HS

THE SYNAPTIC ORGANIZATION OF THE GLOBUS PALLIDUS
A LIGHT AND ELECTRON MICROSCOPIC STUDY

IVAN JAMES LU QUI, B. Sc.

THESIS

Submitted to the Faculty of Medicine of the University of Ottawa in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Department of Anatomy.

Ottawa, September 1966

TABLE OF CONTENTS

INTRODUCTION	1
REVIEW OF LITERATURE	3
MATERIALS AND METHODS	15
OBSERVATIONS	22
DISCUSSION	50
SUMMARY	54
BIBLIOGRAPHY	55

INTRODUCTION

Within the last fifty years the afferent connections of the globus pallidus of various species have been extensively investigated primarily with the aid of the Marchi technique and more recently with the use of the Nauta silver impregnation method.

There is now general agreement that fibers from the caudate nucleus, the putamen and the subthalamic nucleus all project to the globus pallidus. Some authors also mention nigro-pallidal, cortico-pallidal and thalamo-pallidal connections while others deny their existence.

Available data based on degeneration experiments indicate that there is an orderly topographic distribution of efferent fibers of the caudate nucleus and the putamen in the globus pallidus. On the other hand the present state of knowledge concerning the mode of termination of all the pallidal afferents is very limited. It is almost exclusively derived from normal material.

The Nauta silver impregnation technique stains mainly the degenerated fibers and preterminals. These may appear as pericellular granules or can be seen distributed in the interstitium. It is however only rarely and under optimal conditions the unequivocal conclusions can be drawn as to the axosomatic and/or axodendritic termination of the incoming fibers.

In order to show the intimate relationship between afferent endings on the soma or dendrites of cells, other techniques such as neurofibrillar impregnation methods and possibly electron microscopy must be used.

While electron microscopic studies on the normal pallidum have been carried out recently by Fox et al. ('51), no investigation so far has been done on experimental animals using bouton techniques.

Recent anatomical and physiological investigations into the synaptology of some nuclei of the central nervous system seem to have constituted to a better understanding of the functional significance of those connections.

The globus pallidus occupies a crucial position - anatomically as well as functionally - in the "extrapyramidal" system. Its involvement in certain dyskinesias is well known.

Stimulation or ablation experiments of the pallidum also underlie its physiological importance.

The present study was undertaken to obtain information on the intrinsic organization of the normal globus pallidus and also to see how the incoming degenerating fibers establish synaptic contacts with cell bodies or their processes.

For the investigations of the normal synaptic relations cat and monkey brains were impregnated with both Golgi and electron microscopic methods. ok

Two techniques which complement each other were used for the degeneration studies. The degenerating fibers and the area of their preterminal arborizations were studied with the Nauta technique. The degenerating terminal boutons were impregnated with a modified Bielschowsky method. As a routine the two methods were used on alternate sections of the same brain.

Combining these methods and comparing the results of normal as well as degenerating materials, it was hoped that a differential distribution of axosomatic and axodendritic endings can be ascertained following lesions in various structures which project to the globus pallidus.

REVIEW OF LITERATURE

Intrinsic Organization

Golgi studies by Ramon y Cajal ('11) in the globus pallidus on mammals including man, have demonstrated that this nucleus consists mainly of large neurons which are fusiform or triangular in shape with very long dendrites emerging from their poles. The axons which are very thick and very sinuous display fine collaterals which are ramified and very long.

Foix and Nicolesco ('25) in their study of the cyto-architecture of the pallidum of man agree with Cajal as to the shape of the neurons and the extent of their dendritic pattern. These dendrites would course in a sort of sheath made up mainly of glial cells. On the other hand, the axons are difficult to follow, because they are enmeshed with the dendrites and axons of other cells. These axons can also be seen to form small clusters within the different pallidofugal tracts.

Ariens Kappers ('36) has described the cells of the pallidum as multipolar and motor in type, giving rise to efferent paths.

Up until 1965 very little was known about the normal synaptic relationships within the pallidum. In that year Fox ('65) and his co-workers presented their findings at the International Congress of Anatomists in Wiesbaden, Germany. These findings as yet unpublished were based on studies carried out on normal monkeys, cats and rabbits using Golgi-Fox and electron microscopic methods. In Golgi preparations of the globus pallidus they were able to demonstrate the "fortunate impregnations revealed an excessively rich afferent plexus of extremely delicate fibers which completely ensheath

and outline the cell bodies and dendrites forming longitudinal axo-dendritic and axosomatic connections". These findings were supported by electron micrographs which showed that the neuropil consists principally of long dendrites, which were associated with many synaptic endings which Fox and his co-workers termed "Bouton en passage".

Caudato-pallidal Connections

Fibers from the striatum to the pallidum have been mentioned by numerous authors. Ariens Kappers ('21) stated that fibers from the caudate nucleus to the pallidum arise as neuraxes of larger cells of the former nucleus. These axons first course through the internal capsule before entering the pallidum via the external medullary lamina. It is of interest that Ranson ('41) has denied the existence of such a pathway.

In her study of the corpus striatum of Tamandua tetradactyla, O. C. Smith ('30) described fiber bundles which course in a wide arc from the caudate nucleus across the internal capsule toward the peduncle and entopeduncular nucleus. Beyond this nucleus further ending could not be traced and many of the fibers appeared to end in the entopeduncular nucleus and the substantia nigra.

Papez ('38) showed, in the brain of macacus rhesus that efferent fibers from the head of the caudate nucleus and the adjoining parts of the putamen formed a large funnelshaped 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 stated that many of these fibers seemed to end in the internal part of the pallidum. Fibers from the tail of the caudate nucleus formed like the "spokes of a wheel" also perforate the internal capsule radially to enter the pallidum

from diverse sites. Some of these fibers end in the pallidum, whereas others continue through and finally terminate in the lateral part of the substantia nigra.

Ransom et al. ('41) reported that fine, degenerated fibers revealed by the Marchi technique could be followed in macaca mulatta obliquely through the internal capsule into the external division of the globus pallidus. These bundles, greatly reduced in size, were finally lost in the internal division of the pallidum. They were unable to find any evidence of degeneration in the external medullary lamina.

Mettler ('42) in his studies on the relation between pyramidal and extrapyramidal function, was able to follow degenerated axons with the Marchi technique from the head of the caudate nucleus 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 a group of fibers which separated from the above at the dorsal edge of the globus pallidus, entered its medial segment, and was lost to view.

Glees ('45) while studying the interrelation of the strio-pallidum in the macaque with the aid of the Marchi technique, reported that fibers from the dorsal part of the caudate nucleus pass through the internal capsule and enter the external part of the pallidum after first traversing the external medullary lamina.

He also stated that fibers from the ventral part of the caudate nucleus mingle with fibers of the internal capsule and enter the medial division of the pallidum directly from the internal capsule.

In the normal brain of the gibbon Verhaart ('50) found that most of the striatal fibers seemed to terminate in the rostral half of the

globus pallidus. He also pointed out that 99% of those fibers were one micron or less in diameter with an extremely fine myelin sheath.

These conflicting reports on the anatomical relations between the caudate nucleus and the globus pallidus may be due to the Marchi technique employed by these investigators. There is general agreement that the Marchi method may be used successfully to trace fiber tracts but it should be pointed out that this method fails to stain minute non-myelinated nerve fibers not to mention the endings.

The introduction of an improved silver method for tracing degenerating axons by Nauta and Gygax ('54) provided a unique opportunity for Voneida ('60) to reasses the anatomical relations of the caudate nucleus. Voneida made small, controlled lesions in the head of the caudate nucleus of cats and monkeys and reported that fibers arising in the head of the caudate nucleus pass ventrally, caudally and laterally into the anterior of the internal capsule. These fibers then weaved among the capsular fibers and continued in a ventrolateral and caudal direction. At the level of the anterior commissure the fibers made an abrupt turn medially to enter the medial division of the globus pallidus where many of them ended. The remaining fibers passed through the pallidum and then proceeded caudally along the ventrolateral edge of the cerebral peduncle to end finally in the pars reticulata of the substantia nigra. No evidence of degeneration was reported in the external division of the globus pallidus.

Szabo ('62) using the Nauta technique has also made a detailed study of the topical distribution of the striatal efferents in the monkey. In this study he pointed out that all the lesions made by Voneida ('60) were placed ventro-medially in the head of the caudate nucleus. Since

this area projects specifically to the medial segment of the globus pallidus, it would account for the negative findings of Voneida ('60) who failed to find evidence of degeneration in the external segments. Szabo ('62) also found that the lateral areas of the head of the caudate nucleus project to the external segment of the globus pallidus. In his study fibers from the middle part end in both segments equally, while the most medial caudate regions were connected mainly to the internal segment. He concluded that "this topical organization seems to exist also in the antero-posterior direction".

Knook ('65) using the Nauta technique in his studies of the rat brain also concluded that the striatum projected in a direct and systematic manner, with a rostro-caudal and a medio-lateral arrangement.

Regardless of the differences of opinion concerning the topical arrangement of the caudato-pallidal fibers, all authors nevertheless agree to their existence.

Putamino-pallidal Connections

Putamino-pallidal connections were reported by Papez ('38) who stated that fibers from the lateral portion of the putamen conveyed cone-like into the pallidum, some ending in both segments while others pass down to end in the middle portion of the substantia nigra.

In their investigations on monkeys Ranson et al. ('41) as well as Glees ('45) and Mettler ('45) have utilized the Marchi technique and have agreed independently that the putamen sends fibers to both segments of the pallidum.

Ranson and Clark ('53) have suggested that fibers which arise in

the putamen converge like the "spokes of a wheel" before they end in the external and internal division of the globus pallidus.

Johnson and Clemente ('59) have employed the Nauta technique in their analysis of the fiber connections between the putamen and globus pallidus of the cat, and have showed that fibers from the putamen enter the globus pallidus to end mainly in the internal division and not beyond.

In contrast Nauta and Mehler ('61) have demonstrated clearly that electrolytic lesions made in the putamen caused fiber degeneration not only in the globus pallidus but also the reticular part of the substantia nigra.

Szabo ('62) in a detailed study of the striopallidal connections in the monkey by the Nauta technique reported that the origin, course and destination of the putamino-pallidal fibers have a topical arrangement, in which fibers entering the pallidum maintain their relative positions throughout in a dorsoventral and mediolateral direction. Similar observations have recently been reported in the rat by Knook ('65).

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.

Ranson et al. ('41) employing the Marchi technique in their studies on monkeys described afferents via the inferior thalamic peduncle to the internal segment only of the globus pallidus from the nucleus ventralis anterior of the thalamus.

The existence of these pathways have been confirmed by Ranson and Clark ('53).

Knook ('65) systemtically destroyed the entire thalamus in the rat and failed to find any evidence of degeneration in the entopeduncular nucleus (i. e. internal segments of pallidum). However with the exception of the midline nuclei, ventralis anterior and anterior thalamic nuclei, all the other thalamic nuclei showed degenerative changes which terminated in the external segment of the pallidum.

He warned however, that the absence of a thalamo-entopeduncular connection in the rat does not indicate that this connection is also lacking in the dog and cat, and advised that careful studies should be carried out.

Nauta and Whitlock ('53) 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 these 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 ('56) in their studies on the monkey confirmed that the nucleus centrum medianum of the thalamus projects to the putamen but denied that any fibers enter the globus pallidus from this nucleus.

Johnson ('61) reported that a few fibers from the nucleus medialis dorsalis thalami in the cat terminated in the globus pallidus, but the majority of these fibers ended in the caudate nucleus. They also stated that very few fibers from the centrum medianum entered the globus pallidus via the interior thalamic peduncle, but the main projection seems to terminate mainly in the putamen.

Cortico-pallidal Connections

In an excellent review by Knook ('65) of the present state of knowledge

of cortico-pallidal connections, attention is directed to many authors who have denied the existence of such connections. Among them were Wilson, Edin and Lond (1911-12), Wilson (1913-14), Vogt (1919-20), Bickel (1922) and Jokab (1923).

In a later report Mettler ('35) was unable to find any connections from the occiptal, parietal and temporal regions to the globus pallidus, but demonstrated the existence of fibers from the frontal region to the pallidum.

On the other hand, Verhaart and Kennard ('40) made lesions in areas 4, 6 and 4s (according to the classification of Brodmann) of the cortex in macaca mulatta and failed to obtain evidence which indicated that these areas projected to the pallidum.

In the rat brain, Webster ('61) could find "no convincing sign of axons terminating in the pallidum", while in contrast a later study by Knook ('65) on the rat has demonstrated that "there can hardly be any doubt about fibers ending around the cells of the globus pallidus".

The results obtained from studies on the macaca mulatta by Glees ('45), Ranson and Clark ('53) and Showers ('58) are in agreement with the recently reported observations of Knook ('65).

Subthalamo-pallidal Connections

Subthalamo-pallidal connections were not considered in the present investigation. However a brief description of the authors who described such a connection would suffice for completeness.

Glees and Wall ('46) as well as Whittier and Mettler ('49) maintain that the projections to the pallidum from the subthalamic nucleus are numerous. Knook ('65) also agrees that extensive degeneration can be

seen in the pallidum of the rat brain after lesions in the corpus luysii of the nucleus subthalamicus. He has cautioned, however that this nucleus is very difficult to lesion without concomitant damage to other neighboring nuclei, and that results obtained in this manner may be misleading.

Nigro-pallidal Connections

Nigro-pallidal connections were reported by Ranson et al. ('41) in macaca rhesus. They showed that fine degenerating fibers revealed by the Marchi technique could be followed obliquely through the internal capsule to their terminations in both segments of the globus pallidus.

Similar experiments performed by Fox and Schmitz ('44) have confirmed the course and termination of fibers from the substantia nigra. On the contrary, Rosegay ('44) has shown that massive lesions placed in the substantia nigra and basis pedunculi in the cat failed to produce any significant Marchi degeneration in the globus pallidus.

Carpenter and McMaster ('64) also have called attention to the limitations inherent in the Marchi method. In an attempt to resolve this problem, they made controlled experimental lesions in the substantia nigra of twenty-six monkeys and studied the subsequent degeneration with the aid of the Nauta Gygax method. They concluded that nigro-pallidal fibers after traversing the prerubral area enter the ipsilateral globus pallidus by passing through and around the internal capsule. These fibers are then distributed mainly to the internal segment.

Knook ('65) utilizing the same Nauta technique on rats, was in complete disagreement with the latter authors. He concluded that when the substantia nigra was almost completely destroyed, "no evidence could be obtained of a nigro-entopeduncular projection", but a moderate

number of degenerating fibers could be seen in the external part of the pallidum.

It is apparent from the review of the literature that the diversity of evidence concerning the final termination of the afferent fibers to the pallidum may be largely attributable to the limitations of the methods which have been used for tracing degenerating fibers.

The Marchi method has been of questionable value in determining the orientation of these fibers. The conclusions drawn from its applications have differed not only as to polarity but as to whether any connections really exist. On the other hand, the Nauta technique has produced more consistent results; it is relatively selective for degenerating fibers and also stains unmyelinated fibers and preterminals under study.

A brief summary of the confirmed and probable afferent connections of the globus pallidus is presented in Fig. (1).

It should be noted that in the cat the globus pallidus shows no division in an internal and external segment, but available evidence suggests some similarities between the globus pallidus and the nucleus entopeduncularis, Smith ('30), Fox ('44) and Nauta and Mehler ('61).

According to Nauta and Mehler ('61) the entopeduncular nucleus of cat brain shows the same efferent connections as the internal segment of the globus pallidus in the monkey, and in both cats and rats, it might be considered as the homologue of the internal segment. In this investigation the terms entopeduncular nucleus and internal segment are used interchangebly.

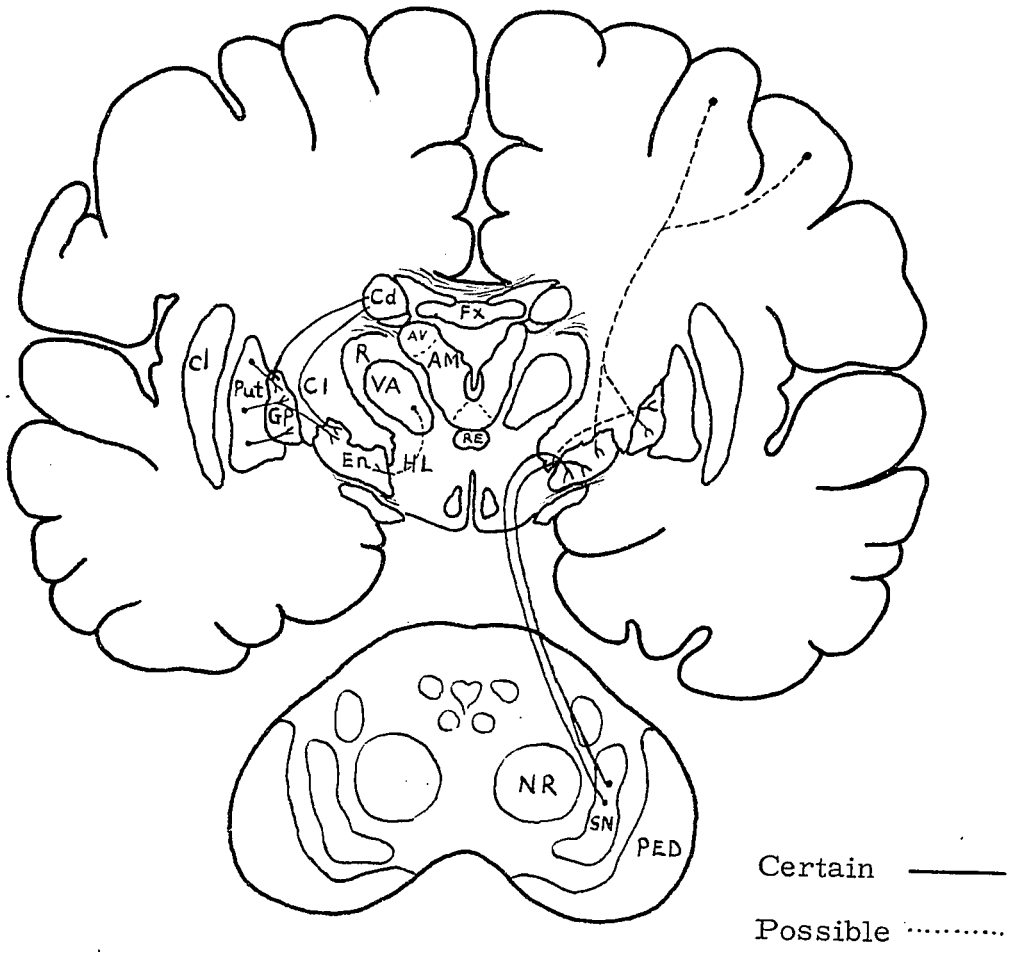


Fig. 1. A diagram of the established and possible afferent connections of the Globus pallidus.

The following abbreviations were used for all figures:

A - axon

AD - anterodorsal nucleus

AM - anteromedial nucleus

AV - anteroventral nucleus

B - bouton

CD - nucleus caudatus

CI - internal capsule

CL - claustrum

D - dendrite

EN - nucleus entopeduncularis

ER - endoplasmic reticulum

FR - frontal

FX - fornix

GP - globus pallidus

HL - hypothalamus lateralis

M - mitochrondia

N - nucleus

NR - nucleus ruber

PED - pedunculus cerebralis

PUT - putamen

R - nucleus reticularis

RE - nucleus reuniens

SN - substantia nigra

SV - synaptic vesicles

VA - nucleus ventralis anterior

MATERIALS AND METHODS

A. Normal Material

I. Golgi-Fox Method ('51)

Six cats and two monkeys were used in this series of experiments. Under deep nembutal anesthesia, they were perfused through the left ventricle using 200 cc. of normal saline followed by 500 cc. of 10% formol. The brains were then removed and placed in 10% formol for further fixation. After a period of three months to one year fixation, the brains were stained according to the Fox modification of the Golgi method. Minor variations of this method were however necessary to achieve satisfactory results.

1. The brains were cut in slices 2-3 mm. thick.
2. Then placed in 6% zinc chromate solution for 48 hours.
3. The slices were then removed and blotted dry.
4. The slices were then suspended by a thread into a large quantity of 0. 75% silver nitrate.
5. After 24 hours, the slices were transfered to a fresh solution of 0. 75% silver nitrate. (The sections were brushed thoroughly before being transfered).
6. The slices were left in the second silver nitrate solution for 24 hours.
7. Steps (2) to (6) were repeated until satisfactory results were obtained. (two and three times)
8. Slices were embedded in celloidin and sectioned at 100-120μ.

II. Electron Microscopy

Three kittens three to six weeks old were used for this method. Under nembutal anesthesia they were perfused through the left ventricle with 100-150 cc. of normal saline followed by 250-300 cc.

of chilled phosphate buffered glutaraldehyde. After this the brain was removed as quickly as possible (fifteen minutes). Using a razor blade thin slices, approximately 3 mm. thick, were cut out and immersed in chilled phosphate buffered glutarldehyde. From each slice, pieces of the pallidum were isolated under the dissecting microscope. After isolation, the pieces were left in the glutaraldehyde solution for two hours at 4°C. then rinsed in phosphate buffer before placing in 2% osmic acid for an additional two hours at 4°C. The tissue was then dehydrated in alcohols and embedded in epon. The ultra thin sections were made with LKB ultratome. The sections were then stained with uranyl acetate for ten minutes at 40°C. rinsed in methanol and counterstained with lead acetate for one minute. The sections were finally studied with an RCA 3 electron microscope.

B. Experimental Materials

Altogether twenty-one cats were operated in this phase of the experiments (see table 1, page 24) but only eleven animals with well localized lesions were used for this study. Lesions were placed stero-taxically in the caudate nucleus, the putamen, the nucleus ventralis anterior of the thalamus and the substantia nigra. In another group of animals various frontal and parietal cortical areas were removed by suction.

All the operations were performed under nembutal anaesthesia and with aseptic precautions. A coagulating current of 3 ma was employed for fifteen seconds through a platinium wire insulated with glass except the tip 1mm. long was used as the electrode.

After the operation a mixture of penicillin and streptomycin was given to each animal locally as well as parenterally. At the end of three to five days of survival, the brains were perfused under deep anesthesia

through the left ventricle using 200 cc. of normal saline and 500 cc. of 10% formalin. The brains were taken out immediatly and stored in formalin (10%) for two weeks to three months.

Frozen sections were cut alternately at 30 micra for impregnation with Nauta-Gygax method and 20 micra for staining with a slight modification of the Bielschowsky-Gross technique. The following modification of the latter technique has given the most consistent result:

1. Wash sections throughly in distilled water.
2. Place sections in 10% silver nitrate for 12-24 hours.
3. Without rinsing, quickly pass sections through 4 dishes of 1:7 formol solution made up with tap water.
4. Rinse briefly 3-4 seconds in distilled water.
5. Place sections in ammonical silver solution made up as follows: to 4 cc. silver nitrate add by drops concentrated ammonium hydroxide (sp. Gr. 28) until a precipitate is formed; then redissolve by adding one drop in excess.
6. Leave sections in above solution until they attain a 'tobacco' colour.
7. Rinse briefly in distilled water to which 3 or 4 drops of ammonium hydroxide have been added.
8. Wash in three changes of distilled water.
9. Place sections in 0.002% gold chloride, acidified with 3 or 4 drops of acetic acid.
10. Leave for 15-20 minutes.
11. Rinse briefly in distilled water.
12. Reduce in 10% sodium thosculphate for 10 seconds.
13. Wash in 3 changes of distilled water.
14. Dehydrate in alcohol, clear in carbolphenol mixture according to Rio-Hortega (48).

15. Mount

The Nauta Gygax method ('54) is well known and will not be detailed here. A modified method by Fink ('66) was used in one case with putamen lesion.

Reports in the literature regarding the use of a bouton method in the pallidum seems to be lacking. This is not surprising since the pallidum, consists mainly of large multipolar cells embedded in a thick matrix of myelinated neuropil - a matrix which makes staining difficult with any histological method and in particular with the Bielschowsky type of stain.

Hoff ('35) using the reduced silver method of Cajal demonstrated the ease with which the spinal cord is stained by this method. However, the distinction between normal and degenerating boutons was not clear, since both normal and degenerated boutons were stained with equal facility.

Gibson ('37) carried out an intensive experimental study of the degenerative changes which boutons in the spinal cord undergo after varying postoperative periods. He showed that the degenerated boutons increased in size from a normal diameter of 2.0 - 5.7 micra five days after the operation. The shape of the boutons also varied from hollow to a solid oval. Phalen and Davenpoet ('37), however, have provided evidence in support of the validity of the method. In their experience normal boutons show considerable variations in their contour, size and appearance and they have concluded that synaptic connections can be located successfully if the degenerating end bulbs are massive in their extent.

Glees ('41) however, in his studies in the lateral geniculate body of the monkey, maintain that the differences are sufficiently clear cut,

at least in this nucleus to enable an exact mapping out of the terminations of transected fiber tracts. He showed that the initial degenerative changes in the terminal boutons are a thickening and heavier staining of the ring formation, associated with a slight enlargement, and a filling of the interior of the ring. As degeneration proceeds the boutons become markedly enlarged to form round oval and elongated fusi form bodies staining a dense black. He noted also that the inital process of degeneration is not confined to the boutons; it also affects the fiber at the site of its terminal division as well as its telodendritic processes. These become irregularly swollen and stain deeply with silver impregnation.

Blackstad, Brodal and Walberg ('51) in their investigation on normal and degenerating terminal boutons in the inferior olive of the cat, point out that conclusive results may be obtained when the concomitant degeneration of the finest terminal fibers is taken into account.

All authors studying normal terminal boutons agree that they frequently are difficult to identify. However, there seems to be general agreement that degenerative boutons stain much more readily than normal ones.

In a more recent publication Walberg ('64) has shown by an electron microscopic technique that the degenerated bouton is much larger than a normal one. He maintains that this increase in size is due to the swelling of the mitochrondia in the degenerating boutons.

Electron microscopic studies carried out by Guillery ('65) on degenerating boutons showed that the increase in size could also be due to an abundance of neurofilaments in the boutons after nerve sectioning.

In the light of these recent findings it would seem that the criteria for determining degeneration by a bouton technique should take into account the following observations:

a. an increase in size of the boutons

b. evidence of degeneration of the finest fibers.

c. the quantitative differences between the normal and degenerated sections of brain tissue understudy.

TABLE 1

LIGHT MICROSCOPY		LESION	STAIN	POST OPERATIVE PERIOD	ELECTRON MICROSCOPY		
Number	Age				Number	Age	
GF-1-6	Kittens (800-900g)	Normal	Golgi-Fox	-----	GEM-1	Kitten 950g.	Normal
GF-7, 8	Monkeys (adult)	Normal	" "	-----	GEM-2	Kitten 1.2kg.	Normal
CDS-7, 8, 15 & 16	Cats (adult)	Cortex	Bielschowsky & Nauta	4 days	GEM-3	Kitten 800g.	Normal
GPS-8	"	Caudate	" " "	4 days			
GPS-4, 9	"	Putamen	" " "	4 days			
GPS-5, 12	"	Caudate	" " "	5 days			
GPS-6	"	V. A.	" " "	3-1/2 days			
GPS-11, 13	"	V. A.	" " "	5 days			
GPS-17	"	S. N.	" " "	6 days			
GPS-19	"	Putamen	" " "	3-1/2 days			
GPS-20	"	S. N.	" " "	5 days			

OBSERVATIONS

Light Microscopy - Normal Material

Golgi-Fox preparations revealed that the cells of the pallidum are mainly large in size and triangular or fusiform in shape as previously described by Cajal ('11). The dendrites are very thick and long and do not seem to possess spines except at their most distal extremities. No attempt was made to follow the axons of these cells. They were very sinuous, and could only be followed for short distances before they became enmeshed in the neuropil.

On the other hand, the afferent fibers display a peculiar fascicle-like formation around the dendrites and soma of these cells.

Many axons are seen to course vine-like along the dendrites making many synaptic contacts along their paths, and ultimately terminating on the soma of the cell.

If the above findings could be viewed in a three dimensional plane, the axons would seem to form a kind of sheath along the length of the dendrites, with many large collaterals given off at all angles. These large collaterals also seem to dichotomize into two or more smaller branches.

These axons arborizations which can be seen throughout the nucleus seem to indicate that a single afferent fiber can establish synaptic contact with the dendrites or the soma of more than one neuron.

In many cases axons can be followed throughout the interstitium without any apparent contact with cells. These finally appear to terminate into a spray consisting of three of four branches and sometimes as many as twenty branches can be seen.

The fact that Golgi preparations only stain one cell in seventy-five made it difficult to determine the synaptic relations of these branches.

Fig. 2a is a photomicrograph of a typical pallidal cell showing an axon coursing parallel to the dendrite and making many synaptic contacts on it before finally terminating on the soma of the cell. A large collateral is also seen to be given off along its course. This collateral further divides into two or three smaller branches.

On the upper left of the photomicrograph the intimate relations of many axons can be seen on another dendritic branch of the cell.

In the lower photomicrograph, Fig. 2b an axon can be seen to give off many collaterals along its course, and at the lower right corner (arrow) one of these collaterals seem to terminate on a dendrite after first dichotomizing into two smaller branches.

Electron microscopy - Normal material

Electron microscopic preparations have revealed a pattern more or less similar to the Golgi-Fox material. The dendrites were recognized by their size, orientation and the presence of dendritic tubules. The neuronal perikarya were identified by their size and shape, the presence of the endoplasmic reticulum and the characteristics of the nucleus. The boutons or presynaptic bags are also recognized by an accumulation of synaptic vesicles and mitochrondia within them. Figs. 4 and 5 show a longitudinal and cross section fo a dendrite respectively, with many boutons attached to its periphery. In some instances, the presynaptic bags could be seen in relation to the neuronal perikarya (Fig. 6). These findings are in agreement with Fox et al. ('65).

On the basis of Gray's ('59) classification these synapses correspond to his type 2 - found in the cerebral cortex. In this type of synapse the percentage length of thickening is small and the pre- and post-synaptic thickenings are of similar dimensions. This type occurs on dendritic

trunks dendritic spines and neuron cell-bodies only.

In addition to the above type of synapse, the present study has also revealed Gray's type 1 synapses which were not proposed by Fox et al. ('65). In this type (Fig. 7) the post-synaptic thickening is more extensive and more pronounced than the presynaptic thickening. Within the extracellular region between the thickened membranes an intermediate band of material can be seen (Fig. 7b) as described by Gray.

The reality of this subdivision into two types was carefully considered by Gray himself. He argued that it is possible that the varying appearances may be caused by different planes of section. However, if this were so, it is unlikely that the two types would be restricted to the dendrites in the one case and to the neuron cell-body in the other. He was unable to find any evidence of type 1 associated with the soma of a neuron. The present investigation is in agreement with this observation. Hamlyn ('62) in his studies on the mossy fibre endings in the hippocampus is also in complete agreement with Gray, but strongly suggests that serial sections should be studied before this question can be resolved.

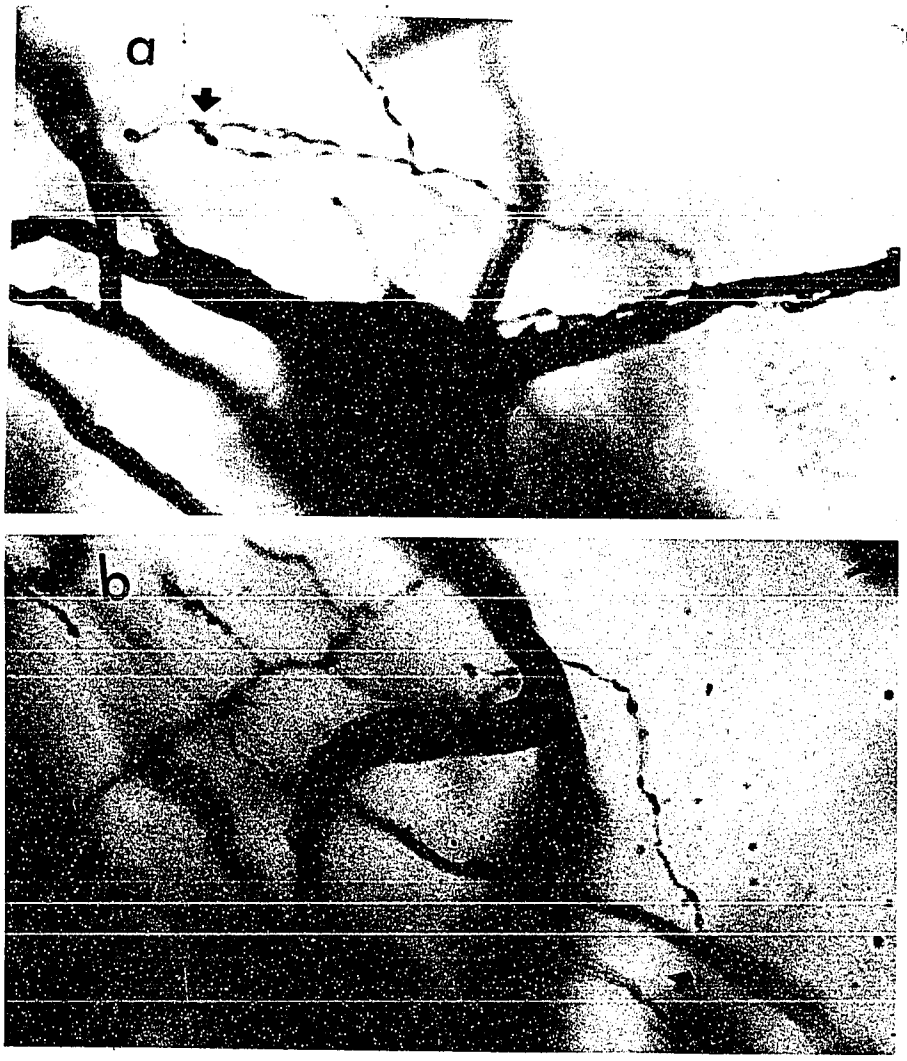


Fig. 2 - Photomicrographs of Golgi sections of the globus pallidus.

a. Typical pallidal cell displaying axo-dendritic and axo-somatic syanpses. 2000X
b. Axon is splitting up into many short branches. At lower right corner one of the branches seem to terminate on a dendrite. 1000X

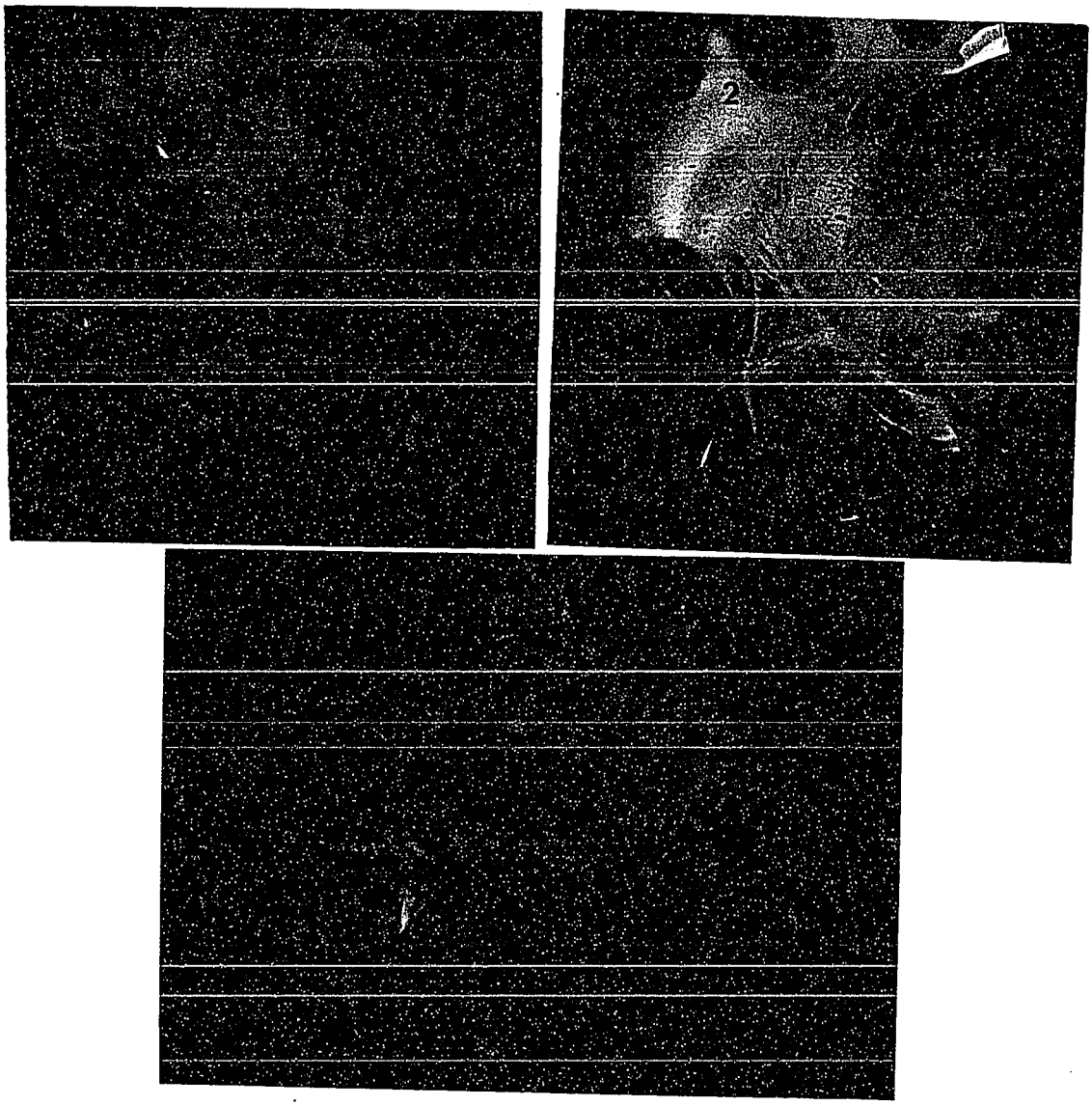


Fig. 3: Photomicrographs of the cat brain showing areas removed for electron microscopy. The blocks have been isolated from the entopeduncular nucleus in GEM-2 and 3. In GEM-1 the area removed was confined to the external segment.

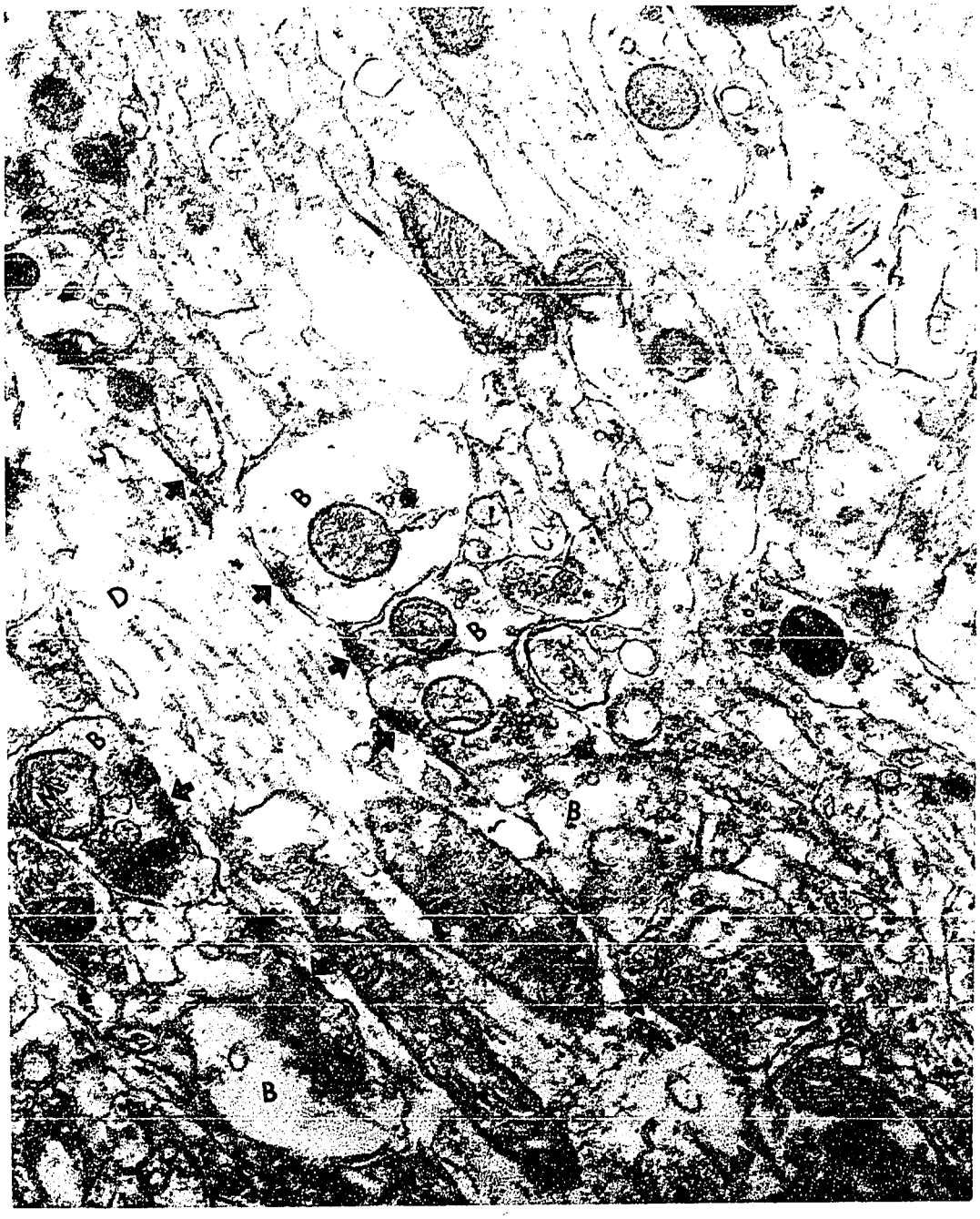


Fig. 4: Electronmicrograph of a dendrite (D) cut longitudinally. Several boutons (B) with characteristic synaptic visicles and mitochrondia can be seen making contacts (arrows)along the whole length of the dendrites on both sides. Gray's Type 2. 29,000X.

Fig. 5: Electronmicrograph of a cross section of a dendrite (D). Many boutons (B) can be seen making synaptic contacts (arrows) around its periphery. 29,000X.

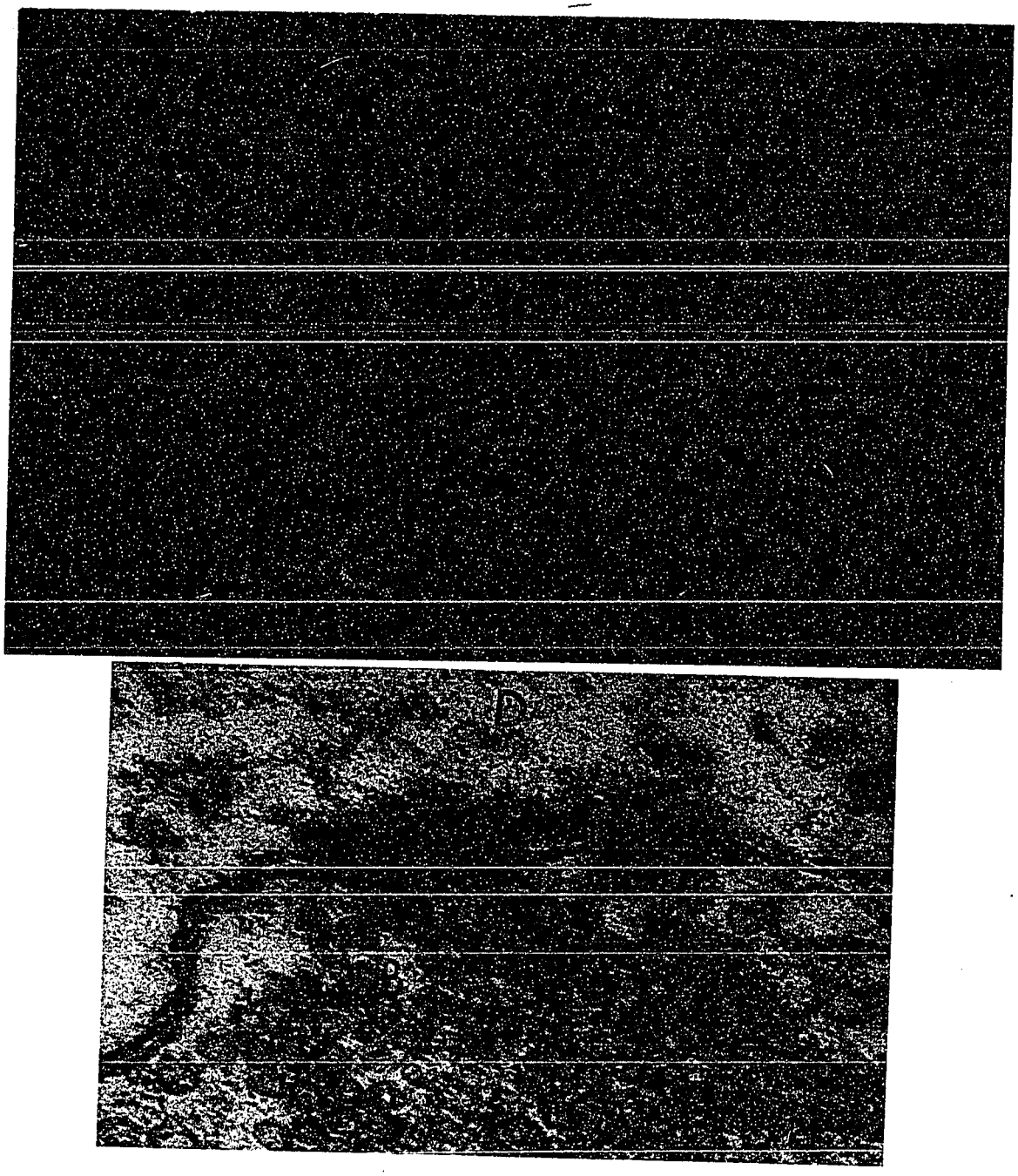


Fig. 7: a. - Electronmicrograph of a type 1 synapse, the bouton (B) is seen to make contact (arrow) with the dendrite (D). Note the darker thickening of the post synaptic membrane. The length of this contact is also much longer than the type 2. 58,000X

b. -Shows the same electronmicrograph as above but at a higher magnification. Arrow shows an extracellular membrane between the pre- and post-synaptic membranes typical of a type 1 synapse. 116,200X

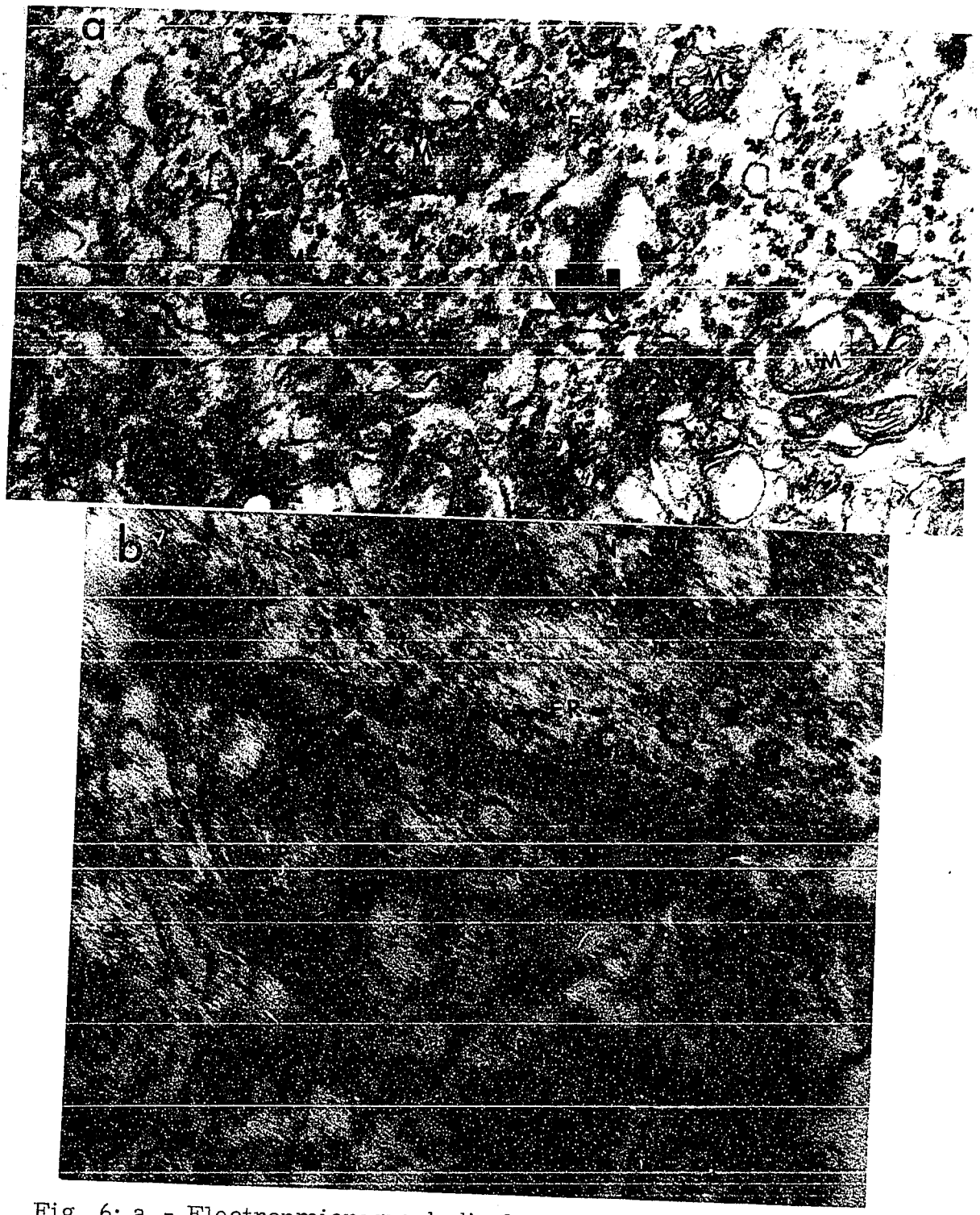


Fig. 6: a. - Electronmicrograph displaying axosomatic synapses. The body of the cell is recognized by the granular endoplasmic reticulum and mitochrondria. Boutons (B) making synaptic contacts on the body can be seen (arrows). Internal segment of globus pallidus 29, 000X.

b. -Electronmicrograph of the external segment of the pallidum, displaying the same type of synaptic contacts as above. 29, 000X

Light Microscopy - Experimental Material

Both lesions and the selective staining of degenerated fibers and terminal boutons were satisfactory in 11 cats.

The course and the apparent terminations of the afferent fibers of the globus pallidus have been well documented. Voneida ('60) in the cat, Papez ('38) and Szabo ('62) in the monkey have detailed the pathways from the head of the caudate nucleus. The pathways from the putamen have been described by Papez ('38), Ranson et al. ('41), Glees ('45) and more recently by Nauta and Mehler ('61) and Szabo ('62). The course from the nucleus ventralis anterior have been described by Ranson et al. ('41) and Ranson and Clark ('53). Finally the pathway from the substantia nigra by Ranson et al. ('41) Glees ('44) and Garpenter ('64).

The present study was limited therefore to a detailed analysis of degeneration as seen within the globus pallidus itself.

The procedure for all lesions were as follows: alternate sections stained by the Nauta-Gygax method were examined, regions showing significant preterminal degeneration were singled out and compared with the corresponding Bielschowsky sections.

A description of each series of lesions is presented below.

Caudate Lesions

Lesions destroying significant portions of the head of the caudate nucleus were produced in three cats. Electrode used to produce these lesions homolaterally were introduced in parasagittal planes in two animals. A contralateral approach was carried out on one animal. Destruction within the caudate nucleus was restricted to the rostral half of the nucleus in one cat, GPS-8. The lesion extended rostrocaudally from Fr. 17.0 to Fr. 13.5 (according to the coordinated in the atlas of

Jasper and Ajmone-Marsan ('54).

However, in one animal GPS-12, the lesion extended caudally to within a few millimeters of the body of the nucleus. The lesion in GPS-5 produced by a contralateral approach, was small and restricted to the centromedial part of the nucleus. Rostrocaudally the lesion extended from Fr. 17.0 to Fr. 14.5.

Electrodes entering the head of the caudate nucleus vertically, first traversed the cortex, the corpus callosum and the fornix before entering the nucleus. In the contralateral approach, the electrode which traversed the cortex continued obliquely through the corpus callosum, the fornix and the septal area before entering the nucleus of the opposite side.

Preterminal Degeneration in cat GPS-5, examination of the Nauta sections indicated that the preterminal degeneration was restricted to the rostral regions of the entopeduncular nucleus. No significant degenerating fibers or preterminal were seen in the external segment.

Individual fibers and preterminals can be seen in Nauta preparations in Fig. 9a. within the nucleus entopeduncularis, some ending pericellularly. Fig. 9b. shows bundles of degenerated fibers and preterminals in the external segment of the pallidum. This heavier degeneration resulted from the extensive lesion in cat GPS-12.

Bouton Degeneration: Terminal boutons revealed by the Bielschowsky method of the corresponding areas show a small group of degenerating boutons on the soma of the cells (see Fig. 10a). Larger clusters can be seen, at a lower magnification Fig. 10b., to cover the majority of the surface area of the soma of the neurons. Fig. 10c. also shows a relatively lesser amount of the proximal end of the dendrites. Smaller number of degenerating boutons were also seen on the distal end of the dendrites. None was seen in the external segment of the pallidum in the

case of GPS-5.

The criteria for determining the degenerated boutons were outlined on page 19-20. The fine degenerating terminal fibers which are difficult to find in this type of staining method were found in a few sections, Fig. 10d. is an example. In general the normal boutons were recognized as small ring shaped structures with an average diameter of 0.8 - 2.1 micra. The degenerated boutons were larger, up to 3.5 micra, they were more darkly stained and possessed in many cases a small degenerating tail.

All silver impregnation methods are unpredictable with regard to the completeness with which they stain fibers and boutons. Blackstad et al. ('51) warned that quantitative estimations must be made with great caution and in his study on the inferior olive of the cat, stressed only the qualitative features.

However, in the pallidum understudy and other areas of the central nervous system, for example, in the optic tectum of birds, the caudate nucleus and the cerebral cortex, it is very difficult to stain boutons under normal conditions. In lesioned animals on the other hand, boutons were stained with great facility, at least in the case of the pallidum.

This observation made it quite easy to compare the numerical differences of the lesioned side of the brain with the normal or unlesioned side. In areas of heavy degeneration as much as 20 - 30 boutons per microscopical field were found. This is in marked contrast to smaller amounts, (3 to 4 per microscopical field) found on the normal side.

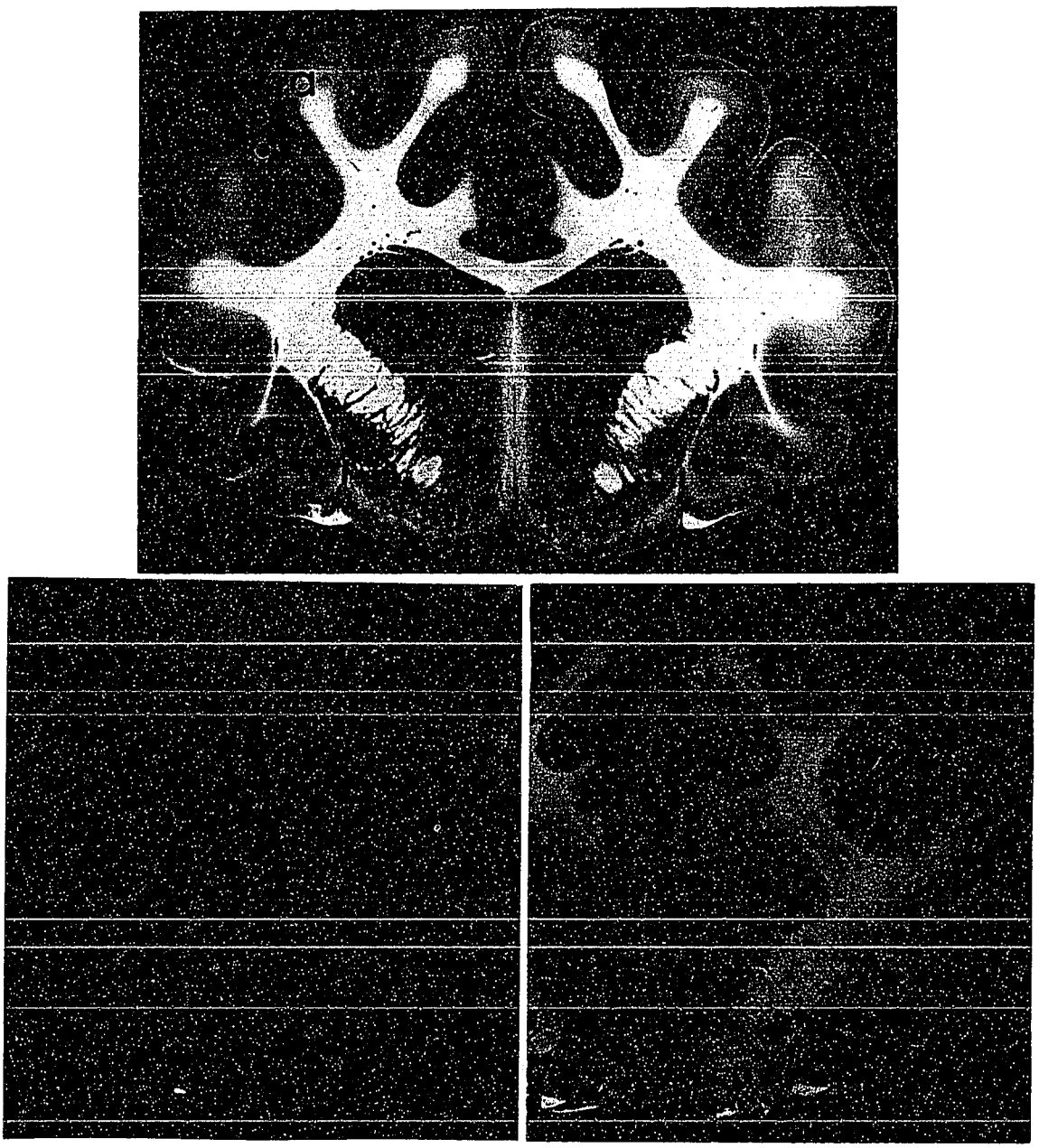


Fig. 8: Photomicrographs of discrete lesions in the caudate nucleus.
a. - Cat GPS-5 Lesion localized to the medio-ventral part of the left caudate nucleus. Electrode inserted contralaterally and involved part of the septal area. 6X.
b. - Cat GPS-12 Two large lesions producing extensive destruction in the right caudate nucleus. Electrode inserted vertically. 6X.
c. - Cat GPS-8 Lesion is confined to the middle third of the head of the caudate nucleus. 6X.

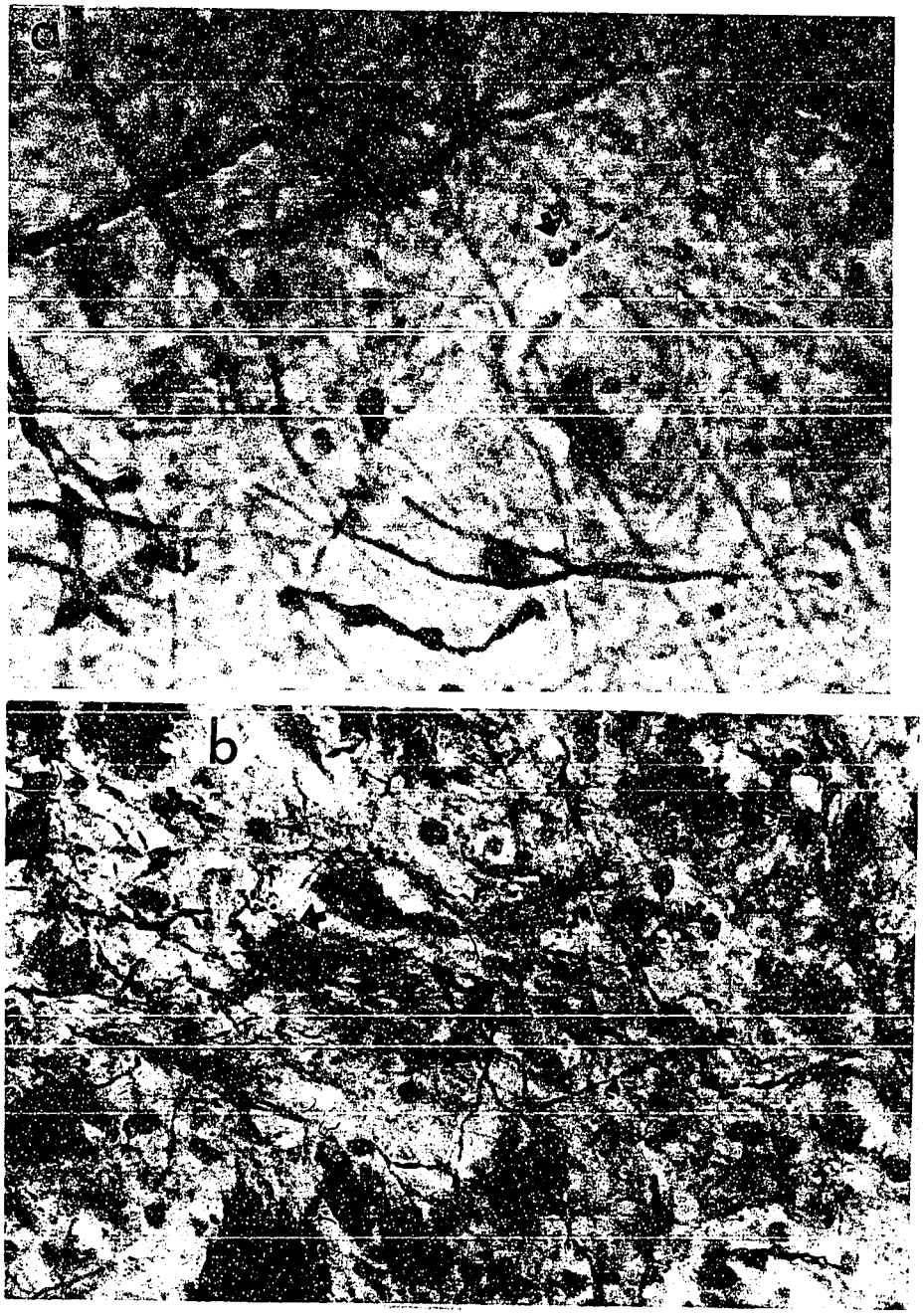


Fig. 9: Photomicrographs of sections of the globus pallidus
a. - Cat GPS-5 Degenerated fibers and preterminals (arrows) in the internal segment of the globus pallidus - Nauta-Gygax, 1750X.
b. - Cat GPS-12 Degenerated fibers and preterminals in the external segment of the globus pallidus. Nauta-Gygax. 800X

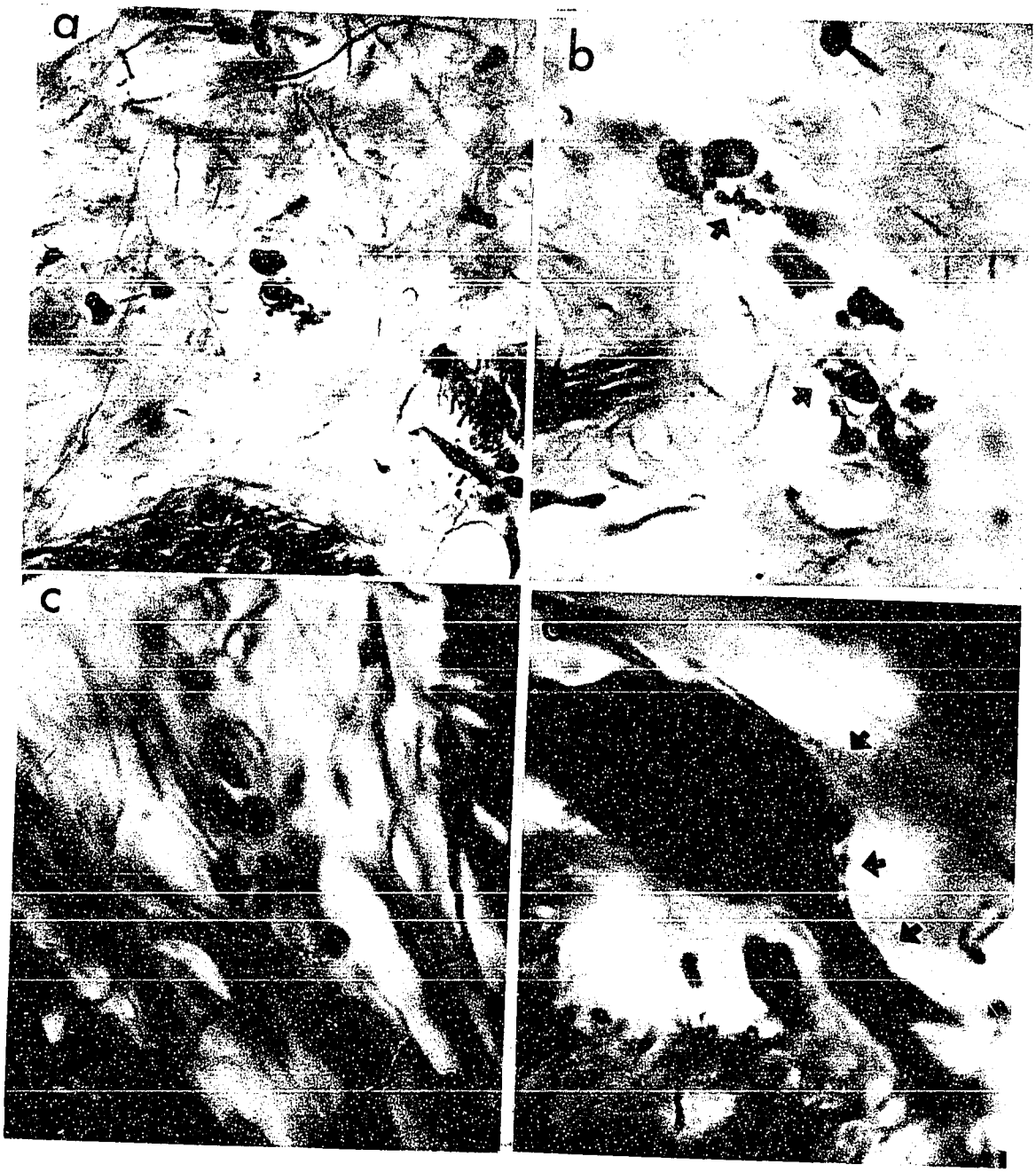


Fig. 10: Photomicrographs of sections from the globus pallidus stained by the modified Bielschowsky method.

a. - Cat GPS-5 Degenerated boutons forming small cluster on the soma of the neuron (arrow). Internal segment of the globus pallidus. 1750X.

b. - Cat GPS-12 Degenerated boutons (arrows) on the cell bodies and proximal end of the dendrites on two cells. External segment. 480X.

c. - Cat GPS-5 Degenerated boutons on proximal end of a dendrite. Internal segment. 1950X.

d. - Cat GPS-8 Two degenerating fibers (arrows) can be seen terminating on bodies of two cells. One cell is barely visible top right hand side. 1950X.

Putamen Lesions.

Successful lesions were produced in this nucleus on three cats. The electrodes used to produce these lesions were introduced in parasagittal planes in two of these animals. In one animal, GPS-7 (Fig. 11b), the lesion destroyed the medial part of the putamen. There was some infringement on the external medullary lamina. The lesion extended from Fr. 13.0 to Fr. 11.0 in a rostro-caudal direction. The electrodes successfully traversed the cortex, the extreme lateral limits of the internal capsule before entering the nucleus.

In the second animal GPS-17 (Fig. 11c), the destruction within the nucleus was limited to the lateral part. The lesion caused damage to the rostral two thirds of the nucleus; the most caudal parts were not damaged. The electrode, after piercing the cortex, travelled through the extreme edges of the internal capsule, the rostral tip of the claustrum and the extreme capsule before entering the nucleus.

In the remaining animal, GPS-4 (Fig. 11a), the electrode was introduced in a rostro-caudal direction at a compound angle, 16° to the vertical plane. The electrode track was seen to pierce the rostral edge of the claustrum and the outer limits of the internal capsule before entering the putamen. The destruction within the nucleus was very small, and was limited to the rostral one third of the putamen.

Preterminal Degeneration: Regardless of the extent of the damage within the putamen, all lesions showed significant degenerating fibers and preterminals in both segments of the pallidum; quantitatively there seemed to be more degeneration in the external segment as compared to the internal part of the pallidum.

In cat GPS-4 the lesion was limited to the rostral half of the putamen. Degenerating fibers and preterminals could be followed into both segments

of the pallidum. However, in this lesion, the majority of the preterminal degeneration seemed to be restricted to the rostral areas of the pallidum.

In cat GPS-11c. a large area of the lateral surface of the nucleus was destroyed. Fig. 12a. shows large degenerating bundles, individual fibres and preterminals within the external segment of the pallidum ; a quantitatively smaller amount of degenerating fibres and preterminals were also seen in the entopeduncular nucleus.

Similar patterns of degeneration was encountered in the remaining animal.

Bouton Degeneration: The corresponding Bielschowsky preparation revealed abundant terminal boutons, again mainly in the external segment. Fig. 12c. shows a degenerating fibre dichotomizing and ending in two boutons; the other degenerating fibre (top right corner) also splits up into three or four branches, one of which can be seen to end in a bouton which makes synaptic contact with the soma of a cell.

Numerical counts made on the degenerated and normal sides show a ratio of approximately fifteen to one. This ratio varied in each case according to the extent of the lesion

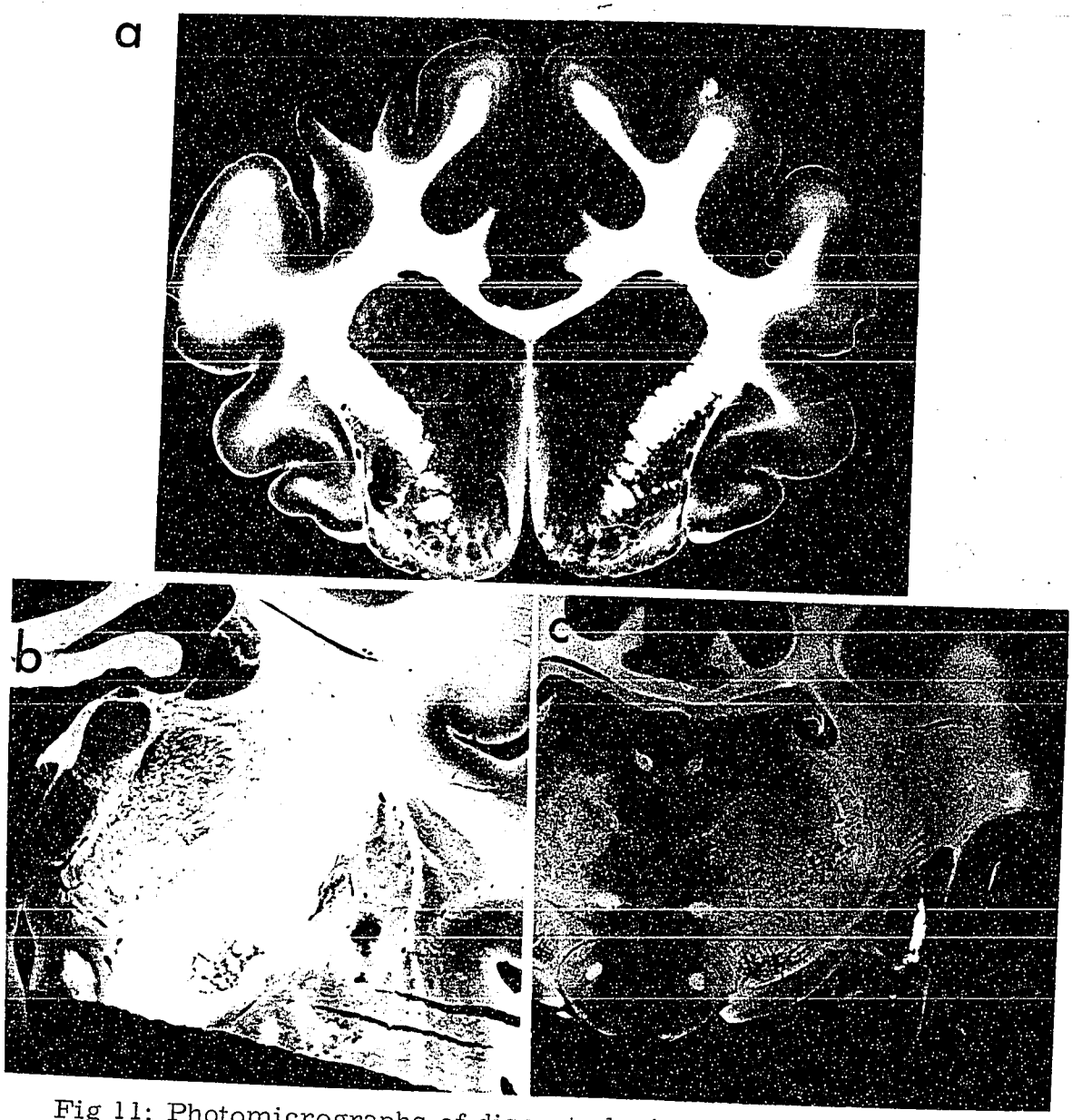


Fig 11: Photomicrographs of discrete lesions placed in the putamen.
a. - GPS-4 Electrode inserted at a compound angle, the lesion destroyed a large section of the rostral part of the putamen, 6X.
b. - GPS-9 The lesion was confined to the medial part of the putamen and involved the external medullary lamina. 6X.
c. - GPS-19 The lesion destroyed a large area on the lateral edge of the putamen and involved the capsula extrema and part of the claustrum. 6X.

Fig. 12: Photomicrographs of sections of the globus pallidus.

a. - Cat GPS-19 Degenerated putamino-pallidal fibers forming two fascicles in the external segment of the globus pallidus Nauta (Fink modification). 640X.

b. - Cat GPS-19 Degenerated terminal boutons (arrows) in the external segment of the pallidum. In the centre a bouton with degenerating tail is seen lying in contact with the cell body. Bielschowsky. 1, 900X.

c. - Cat GPS-9 Degenerating fibers and boutons arrows seen in the external segment of the pallidum. On the top a degenerated fiber divides into three terminal branches, an enlarged terminal bouton is seen on one of these. On the bottom a degenerated fiber dichotomizing into two terminal branches. 1, 900X

Ventralis Anterior Lesions.

Three lesions were successfully performed in this nucleus. The electrodes were introduced in parasagittal planes; in all cases the tracts from these electrodes traversed the cortex, infringed upon the lateral edge of the corpus callosum, pierced the nucleus anterior dorsalis, and the nucleus anterior medialis before entering the nucleus ventralis anterior. In one animal GPS-6 the lesion was quite small and was restricted to the central area of the nucleus; (slight damage to the nucleus ventralis lateralis caudally was caused by the lesion). In GPS-11 extensive damage was done to the nucleus ventralis lateralis and only slight damage to the nucleus ventralis anterior.

In GPS-13 the lesion caused destruction of the entire nucleus and extended rostrally into the nucleus reticularis, and caudally into part of the nucleus ventralis lateralis.

Preterminal Degeneration: No significant degeneration by the Nauta technique could be followed into the pallidum from the two cats with small lesions. However, in the cat GPS-13 with the massive lesion, degenerating fibers could be followed ventrally in the inferior thalamic peduncle, in agreement with Ranson, Ranson and Ranson ('41). Within the pallidum on the other hand, only a small number of degenerating fibers were seen as shown in Fig. 14d, scattered throughout the nucleus.

Bouton Degeneration: A corresponding small number of boutons in various stages of degeneration were however seen. These are shown in Fig. 14a-b.

It is significant to note, however, that no clusters or any evidence of degenerating boutons were seen on the cell bodies and all the boutons within the nucleus were seen either on dendrites or within the interstitium.

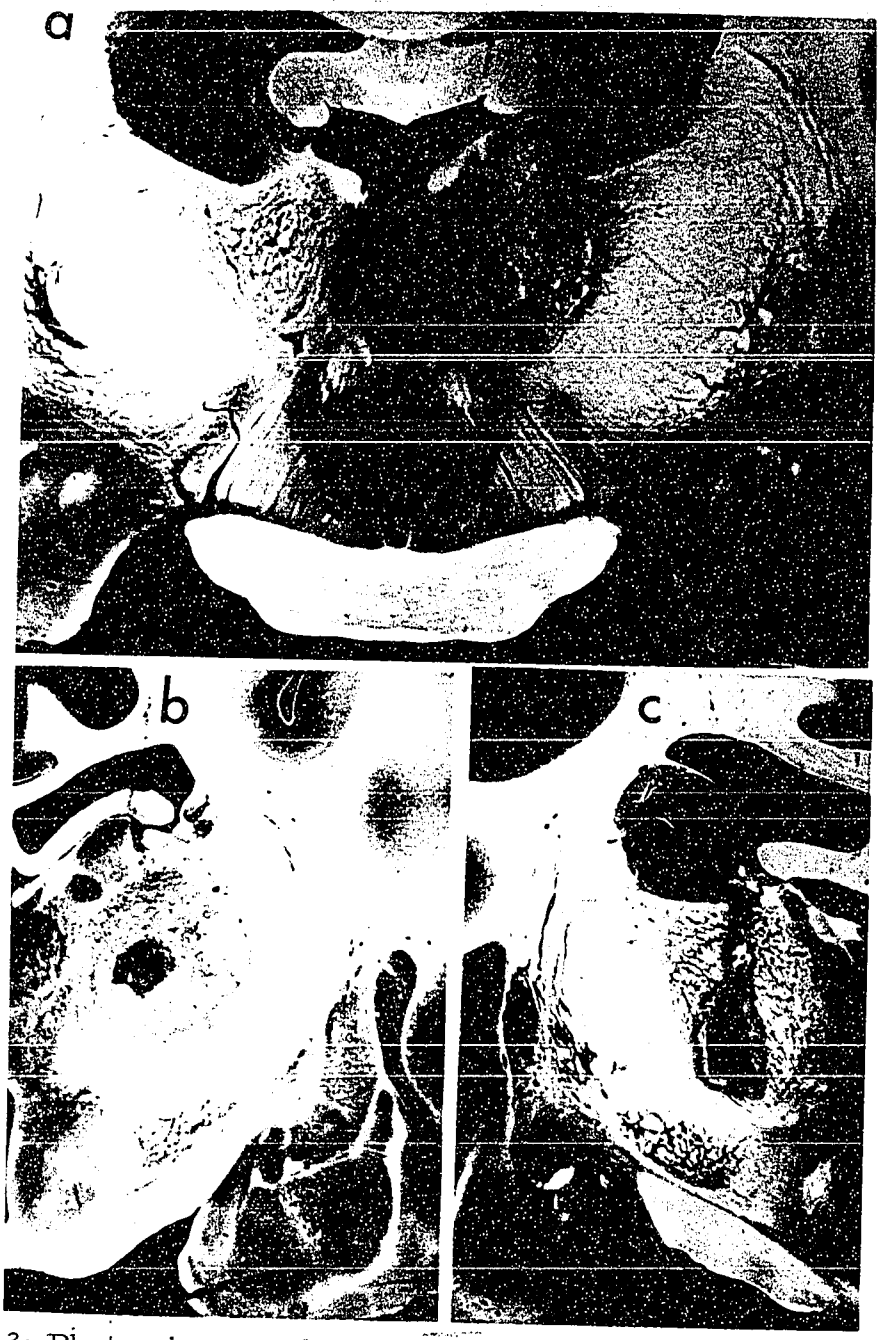


Fig. 13: Photomicrographs of discrete lesions in the nucleus ventralis anterior.

a. - Cat GPS-13 Two large lesions producing extensive destruction in the right ventralis anterior, and involved a large part of the nucleus reticularis rostrally and to a lesser extent the nucleus ventralis lateralis dorsally. 6X.

b. - Cat GPS-6 The lesion was confined to the central part of the right ventralis anterior. =6X.

c. - Cat GPS-11 The lesion destroyed a small portion of the left ventralis anterior and involved a larger area of the nucleus ventralis lateralis ventrally and caudally. 6X.

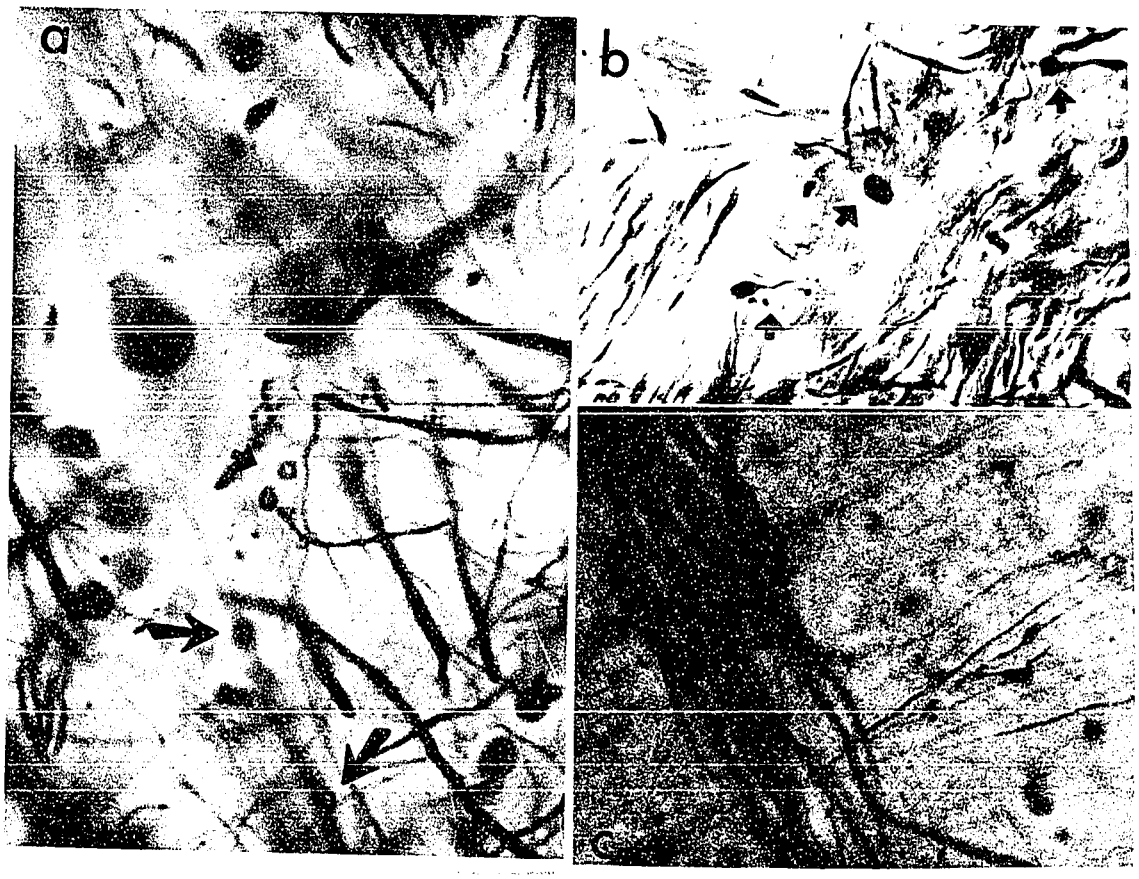


Fig. 14: Photomicrographs of sections from the globus pallidus
a. - Cat GPS-13 Two degenerated boutons (large arrows) seen on the dendrite of a pallidal cell. Two other boutons can be seen in the interstitium near to the soma of the cell, Bielschowsky, 1900X.
b. - Cat GPS-13 Three boutons in various stages of degenerations are seen (arrows); at the bottom a bouton with a degenerating tail, Bielschowsky, 1850X.
c. - Cat GPS-13 Preterminal degeneration in the internal segment of the pallidum, Nauta. 800X.

Substantia Nigra Lesions.

Successful lesions were made in this nucleus in two cats GPS-17 and GPS-20. The electrodes were introduced in each case in a vertical plane. Two lesions were performed in each case, one lesion was an attempt to destroy the medial part, and the other to destroy the lateral segment. In one cat GPS-17 the first lesion destroyed the entire medial segment in the caudal half of the nucleus. The second lesion destroyed a small segment in the ventral portion of the middle one third of the nucleus. The electrode also caused some damage to the cerebral peduncle. In cat GPS-20, one of the two lesions was confined to the middle third of the nucleus, but the second lesion caused damage mainly to the cerebral peduncle.

The electrode track in both cats traversed the cortex the corpus callosum, the fornix and the nucleus lateralis posterior, continued through the pretectal area, and the lateral edge of the red nucleus before entering the substantia nigra (Fig. 15).

Preterminal Degeneration: Nauta sections (Fig. 16a-b) conclusively reveal a substantial amount of degenerating fibers and preterminals within the internal segment of the pallidum. In agreement with Carpenter ('64) degeneration was restricted to the dorsal part of the nucleus adjacent to the internal capsule. These degenerating fibers and preterminals did not seem to arborize about neurons but seemed to become scattered in the neuropil. The heaviest degeneration was seen in the most caudal sections of the entopeduncular nucleus.

Examination of the external segments revealed little degeneration, an occasional degenerated fiber was seen among the normal fibers.

Bouton Degeneration: As in the case of lesions in the nucleus ventralis anterior, the degenerating bouton seen in the Bielschowsky sections were

confined to the dendrites, and in the majority of cases they were scattered in the interstitium. The small clusters of boutons seen on the cell bodies in the case of caudate and putamen lesions were not found. Fig. 16c-d show a degenerating terminal fibers and boutons with degenerated tails, typifying the mode of terminations of nigropallidal fibers.

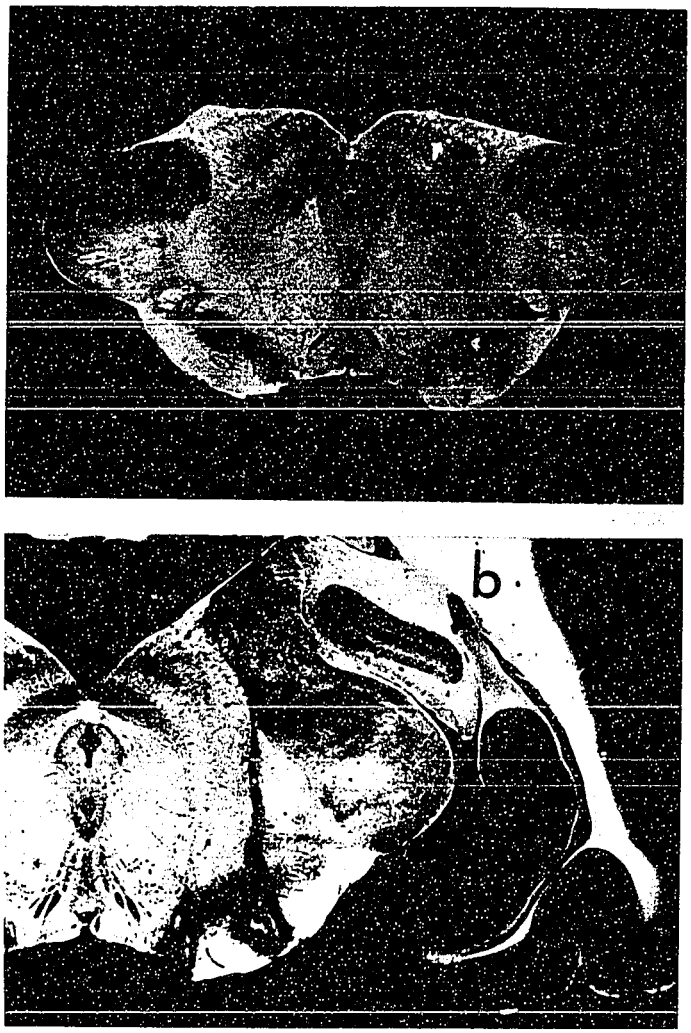


Fig. 15: Photomicrographs of lesions in the substantia nigra.
a. - Cat GPS-20 One of the lesions was restricted to the middle third of the substantia nigra. The other electrode destroyed part of the cerebral peduncle. 6X.
b. - Cat GPS-17 Lesions destroyed the medial and latero-ventral part of the substantia nigra, and, involved part of the cerebral peduncle. 6X.

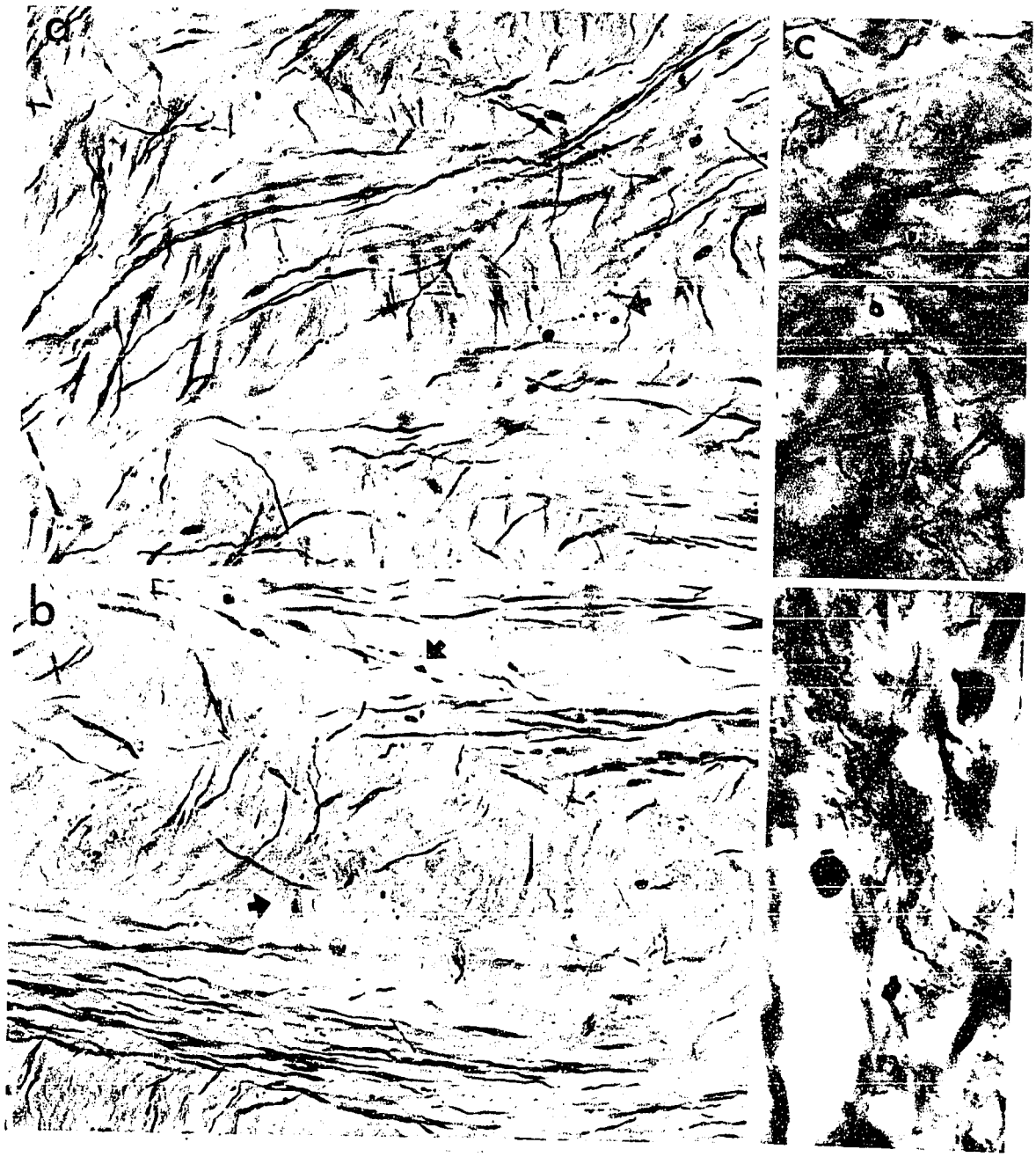


Fig. 16: Photomicrographs of sections of the globus pallidus.
a. - Cat GPS-17 Preterminal degeneration seen in the dorsal part of the entopeduncular nucleus, Nauta. 700X
b. - Cat GPS-17 Degenerating fiber coursing into the entopeduncular nucleus from the internal capsule. Some preterminal degeneration (arrows) are seen at bottom, Nauta. 700X
c. d. - Cat GPS-17 Terminal fibers and degenerating boutons are seen in the interstitium of the entopeduncular nucleus, Bielschowsky, 1200X.

Cortical Ablations.

Various frontal and parietal cortical areas were removed by suction. The frontal ablations were performed on five cats Figs. 7 - 11 and parietal areas on three cats. (Figs. 15-17)

In the first series of ablations, the areas destroyed included the gyrus proreus, the anterior and posterior sigmoid gyri.

In the second series of experiments, the areas destroyed included the anterior and post suprasylvian gyri, with some involvment of the ectosylvian gyrus and the anterior lateral gyrus extending into the posterior lateral gyrus.

Preterminal and Bouton Degeneration: Nauta and Bielschowsky preparations failed to show any significant terminal or preterminal degeneration within the globus pallidus.

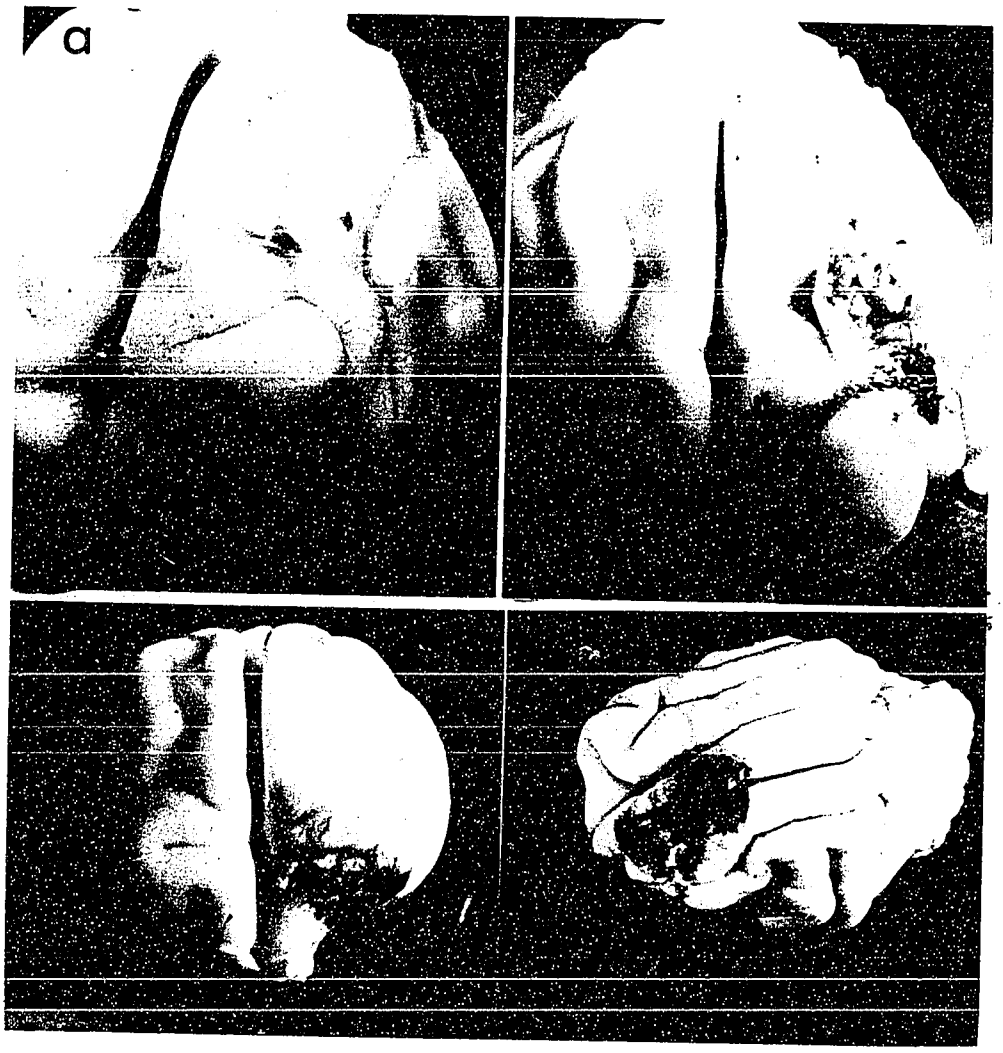


Fig. 17:. Photomicrographs of ablations in the cerebral cortex.
a. - Cat GPS-7 Ablation destroyed the anterior and posterior sigmoid gyri.
b. - Cat GPS-8 Ablation destroyed the anterior and post sigmoid gyri and involved part of the gyrus proreus.
c. - Ablation caused destruction to the anterior supra sylvian gyri. 8X.
d. - Ablation caused damage to the anterior and posterior sigmoid gyri, the anterolateral gyrus and involved part of the anterior suprasylvian gyrus. 5X.

DISCUSSION AND CONCLUSIONS

The main purpose of the present investigation was to study in as great detail as possible the mode of termination of the afferent fibers to the globus pallidus.

The complexity of the problem made it necessary to study the intrinsic organization of the nucleus in normal material. In this respect studies were carried out on the light and electron microscopic level. Golgi preparations have revealed the intricate formations of axons around the neurons. These axons seem to make many synaptic contacts on the dendrites before terminating on the soma of the cell.

The electron microscopic study has confirmed the general synaptic relations as shown by the light microscope. This investigation also revealed that there may be two types of synaptic contacts within the pallidum, type 1 and 2 as classified by Gray ('59) in his studies on the axosomatic and axodendritic synapses of the cerebral cortex.

Caudatopallidal connections

After sterotaxically placed lesions in the head of the caudate nucleus, degenerating fibers and preterminals can be seen by the Nauta technique to enter and terminate in both segments of the pallidum. This is in agreement with numerous authors cited previously. Although it is difficult to determine by this method the actual mode of termination of these fibers, a careful examination of many sections clearly shows a pericellular arrangement.

The corresponding Bielschowsky sections have shown that these fibers terminate mainly on the soma of the cell and on the proximal ends of the dendrites. Many boutons can be seen forming small clusters on the cell bodies and on the proximal end of the dendrites.

Quantitative estimates of degenerating boutons are difficult with any silver impregnation methods. However, in this study the differences in number of degenerating boutons on the lesioned side of the brain compared to the normal or unoperated side were sufficiently marked to permit the conclusion that the fibers ended in this nucleus.

Putaminopallidal Connections

The present investigation confirms the findings of previous authors with regard to the site of termination of fibers from the putamen to the pallidum.

The Nauta technique (Fink's modification) has shown quite clearly the abundance of degenerating fibers and preterminals entering the pallidum. Many of these fibers are seen to terminate around the cells in both segments of the nucleus.

The mode of termination as revealed by the Bielschowsky sections seems to be similar to that established by the caudate connections.

Many of the sections have revealed that the incoming fibers make axosomatic and axodendritic contacts but mainly of the former type. In many sections the degenerating fibers are seen to dichotomize into two or more branches before terminating on the cell bodies or dendrites. Quantitatively it would appear that more fibers end in the external than in the internal segment.

Connections from the Nucleus Ventralis Anterior

Stereotaxic lesions of various sizes were placed in this nucleus. Brains with small lesions did not reveal any conclusive evidence of degeneration either by the Nauta technique or by the Bielschowsky method. Even in a case in which the entire nucleus was destroyed only

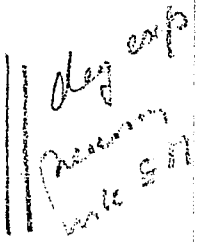
a very small number of fibers and boutons could be seen. The boutons nevertheless displayed all the evidences of degeneration. The numerical count was in the ratio of 3:1 and strongly suggests that at least a small percentage of fibers from the nucleus ventralis anterior projects to the pallidum. They are mainly seen on dendrites or in the interstitium, indicating axodendritic contacts.

Nigro-pallidal Connections.

The results of the present study indicate that relatively discrete stereotaxic lesions destroying significant portions of the substantia nigra in the cat produce terminal degeneration within the globus pallidus, particularly in the internal segment. These findings are in agreement with those of Ranson et al. ('41), Glees ('44) and Gardner ('64). The Nauta sections have shown that these fibers end mainly in the dorsal part of the nucleus bordering the internal capsule. Furthermore these fibers, in agreement with the observations of Gardner ('64) do not appear to end around the cells of the pallidum, but are scattered in the interstitium.

The Bielschowsky preparations also display a significant number of degenerating boutons.

In contrast to the fiber systems of the caudate nucleus and the putamen, the nigropallidal fibers appear to terminate mainly on the dendrites of the cells.



It should be emphasized that none of the cortical lesions resulted in any detectable fiber degeneration in the globus pallidus. These negative findings are in good accord with similar reports by some authors.

The sites of termination of the afferent fibers of the pallidum

established in this study on the whole agree well with those found by earlier workers using various experimental animals. However the use of a bouton staining technique makes it possible to determine the mode of termination more precisely than has been done so far with the Marchi and Nauta methods.

The physiological implication of these findings have not been considered in this study. However, the views expressed by some authors seem to correlate well with the anatomical findings.

Eccles ('64) has considered the possibility that type 1 contacts are excitatory and type 2 inhibitory. Kappers et al. ('36) in reviewing the functional aspect of the corpus striatum of vertebrates claimed that the caudate nucleus exerts an inhibitory influence on the globus pallidus. If these views are correct then the observations that fibers from the caudate nucleus and the putamen make type 2 contacts with the pallidum will lend anatomical support to the above-mentioned physiological hypothesis.

Gardner ('64) in his study of the functional aspect of the substantia nigra suggests that the influences of this nucleus upon motor function and other portions of the neuroaxis are probably mediated by the globus pallidus and relay nuclei of the mesencephalon.

Apart from this observation there is no physiological evidence available in the literautre to suggest that the nucleus ventralis anterior or the substantia nigra may have an excitatory influence on the globus pallidus.

SUMMARY

1. The intrinsic organization of the pallidum was first investigated by the Golgi-Fox method on several cats and monkeys.

2. An electron microscopic study was also undertaken on three cats. The results obtained confirm the general synaptic organization seen in the Golgi preparations. Two types of synaptic contacts are observed in the pallidum, type 1 and 2 according to the classification of Gray ('59).

3. Stereotaxic lesions were placed in the caudate nucleus, the putamen, the nucleus ventralis anterior and the substanita nigra. The ensuing preterminal and terminal degenerations were studied by the silver impregnation methods of Nauta and a modified Bielschowsky technique. Special emphasis was placed on the mode of termination of these fibers terminating in either segment of the globus pallidus.

4. The fibers from the caudate nucleus and the putamen terminate mainly as small clusters on the cell bodies and proximal ends of the dendrites.

5. Nauta sections of lesions made in the nucleus ventralis anterior and the substantia nigra show that degenerating fibers do not end on the cell bodies, but seem to be scattered in the interstitium. Bielschowsky preparations also confirm that the boutons make synaptic contacts primarily on the dendrites of the cell; many of these boutons are also seen among the neuropil.

6. From these studies it appears that those frontal and parietal areas of the cortex which were investigated do not project to thepallidum.

BIBLIOGRAPHY

Bickel, G., Quoted by H. L. Knook 1965 The fiber-connections of the forebrain, Van Gorcum and Co. N. V.

Blackstad, Th. W., Brodal, A. and Walberg, F. 1951 Some observations on normal degenerating terminal boutons in the inferior olive of the cat. Acta. Anat. (Basel) 11:461-477.

Cajal, S. Ramon y. 1911 Histologie du systeme nerveux de l'homme et des vertebres. Paris, Maloine (Editeur).

Carpenter, M. B., and McMasters, R. C. 1964 Lesions of the substantia nigra in Rhesus monkey. Efferent fiber degeneration and behavoural observations. Am. J. Anat., 114:293-320.

Eccles, J. C. 1964 The physiology of Synapses, Springer, Berlin.

Fox, C. A. Ubeda-Purkiss, M., Ihrig, H. K. and Biagiolo, D. 1951 Zinc chromate modification of the Golgi technique. Stain Tech., 26:109

Foix, C. H. et Nicolesco, J. 1925 Anatomie cerebrale. Les noyaux gris centraux et la region mesencephalo sous-optique. Masson et Cie, editeurs. Paris.

Fox, C. A., Hillman, D. E., Sether, L. A. and Siegesmund, K. A. 1965 The fine structure of the globus pallidus. 8th International congress of anatomizts. Wiesbaden, Germany.

Fox, C. A. and Schmitz, J. T. 1944 The substantia nigra and the entopeduncular nucleus in the cat. J. Comp. Neur., 80:323-334.

Fink, R. ('66) personal communication.

Gibson, W. C. 1938 Degeneration of the bouton terminaux in the spinal cord. Arch. Neur. Psychiat., 38:1145

Glees, P. 1941 The termination of optic fibers in the lateral geniculate body of the cat. J. Anat., 75:434-440.

Glees, P. 1944 The anatomical basis of cortico-straite connections. J. Anat., 78:47-51.

Glees, P. 1945 The interrelation of the strio-pallidum and the thalamus in the macaque monkey. Brain, 68:331-346.

Glees, P. 1946 Terminal degeneration within the central nervous system as studied by a new silver method. J. Neuropath. Exp. Neurol., 5:54-59.

Glees, P. and Wall, P. D. 1946 Fiber connections of the subthalamic region and the centromedian nucleus of the thalamus. Brain, 69:195-208.

Gray, E. G. 1959 Axosomatic and axodendritic synapses of the cerebral cortex. J. Anat., 93:420-433.

Guillery, R. W. 1965 Some electron microscopical observations of degenerative changes in central nervous synapses. Progress in Brain Research, 14:57-76.

Hoff, E. C. 1935 Corticospinal fibers arising in the pre-motor area of the monkey: distribution of bouton terminations. Arch. Neurol. and Psychiat., 33:687-697.

Jakob, A. Quoted by H. L, Knook 1965 The fibre connections of the forebrain. Van Gorcum & Co. N. V.

Jasper, H. H. and Ajmone-Marsan, C. 1954. A stereotascic atlas of the diencephalon of the cat. National Research Council of Canada, Ottawa, Canada.

Johnson, T. N. and Clemente, C. D. 1959 An Experimental study of the fiber connections between the putamen, globus pallidus, ventral thalamus and midbrain termentum in cat. J. Comp. Neur., 113:83-101.

Kappers, C. U. Ariens, Huber, G. C. and Crosby, E. C. 1936 The comparative anatomy of the nervous system of vertebrates, including man. The MacMillan Co., N. Y.

Knook, H. L. 1965 The fiber-connection of the forebrain, Van Gorcum, N. V.

Mettler, F. A. 1935 Corticifugal fiber connections of the cortex of macacamulatta. The occipital region. J. Comp. Neur. 61:221-256.

Mettler, F. A. 1935 Corticifugal fiber connections of the cortex of macaca mulatta. The frontal cortex. J. Comp. Neur. 62:509-542

Mettler, F. A. 1935-36 Corticifugal fiber connections of the cortex fo macaca mulatta. The parietal region. J. Comp. Neur. 62:263-292.

Mettler, F. A. 1935-36 Corticifugal fiber connections of the cortex of macaca mulatta. The temporal region. J. Comp. Neur. 63: 25-48.

Mettler, F. A. 1942 Relation between pyramidal and extrapyramidal function. Assoc. Res. Nerv. Ment. Dis., 21:150-227.

Mettler, F. A. 1945 Fiber connections of the corpus striatum of the monkey and baboon. J. Comp. Neur. 82:169-204.

Nauta, W. H. and Gygax, P. A. 1954 Silver impregnation of degenerating axons in the central nervous system, A modified technique. Stain Tech., 29:21-93.

Nauta, W. J. H. and Mehler, W. R. 1961 Some efferent connections of the lentiform nucleus in monkey and cat. Anat. Rec., 139:260.

Nauta, W. J. H. and Whitlock, D. G. 1954 An anatomical snalysis of the non-specific thalamic projection system. Brain Mech. and conscious. A symposium organized by the council for interant. organ. of med. science. Oxford pp. 81-116.

Papez, J. W. 1938 Reciprocal connections of the striatum and pallidum in the brain of pithecus macacus rhesus. J. Comp. Neur., 69:329-349.

Phalen, G. S. and Davenport, H. A. 1937 Pericellular end-bulbs in the central nervous system of vertebrates. J. Comp. Neur., 68:67-82.

Ranson, S. W. and Clark, S. L. 1953 The antomy of the nervous system, Its development and function. W. B. . Saunders & Co., Philadelphia & London.

Ranson, S. W. Ranosn, S. W. Jr. and Ranson, M. 1941 Fiber-connections of corpus striatum as seen in Marchi preparations. Arch. Neurol. Psychiat., 46: 230-249.

Rio-Hortega, P. Del Quoted in Mikroskopische Technik. Romeis, B. ed. 1948 page 361.

Rosegay, H. 1944 An experimental invesitgation of the connections between the corpus striatum and substantia nigra in the cat. J. Comp. Neur. 80:293-322.

Showers, M. J. 1958 Correlation of medial thalamic nuclear activity with cortical and subcortical neuronal arcs. J. Comp. Neur., 109:261-315.

Smith, O. C. 1930 The corpus striatum, amygdala and stria terminalis of tamandua tetradactyla. J. Comp. Neur., 51:65-127.

Szabo, J. 1962 Topical distribution of the striated efferents in monkey. Exp. Neurol., 5:21-36.

Verhaart, W. J. C. 1950 Fiber analysis of the basal ganglia. J. Comp. Neur., 93:425-440.

Verhaart, W. J. C. and Kennard, M. 1940 Corticifugal degeneration following thermocoagulation of areas 4, 6 and4-s in Macaca mulatta. J. Anat., 74:239-254.

Vogt, C. O. Quoted by Knook, H. L. 1965 The fiber-connections of the forebrain. Van Gorcum and Co. N. V.

Voneida. J. J. 1960 An experimental study of the course and destination of fibers arising in the head of the caudate nucleus in the cat and monkey. J. Comp. Neur., 115:75-87.

Walberg, F. 1964 The early changes in degenerating boutons and the problem of argyrophilia. Light and Electron-microscopic observations. J., Comp. Neur., 122:113-137

Whittier, J. R. and Mettler, F. A. 1949 Studies on the subthalamus of rhesus monkey. I Anatomy and fiber connections of the subthalamic nucleus of luys. J. Comp. Neur. 90:281-318.

Wilson, S. A. K., Edin, B. S. and Lond, M. R. C. P. 1911-1912 Quoted by H. L. Knook 1965 The fiber connections of the forebrain Van Gorcum and Co., N. V.