations of rings of myelin-like figures appear within the terminal (Dt).

Plate 41, Figs. 29 and 30 show small degenerating terminals (Dt) at a later date. These terminals were seen on small dendritic processes (Dp) and around small protuberances (Sp) emanating from these dendrites.

Again, only finely myelinated and unmyelinated fibers showed signs of degeneration.

Plate No. 41

(28-30) Shows various degenerating terminals seen in the entopeduncular nucleus following lesions in the nucleus ventralis anterior of the thalamus.

(28) Early evidence of terminal degenerating (Dt) seen three days postoperatively. Note also axon to axon (T_5 and T_6) contact (large arrows) both terminals appear normal, (42,000X). In (30) only a few synaptic vesicles remain in the bouton. Seven days post-operatively. 38,000X. Fig. 29, 30,000X.

28

29

30

Discussion and Conclusions

1. Synaptic Organization of the Pallidum

The cytoarchitectonic studies made with the Nissl method in the present investigation have served to delimit, in as great detail as possible, the relationships between the nucleus basalis of Meynert and the globus pallidus in the cat. They were most valuable in attempting to correlate certain features of the neuropil observed in Golgi sections, with the presence of different types and sizes of cells, in both the cat and the monkey.

The entopeduncular nucleus of the cat has now been accepted as a homologue of the internal segment of the globus pallidus in primates, mainly on the basis of the similarity of their afferents, Nauta (1954). This study has shown that the morphology of the cells in these two nuclei in the two species is also similar. They both contain neurons of the large type.

Ironically however, the globus pallidus (pallidum externum) of the cat can only be compared to the external part of the pallidum of primates with some reservations, at least morphologically. It has been found in the present study that the globus pallidus of the cat is not as extensive as previously shown in most of the atlases of the cat's brain.

This study shows that in the cat the globus pallidus appear to be more extensive in regions rostral to the optic tract, than caudally. At approximately the rostral one third of the latter tract, cells of the nucleus basalis begin to prevail over those of the globus pallidus. At the most caudal level, basalis cells are still predominant and only a few pallidal cells are seen at this level, intermixed with cells of the nucleus basalis. These observations were subsequently supported by the Golgi preparations.

The findings have emphasized, the intimate interrelationship of these two nuclei. It is not surprising therefore that Kodama (1926) considered the nucleus basalis to be a third part of the globus pallidus.

The modifications of the Golgi method by Fox (1951) and Ramon-Moliner (1958) was instrumental in working out in greater detail the dendritic arborizations of the cells, and some additional patterns of terminations of the afferent fibers of the pallidum not reported by previous investigators.

These Golgi methods have shown that the cells on the borders of the nucleus are in general arranged in orderly rows (unlike the random orientation of the cells within the nucleus). This arrangement of the cells seem to conform to the general outline of the nucleus. The dendrites of these cells surrounding the nucleus are so arranged that secondary branches extend from the main dendrites and protrude for

varying distances in the main fiber systems surrounding the nucleus. In this way the dendritic branches serve as 'guidelines' to the incoming fibers. On the other hand, the possibility of other fiber systems which course through these large fiber tracts may also be assumed to make contact with these dendrites. For example, cells of the pallidum externum and internum, in addition to sending dendritic branches into the internal capsule and the external and internal medullary laminae, send branches also to the ansa lenticularis. Efferent fibers therefore, from the external part of the pallidum coursing through this fiber tract may be assumed to make contact with the dendritic branches of the internal part which reach out into the ansa lenticularis.

Knook (1965), maintains that some efferent fibers from the amygdala course through the ansa lenticularis. Whether these fibers make synaptic contact with the pallidal dendrites within the ansa lenticularis cannot be confirmed, but such a possibility cannot be ruled out. Also, the neurones of both the pallidum and the nucleus basalis are so intricately interrelated that it is not surprising that inter neuronal connections could exist between these two nuclei.

Observations from the Golgi methods have also indicated that synaptic terminals of axons within the pallidum are in the form of small, club-shaped, short sprouts attached to short collateral branches (Pl. 21, Fig. C).

These terminals, have been found to contact both dendrites and cell bodies.

Electron microscopic observations have shown that terminals completely surround and make synaptic contact with the dendrites of cells covering their entire surface. The terminals on the cell bodies, on the other hand, seem to be restricted mainly to the area on the soma of the neurons adjacent to the dendritic trunks, leaving many areas on the cell soma devoid of synapses (Plates 24, 25 and 38).

Mori (1966) observed in the pallidum of the rat, that there are many different types of endings that synapse on the same neuron. He also showed that these terminals did not have a dual synaptic arrangement with other cells such as is found in the striatum. He then, concluded that the fibers which entered the pallidum are "plural axons, one neuron".

The present study with the Golgi method does not lend support to this statement. At least not in the cat and monkey. As seen in Pl. 19, Fig. E, several afferent fibers can be seen to course along a dendrite and make many synaptic contacts. At two definite points (1)(2) the same fibers branch off in different directions. It is assumed that the subsequent course of these fibers is along other cells or their processes. Again afferent fibers while coursing in the pallidum give off small collaterals to other cells (Pl. 26, Fig. R). In this manner one incoming axon

can make synaptic contacts with a number of cells.

In the electron micrographs the synaptic terminals on dendrites in the pallidum of the cat appear as three definite types. These types may be classified according to the kind of synaptic vesicles in them. Types (t_1 and t_2) as shown in Pl. 29, Fig. D, already reported by Mori (1966) in the rat is in general the types most frequently found in the pallidum and in other areas of the central nervous system.

In addition some synaptic terminals contain large vesicles with an electron-dense central core (Pl. 29, Fig. E). This type of vesicle was reported to be present in autonomic axons by Wolfe (1961), Urchizono (1967). Evidence has also been given supporting the association of dark-core vesicles with the presence of catecholamines; Wolfe et al (1962) concluded that norepinephrine resides in the electron-dense core of the granulated vesicles in the pineal gland of the rat and that the presence of these vesicles can be used as one criterion for the identification of adrenergic sympathetic axons in electron micrographs.

The occurrence of synaptic terminals in the pallidum containing both agranular and granular vesicles is not completely understood in the light of the present investigations.

The swellings on all types of fibers in Golgi preparations have also been observed in our electron micrographs and interpreted as boutons en passant. However, as

noted previously these swellings do not necessarily represent regions of synaptic contacts since axons in the middle of small fiber bundles always have them. Corresponding swellings in the electron micrographs did not always present membrane specialization, though they show concentration of vesicles. Images of beaded axonal appearance similar to the swellings of Golgi preparations have been related to axoplasmic transport (OCH, 1965). It is equally possible, even likely, that these bulbous enlargements are "boutons en passant," but this can be only ascertained by serial sections.

In the globus pallidus and in the entopeduncular nucleus, Gray's type 2 synapses are frequently seen on dendritic trunks and on cell somas. In these types of contacts, the thickening of the synaptic membrane is not so prominent as in the type 1 contacts. Mori (1966) noted that the two synaptic membranes of most of the synapses in the pallidum of the rat, are not prominent and are generally so close together that the synaptic cleft has almost the same width as the intercellular space (about 200Å), that is, it is narrower than the normal axo-dendritic synaptic cleft (250-300Å).

These characteristics of the fine structure of the pallidal synapses, suggested to the above author, that they are more suitable for electrical transmission than for chemical transmission.

2. Mode of termination of the different afferent systems to the globus pallidus

The present electron microscopic study, on the degeneration pattern following lesions in the substantia nigra, have confirmed the existence of terminal degeneration in the entopeduncular nucleus of the cat, as was seen previously with the Nauta and Bielschowsky techniques (Thesis, 1966).

A detailed examination of these terminals indicate that they end mainly on dendrites and possibly on dendritic spines, which are not abundant on pallidal neurons. Terminal degeneration however can be seen also on the most proximal and of the dendrite, but not on the soma of cells. Since no indication of degenerating myelinated axons were observed in the neuropil of the entopeduncular nucleus, as a result of the lesions placed in the substantia nigra. The nigro-pallidal fibers are assumed to be of fine calibre and mainly finely myelinated or unmyelinated.

In contrast to this, lesions in the caudate nucleus and in the putamen have resulted in degenerating terminals which end on both the dendrites and the soma of the cells. Degenerating myelinated and unmyelinated axons were also frequently seen throughout the neuropil of the pallidum.

One of the problems, that have been discussed recently, is the nature of the mechanism of caudate action on the entopeduncular nucleus and on the globus pallidus.

Malliani and Purpura (1967) who, following stimulation of the head of the caudate nucleus in the cat record inhibitory postsynaptic potentials (IPSP) intracellularly from neurons in the medial part of the pallidum and in the entopeduncular nucleus. Such IPSP's were frequently preceded by short latency EPSP's but the latter, they noted did not always elicit discharge.

There are at least three possibilities that can account for an anatomical substrate for an inhibitory mechanism in the pallidum.

The first possibility refers to the existence of short axoned or Golgi type II cells located within the nucleus. In this regard, after repeated observations in several Golgi sections of the cat and monkey, no evidence could be found to positively identify any of the cells within this nucleus as belonging to the above types.

Another possibility is the existence of axo-axonal junctions, which according to Eccles (1964) and Walberg (1965) are responsible for presynaptic inhibition. Occasionally in this study axon to axon contacts have been found between adjacent boutons (Plates 27 and 41). These contacts as already explained above bear more resemblance to desmosome-like attachments than to true synaptic contacts. (Note

observations on Gray's type 2 synapses.) Unless further electron microscopic study can reveal the true nature of these synapses within the pallidum; these images should be interpreted with extreme caution.

Eccles (1964) has postulated that inhibitory synapses located on the soma of cells are optimally placed for controlling the generation of impulse discharge. He and his collaborators have found that this is the case in the inhibitory synapses of the axons of basket cells in the cerebellum. The observation derived from our electro micrographs indicate that the caudate and putamen fibers make both axo-somatic and axo-dendritic synapses on pallidal neurons, indicating that these fibers may be both excitatory and inhibitory in function, according to the criterion proposed by Eccles (1964). With the absence of interneurons in the pallidum, it is difficult to visualize this dual function of striatal fibers.

In this study it was shown that several kinds of axons are very closely packed together in small clusters and on all parts of the cytoplasmic membrane of the pallidal neuron. These accumulations of terminals are also very neatly surrounded by several folds of glial processes. A similar involvement of axons and glial processes exist in the glomeruli of the lateral geniculate body (Szentagothai, 1966). The latter author considered the possibility that the excitation of some of the axon terminals could influence

the functional state of the others. He hypothesized that "it was not inconceivable that the optic endings could be depolarized without any specific ultrastructural devices, just by being immediately surrounded at by far the larger part of their surface by other axon endings."

But before speculating on similar events occurring in the pallidum, it is probably more advisable to wait until more physiological information is available.

SUMMARY

1. The cytoarchitectonics of the globus pallidus and the nucleus basalis of the cat were studied. A modified map of these results were presented.

2. Cytoarchitectonic studies have also revealed that in certain levels of the pallidum of the monkey, the concentration of pallidal cells appear to be greatest on the dorso-lateral aspects and least on the ventral borders of both the external and internal segments.

3. Ramon-Moliner modifications of the Golgi method were used to study in greater detail the orientation and dendritic arborizations of the cells of the pallidum of the cat and monkey.

4. The Golgi and electron microscopic methods have revealed the existence of some additional patterns of terminations of the pallidal afferents not described by any previous investigator.

The main mode of termination as seen by the Golgi method can be classified as follows:

(A) Short collaterals - these collaterals may be seen to extend off at right angles to the main branch or they may be given off as a short stalk which remain quite close to the main fiber, Pl. 21, Fig. E.

(B) Axons that terminate as small clusters.

(C) Several different axons may make contact with

one neuron, but in so doing they may give off several collaterals to other neurons.

(D) Axons that make "en passant" contacts; first reported by Bielschowsky (1909).

(E) Small bundles of fibers which appear to originate from the putamen, course through the pallidum, making contact with many cells. These fiber bundles bifurcate and travel in all directions, apparently following the course of the dendrites.

(F) Axon to axon contacts has been observed occasionally in the neuropil of the entopeduncular nucleus and in the globus pallidus of the cat.

It was emphasized however, that these images should be interpreted with extreme caution. Tridimensional reconstruction is necessary in order to trace these profiles through trajectories long enough to decide about their nature.

5. Following sterotactically placed lesions in the substantia nigra, degenerating terminals were seen to make synaptic contacts mainly on dendrites in the entopeduncular nucleus of the cat. No evidence of axo-somatic contacts could be found. Judging by the absence of degenerating myelinated fibers, it was assumed that nigro-pallidal fibers are mainly non-myelinated or finely myelinated in character.

6. Lesions placed in the nucleus ventralis anterior of the thalamus showed terminal boutons ending mainly on dendrites and dendritic spines similar to lesions placed in

the substantia nigra. The axons showing evidence of degeneration were restricted mainly to the very finely myelinated and unmyelinated fibers. No convincing evidence could be found to indicate that the nucleus ventralis anterior of the thalamus sends fibers to the globus pallidus.

7. Lesions in the caudate nucleus and putamen produced similar degenerating patterns. These fibers made both axo-somatic and axo-dendritic synapses. These findings were considered in relation to recent neuro physiological observations.

8. Three definite types of boutons containing different kinds of synaptic vesicles were found in the entopeduncular nucleus and in the globus pallidus of the cat. A possible fourth type was also seen, which consisted of mixtures of synaptic vesicles of the former three types. The functional significance of these different kinds of synaptic endings remain obscure.

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