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Degeneration and Electron Microscope Analysis of the Synaptic Glomeruli in the Lateral Geniculate Body

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Summary. Optic fibers of retinal origin terminate in the lateral geniculate body exclusively in the so called glomerular synapses. They can be recognized on the basis of their unusually large irregular mitochondria having very few cristae. In the cat the structure of the optic terminal profiles is rather dense. The majority of terminals in most glomeruli originate from axons of other source. Relatively large axon terminal profiles of unusually light structure cannot be brought to degeneration by any interference with extraneous pathways. From Golgi information it becomes obvious that they originate from local Golgi 2nd type neurons. Small rather dense axonal profiles of the glomeruli can occasionally be traced back by degeneration to the occipital cortex (parastriate), although most of the descending cortical afferents of the lateral geniculate body terminate outside the glomeruli on more proximal parts of the dendrites. — Axo-axonic synapses are very frequent. If an optic terminal is involved, it appears that by structural standards it is "presynaptic" to the non optic. As judged, however, from the numerous axo-axonic contacts persisting after enucleation, many of the contacts are established between non optic axon terminals. — The progress of secondary degeneration and particularly the removal from the glomeruli of degeneration fragments is unexpectedly rapid. — The possible functional significance of these findings, especially also with regards to presynaptic inhibition, is discussed.

Key Words: Lateral geniculate body — Glomerular synapses — Synaptic degeneration ultrastructure — Golgi neurons.

Introduction

Complex synaptic arrangements in the LGB, resembling the glomerular synapses of the cerebellar cortex, have been described first in the cat (SZENTÁGOTHAI 1962, 1963), and it has been assumed on the basis of some preliminary degeneration findings, that the main axon terminals of these glomeruli are from fibers of retinal origin. Similar synaptic arrangements have been described in the monkey by COLONNIER and GUILLERY (1964) and large relatively pale profiles have been identified by secondary degeneration as of optic origin. The structure of the cat geniculate glomeruli has been clarified in more detail by the studies of PETERS and PALAY (1965)* stressing the central position of a relatively large

* A detailed account of these studies has appeared shortly after this manuscript has been sent for printing. [A. PETERS and S.L. PALAY: The morphology of laminae A and A_1 of the dorsal nucleus of the lateral geniculate body of the cat. J. Anat. (Lond.) **100**, 451—486 (1966).] In spite of considerable differences in approach — the present paper being based mainly on experimental degeneration — the conclusions reached in both investigations show fair agreement.

terminal, the more peripheric distribution of smaller endings and the separation of the glomeruli from their environment by a glial sheath.

Although some preliminary information on the probable origin of non optic synapses in the geniculate glomeruli has been given already (SZENTÁGOTHAI 1962, 1963), this question is far from being sufficiently clear, particularly with respect to short local neurons, repeatedly mentioned in the literature to occur in the LGB. Also the identification of optic terminals by secondary degeneration, and particularly the explanation of persisting synaptic glomeruli after transection of both optic nerves is not entirely satisfactory. This paper presents the results of further investigation into the structure of the geniculate glomeruli, using Golgi studies mainly for observations of the Golgi 2nd type neurons and their connexions, and EM studies after destruction of various pathways afferent to the LGB.

Material and Methods

Adult cats were used in this study. For investigation on the light microscopy level, mainly of Golgi 2nd type cells, the perfusion Kopsch method developed by SZENTÁGOTHAI (1963) was used as described in more detail in a recent paper by TÖMBÖL (1967). Although most neuronal elements, both nerve cells and axon ramifications, may be well stained in one series or the other, the procedure is particularly suited for Golgi 2nd neurons. — For EM studies the LGB was exposed under deep ether anesthesia and a sagittal incision flooded with cold (4°C) buffered 3.5% glutaraldehyde solution. Excess of glutaraldehyde solution and blood was continuously removed and new fixative steadily dropped on during the excision procedure. Small tissue blocks were cut out from the exposed surface by sharp scissors and immersed in the same fluid. The brains were then fixed in formol solution and the exact site of the blocks controlled on frozen sections. — After one hour glutaraldehyde fixation the tissue blocks were washed in Milloning-phosphate buffer and postfixed for one hour in cold buffered 1% osmic acid solution, dehydrated in alcohol and embedded in Araldite (Fluka). — Ultrathin sections (LKB Ultratome) were stained with lead citrate after Reynolds and investigated on the Tesla Table and the Hitachi Electron microscopes.

For degeneration purposes adult cats with both optic nerves operatively transected were kept alive 2, 3, 4, 5 and 7 days, and the LGB prepared for EM investigation as above. — In order to investigate the terminations in the LGB of recurrent cortical pathways cortical regions surrounding the striate region were removed superficially by suction. The striate region was left intact in order to avoid retrograde degeneration of LGB neurons. This was considered permissible as according to WIDÉN and MARSAN (1961) and SZENTÁGOTHAI (1963) the strongest recurrent cortical connexions of the LGB originate from the surrounding of area 17. Animals with cortical lesions were let to survive for 5 days and material fixed in the same manner. — Some of the degeneration material was fixed by the perfusion method of HOLT and HICKS (1961), but the results were essentially not different and less good than with the glutaraldehyde fixation given above.

EM studies on chronic degeneration meterial from earlier series both of optic nerve transected and animals with cortical lesions (2 months survival time) was also used, but as the results reach beyond the scope of this paper, some observations will only be mentioned briefly. One of the present authors (J.H.) will discuss this material from other aspects in the near future.

Results

1. Golgi 2nd type neurons of the LGB

The perfusion Kopsch procedure is particularly suited for elective staining of Golgi 2nd type neurons in all parts of the thalamus and the geniculate bodies. It is particularly important that the staining is successful in adult animals, so that the dendritic tree — not characteristic enough in young animals as generally used

for the classical Golgi procedures — can be visualized in its mature form. — Fig. 1 shows a photograph and a detailed camera lucida drawing of its dendritic and axonal arborization, as well as of some other terminal axon arborizations. As practically only Golgi 2nd type neurons are stained in this material the other

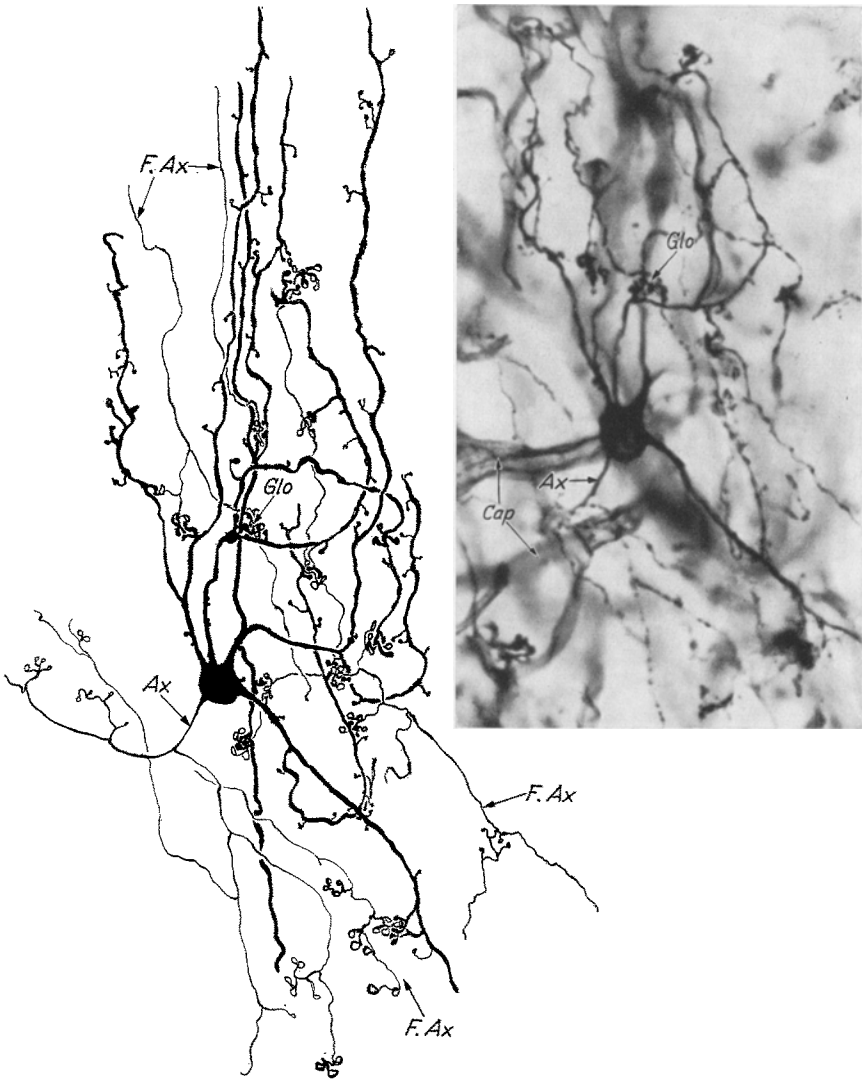
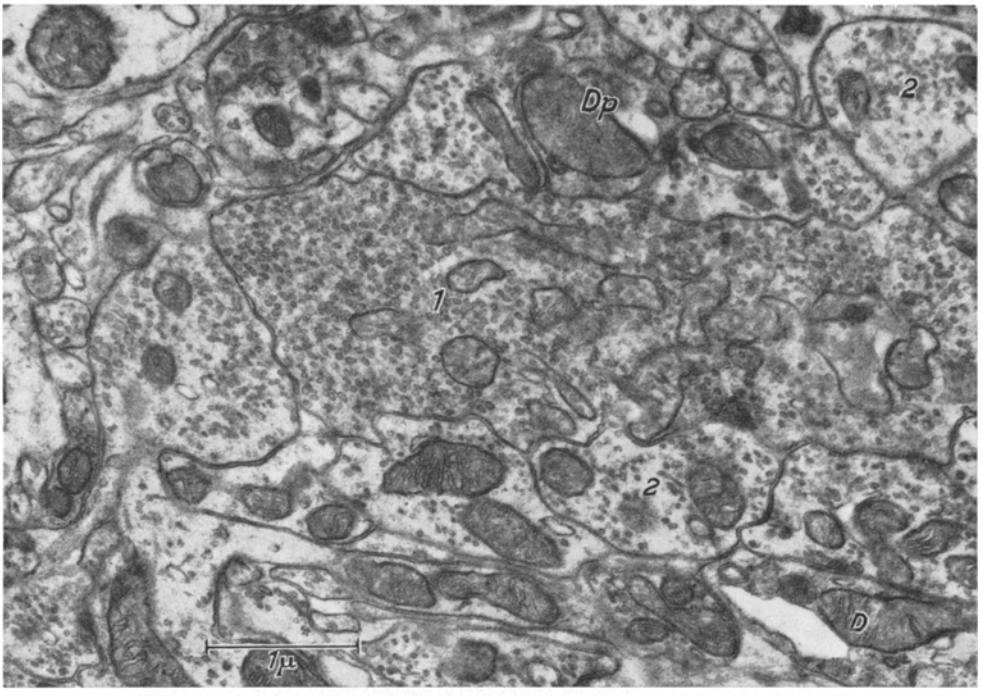


Fig. 1. *Characteristic Golgi 2nd type neuron in the LGB of an adult cat in photomicrograph (right above), and the main connexions shown in a camera lucida drawing (left below).* Own axon (Ax) ramification and parts of axon ramifications of foreign Golgi 2nd type cells (F. Ax) can be distinguished. The latter engage in glomerular (Glo) synaptic formations with "drumstick" side branches of the dendrites. Capillary network (Cap) is stained. — Perfusion Kopsch procedure

axons are from neighbouring Golgi cells. Both the spheric or ovoid cell body and the "wavy" character of the dendrites is highly characteristic of the Golgi cells, contrasting sharply with the larger angular bodies and radiate — almost rigid —

Fig. 2



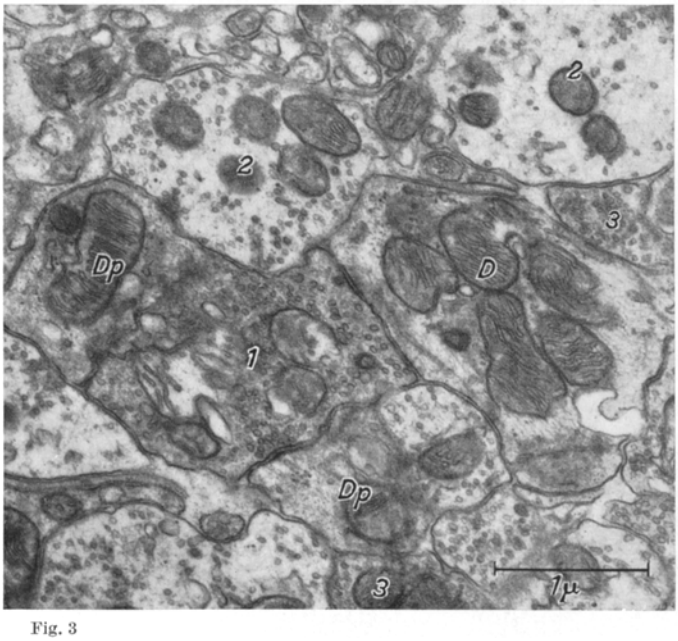


Fig. 3

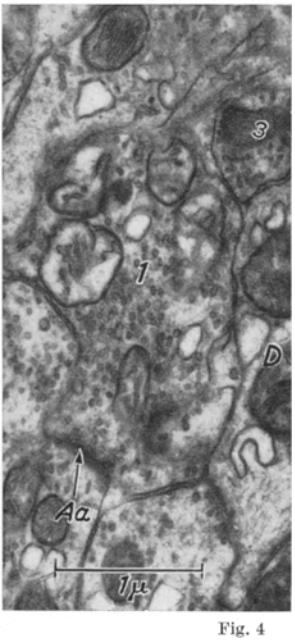


Fig. 4

Figs. 2, 3, 4. *Glomerular synaptic complexes of adult cat* with various kinds of axonal and dendritic profiles (D or Dp = dendritic protrusion) are closely packed together. Even at this low power various kinds of axon endings can be distinguished: (1) large dense profiles generally of central position, having large irregular mitochondria with very few cristae, (2) pale almost empty profiles with small dark mitochondria and (3) small dense profiles with densely cristated mitochondria. — In Fig. 4, axo-axonic (Aa) contact with (1) type profile being "presynaptic".

dendritic tree and "tufted" branching pattern of the geniculo-cortical relay (GCR) cells.

(A detailed description of the cell types — as shown by this procedure — is outside the frame of this study. A general description of the two typical cell types and their connexions in the main specific nuclei of the thalamus has been already

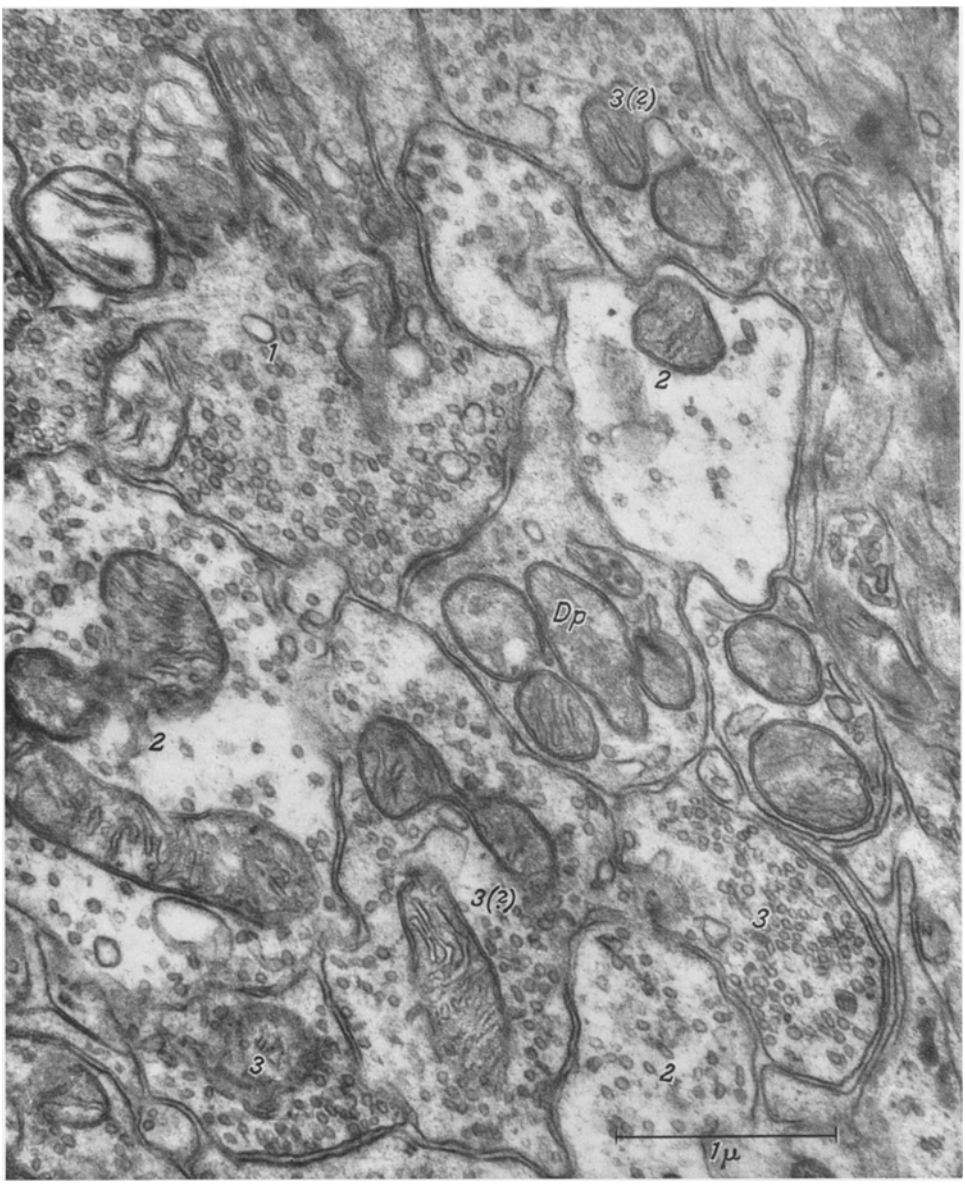


Fig. 5. As Figs. 2 and 3 with higher power. Type (1) axon terminal can be easily recognized at upper left, having synaptic contacts with dendritic protrusion (Dp). The density of type (1) profile is both due to dense plasma background and numerous synaptic vesicles, whereas lightness of (2) type profiles to light plasma and very few vesicles. The small (3) type profiles are often ambiguous (3 ?), but can on the basis of their dense mitochondria always be distinguished from type (1)

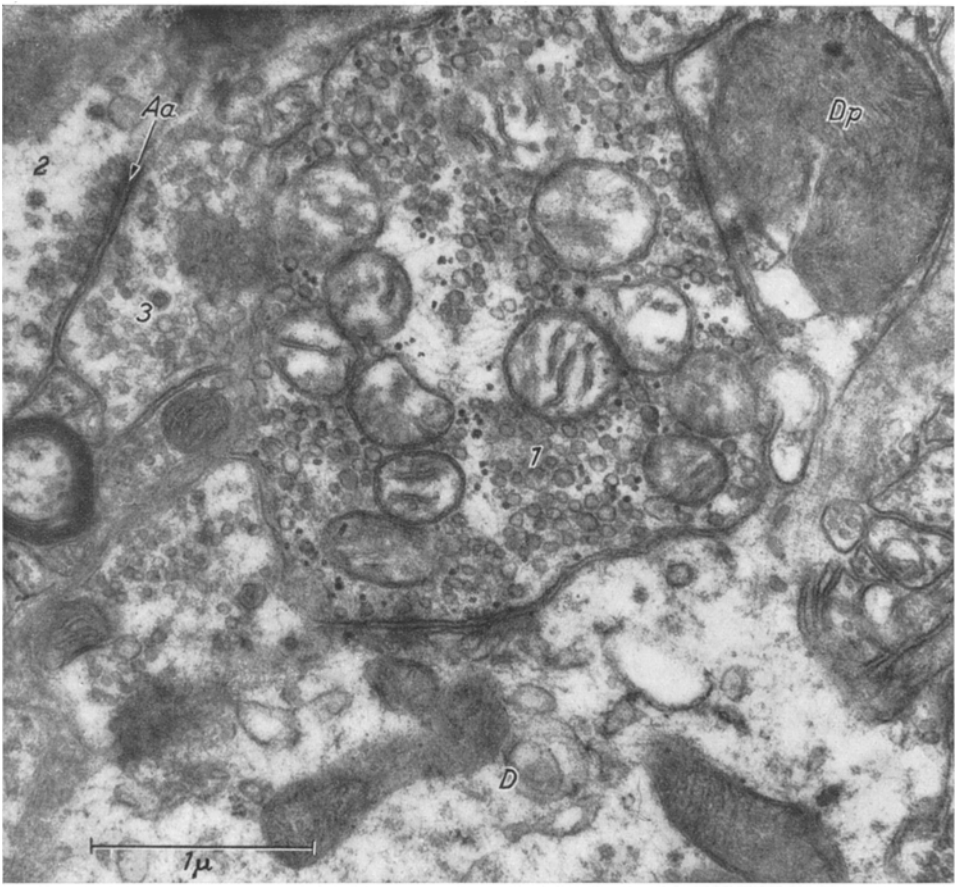
given by one of us [T.T., 1967]; a further discussion of the LGB cell types will be combined in near future with a topographic study of optic and other axon ramifications). — The large number and even distribution of the Golgi neurons is striking, their axon ramifications alone — although in most cases arborizing in close neighbourhood of the cell body — build up a continuous dense axonal feltwork in the LGB neuropil. As seen from both the microphotograph and drawing of Fig. 1 the terminal branches of the Golgi axons terminate in grape-like clusters of delicate end-rings. These clusters interdigitate in glomerular formations both with the fine "spine-like" drumstick shaped side-branches of Golgi cell dendrites and with the spheroid protrusions of GCR cell dendrites described already by SZENTÁGOTHAI (1963). Thus the Golgi cell axons get into contact with the dendrites of other Golgi cells as well as with those of GCR neurons. — The optic afferents recognizable on the basis of their much larger caliber (SZENTÁGOTHAI 1963) establish synapses with both GCR and Golgi neuron dendrites. — Recurrent cortical fiber terminals can be identified with high probability with delicate straight fibers giving short terminal side branches along their entire course and making single contacts indiscriminatively with all dendrites they happen to cross (TÖMBÖL 1967).

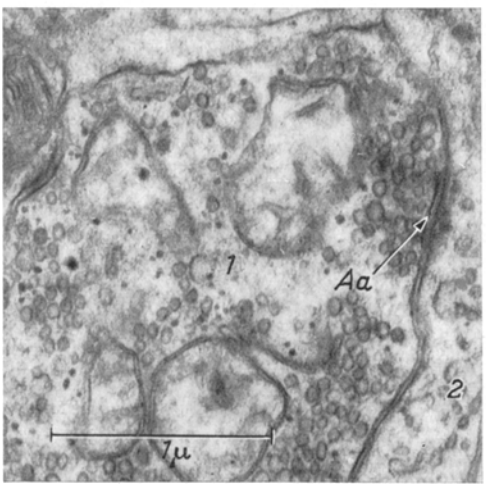
2. Synaptic glomeruli

The normal all-over appearance of synaptic glomeruli is shown on low power electron micrographs in Figs. 2, 3 and 4. As described already by SZENTÁGOTHAI (1962, 1963) and PETERS and PALAY (1965) they consist of various axonal profiles, with larger ones more often localized in the center, and smaller ones as well as dendritic profiles situated on the periphery of the glomeruli. It is relatively rare that a larger dendrit itself enters a glomerulus. The dendritic profiles are generally bulges or terminal side branches of the dendrites, containing one or several large densely cristated mitochondria. — Three different kinds — at least — of axonal terminals can be recognized in the glomeruli of the cat (Figs. 3, 5): 1. One kind mostly occupying the center of the glomerulus is large, irregularly shaped, relatively dark in consequence of its dense filamentous plasma structure and numerous synaptic vesicles, and particularly characteristic large irregular mitochondria with unusually few cristae. 2. Another type is also relatively large — although smaller than (1) — with very light plasma structure and few irregularly distributed vesicles, its mitochondria are dark in consequence of densely arranged cristae. 3. Smaller profiles, often darker than (1) with dense plasma structure, and densely cristated dark mitochondria. Transitory forms are, of course, frequent which cannot be simply classified into one of these groups, but there is no sufficient reason to make further subdivisions. — Axo-axonic contacts with specialized regions of attachment: i. e. accumulations of synaptic vesicles on one side and a thickened part of the membrane on the other side of a widened part of the synaptic cleft are seen quite often. If a type (1) ending is involved it is always that which has an accumulation of vesicles (Fig. 4).

Degeneration studies: Figures 6 and 7 show the early changes that can be observed already on the second day and more clearly on the 3rd day after transection of the optic nerve. They occur exclusively in the large terminals (1) with the characteristic irregular sparsely cristated mitochondria. Synaptic vesicles become less

Fig. 6





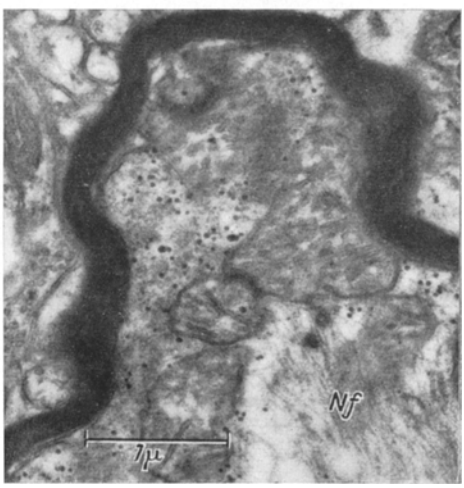


Fig. 7

Fig. 8

Figs. 6, 7, 8. *Early (2nd and 3rd day) changes after optic nerve transection.* Dense (probably glycogen) granulae appear at this stage exclusively in type (1) axon terminals. The synaptic vesicles become more irregular in size and begin to be lumped together at the periphery. Axo-axonic contacts (Aa) — on Fig. 6 between (2) and (3) type profile, and in Fig. 7 between (1) and (2) type ending. D = dendrite, Dp = dendritic protrusion. — In Fig. 8 the similar early changes in myelinated optic fiber are shown, with hypertrophic neurofilaments (Nf) in the center and mitochondria, dense granula and other dense material gathered at the periphery

Fig. 9

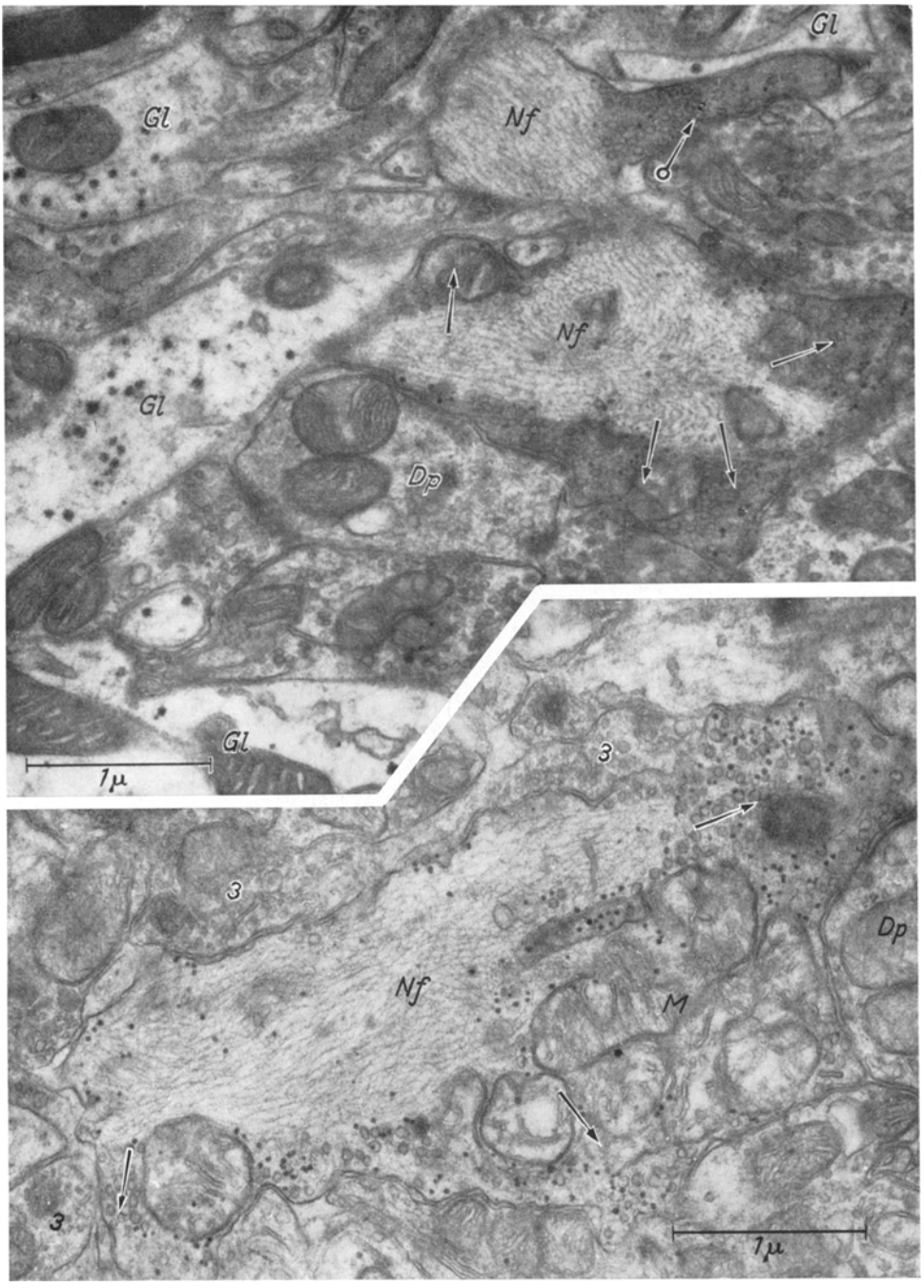


Fig. 10

Figs. 9, 10. *Neurofilament hypertrophy and beginning fragmentation stage (4th day) of optic afferent degeneration.* The outlines of the terminals have become irregular, their centers are occupied by strangely hypertrophic neurofilament (Nf) material, while at their periphery mitochondria, dense granulae and synaptic vesicles are forming dense irregular masses (arrows). The degenerated terminals can be recognized on the basis of their mitochondria (M) as type (1) endings (Fig. 10). The other non degenerated terminals are sufficiently changed to make their recognition ambiguous. Glial processes (Gl) containing dense glycogen bodies invade the glomerulus. Finger shaped dense protrusion of degenerated optic ending at upper right of Fig. 9 (ringed arrow) is already surrounded by small glial profile (Gl)

Fig. 11

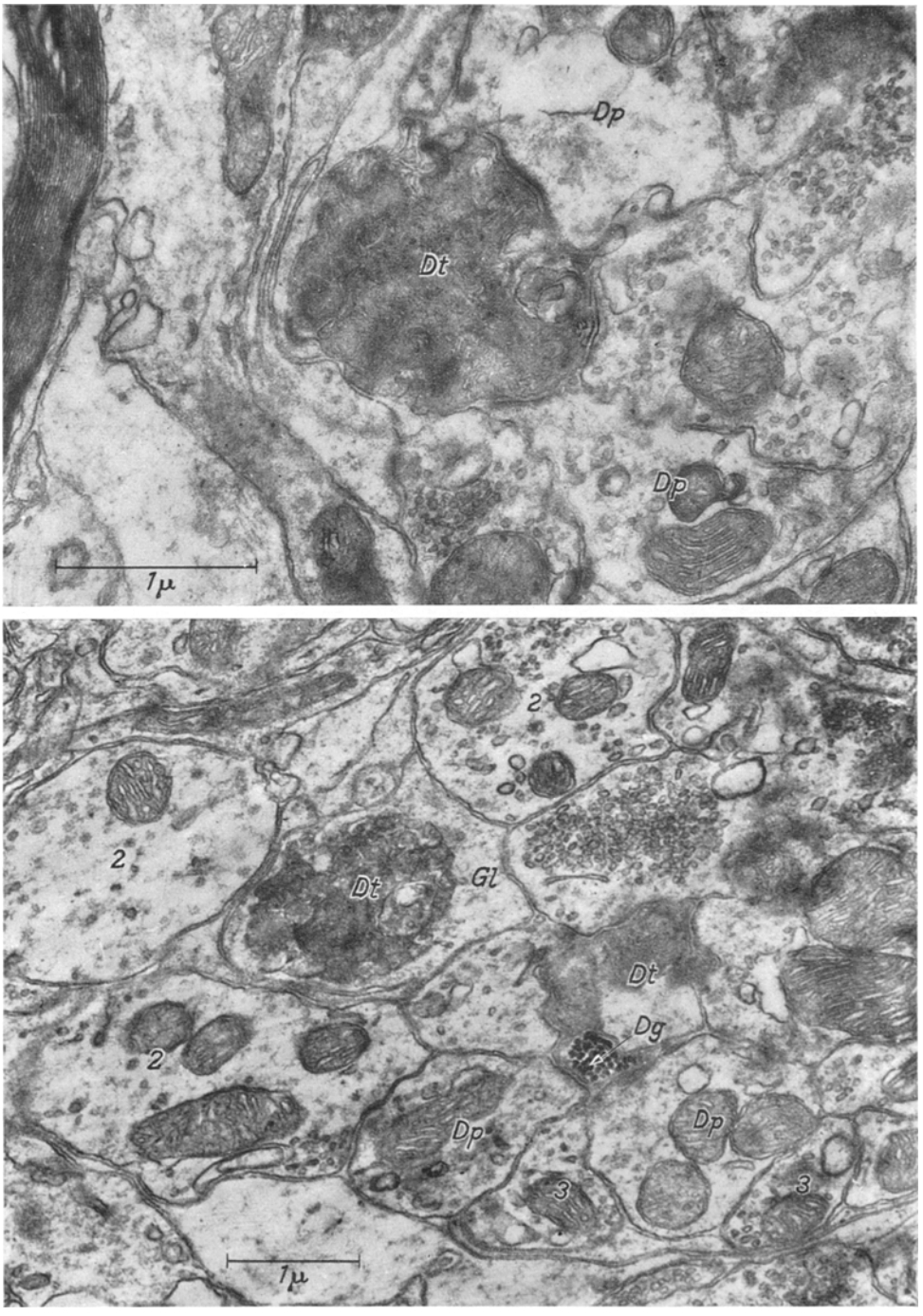


Fig. 12

Figs. 11, 12. *Dense fragment* (WALBERG) *stage (5th day) of optic terminal degeneration.* The degeneration remnants of optic terminals (Dt) are irregular dense bodies in which the original mitochondria and synaptic vesicles can hardly be recognized, occasionally there occur clusters of dense granula (Dg). The degenerated fragments are often surrounded by glial profiles (Gl). From the persisting elements of the glomeruli dendritic protrusion profiles (Dp) and axon endings can be seen, some of which can be recognized as type (2) others as type (3) terminals, most of them are ambiguous, but certainly not type (1)

Fig. 13

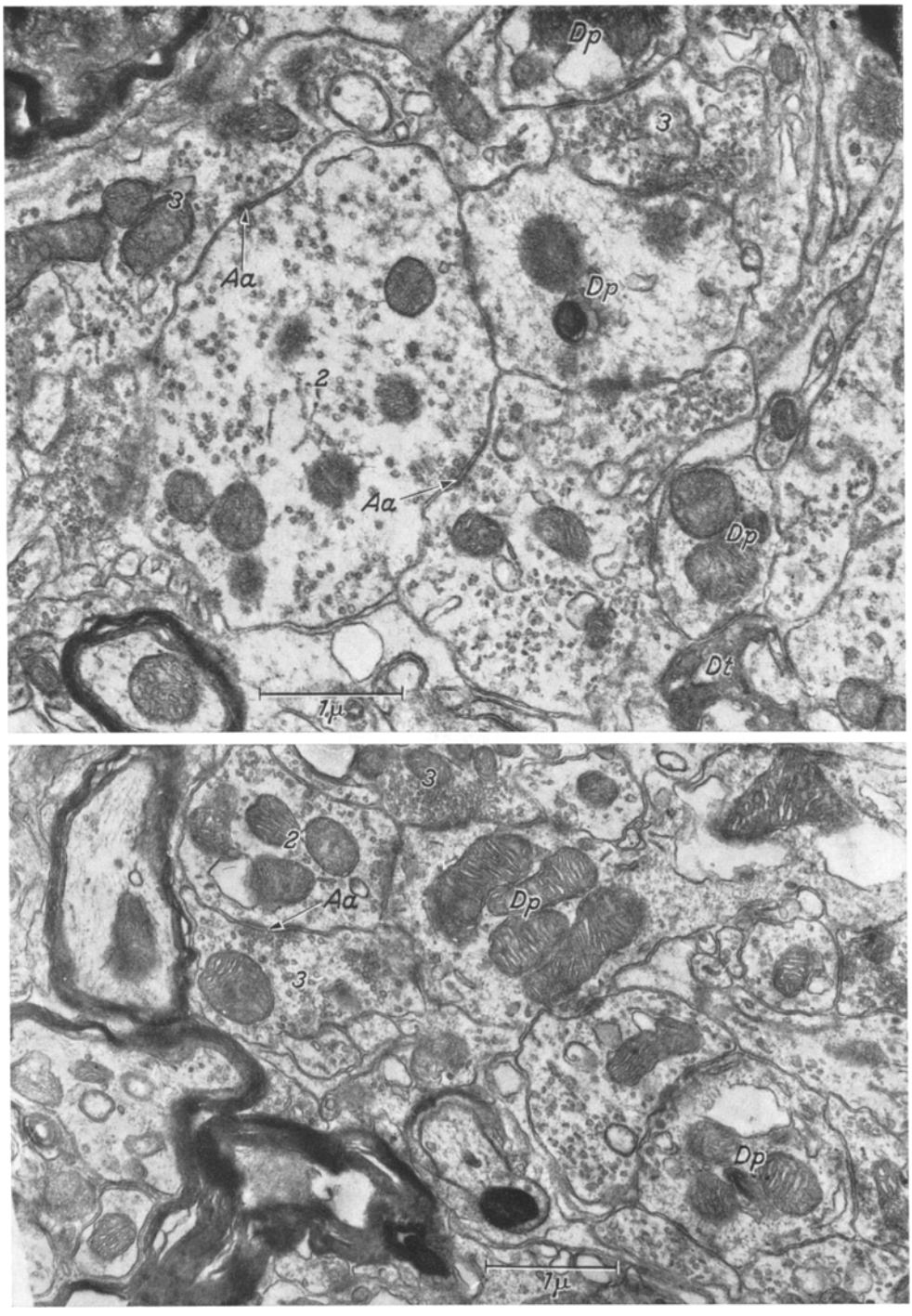


Fig. 14

Figs. 13, 14. *Deafferented glomeruli (5th and following days) reconstituted after removal of optic terminal fragments.* In Fig. 13 degenerated fragment (Dt), in advanced stage of decomposition, can still be seen at lower right. Degenerated optic fibers can be seen at upper left of Fig. 13 and lower left of Fig. 14. The glomeruli contain only dendritic protrusions (Dp) and type (2) and (3) axon terminals that often seem to be slightly hypertrophic. Axo-axonic contacts (Aa) between (2) and (3) type endings persist, but often the distinction between the two endings is ambiguous

Up: Fig. 15

Middle: Fig. 16

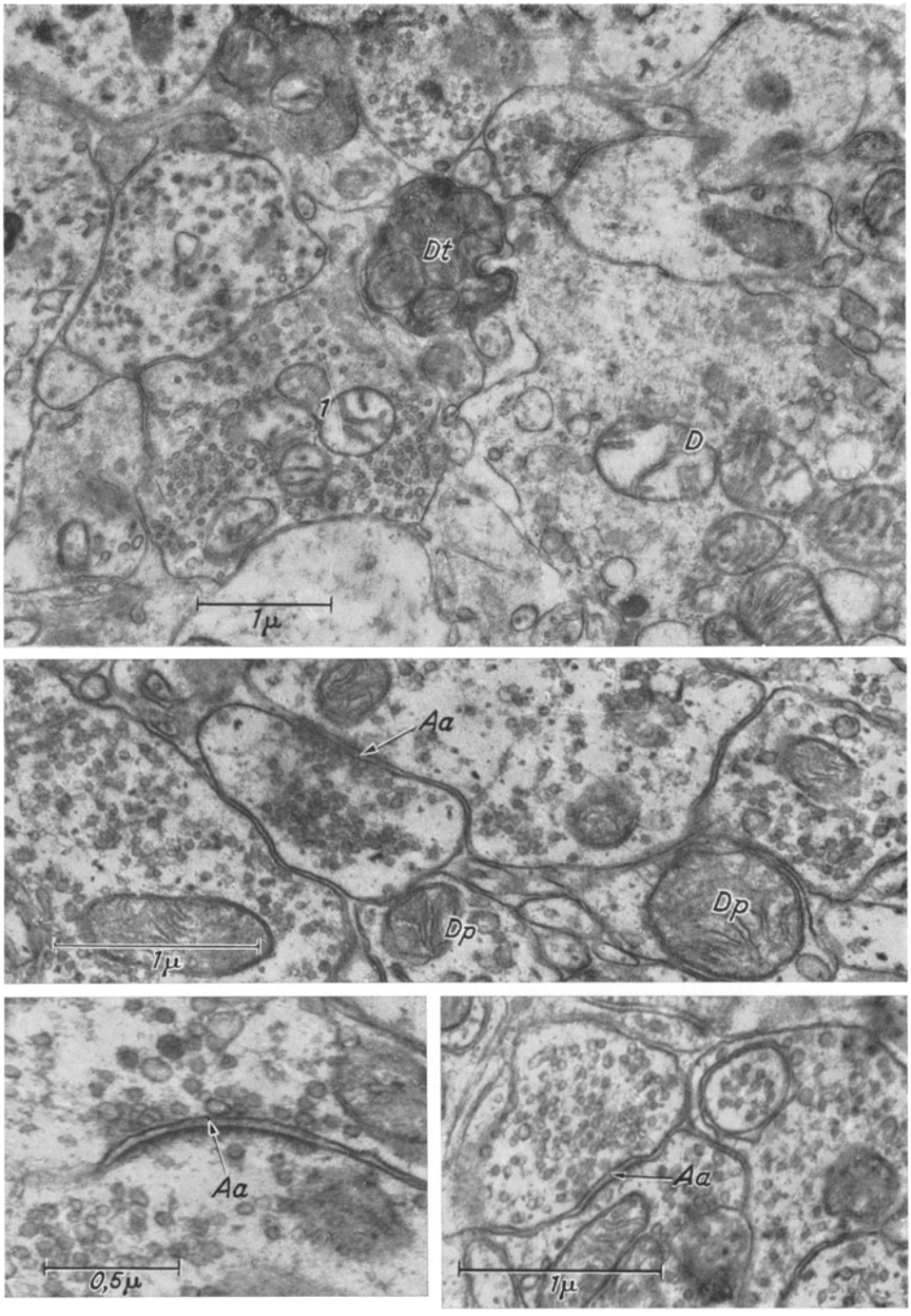


Fig. 17

Fig. 18

Fig. 15. *Dense degenerated terminal (Dt) 5 days after cortical ablation in the occipital lobe (area surrounding striate region).* Although attached to a tangentially cut glomerulus this terminal primarily contacts larger dendrite (D). Intact type (1) axon terminal can be recognized

Figs. 16, 17, 18. *Chronically deafferented LGB (optic nerves cut and parastriate cortex removed ipsilaterally) with persisting axo-axonic contacts (Aa).* Due to complete atrophy of many — particularly the GCR — neurons dendritic profiles (Dp) are rare, but glomeruli consisting mainly of Golgi 2nd type axon terminals and probably of afferents from other cortical regions and of subcortical afferents from unknown source persist indefinitely

uniform in size, they tend to accumulate at the periphery of the terminal. In the center the first signs of hypertrophy of neurofilaments can be observed. The most apparent early sign of degeneration is the appearance of granula of high density resembling glycogen bodies. Such granula can be observed very occasionally in normal synaptic terminals of the spinal cord as well as in mossy fiber rosettes of the developing cerebellum, so that they cannot be considered as general signs of degeneration. In the LGB, however, no granula have been ever observed in normal axon terminals. — Essentially the same changes can be observed in some myelinated axons (Fig. 8), obviously preterminal parts of optic fibers.

On the 4th day the changes are even more pronounced (Figs. 9, 10). The central parts of the terminals show the strong hypertrophy of neurofilaments, as described by GRAY (1964) and COLONNIER and GUILLERY (1964). The synaptic vesicles and degenerating mitochondria as well as the dark granula are accumulated at the periphery forming irregular protrusions of the ending (Figs. 9 and 10). Glial processes with glycogen bodies can be seen to enter the glomeruli and to surround mainly the dense protrusions of the degenerating terminals (Fig. 9).

By the end of the 5th postoperative day the picture has again changed considerably. The degenerated optic terminals are transformed into dense irregular profiles, completely surrounded by glia, in which remnants of mitochondria, of synaptic vesicles and of granula are lumped so completely together that they can hardly be recognized (Figs. 11, 12). This characteristic picture of degenerated synaptic terminals was described first by WALBERG (1964, 1965) in various regions of the CNS. — Beginning with the 5th postoperative day and after an increasing number of glomeruli is found either devoid of degenerated fragments, or with their last remnants seen to be removed to their periphery (Figs. 13, 14). — After bilateral transection of both optic nerves, from the 6th day not a single type (1) terminal is found in the geniculate glomeruli. The (2) and (3) type axon terminals do not show degenerative changes after optic nerve transection and persist indefinitely. If the optic (1) terminals were not recognized on the basis of their characteristic mitochondria, and the unexpectedly quick removal from the glomeruli of their degeneration fragments were not known, one might be easily misled — by the many seemingly intact glomeruli encountered from the 6th day — into believing that no change happened at all. An enlargement of the persisting axon terminals, taking place after removal of the optic terminal fragments, may be particularly deceptive. In spite of that the all-over size of the glomeruli appears to decrease. — Degenerated dense fragments can be observed, of course, much later than the 6th postoperative day, but these are the remnants of the preterminal fibers — mostly myelinated — corresponding to the many fragments found in the Nauta picture even several weeks after optic nerve transection.

Axo-axonic contacts persist in large number even two months after bilateral optic nerve transection (Figs. 16, 17 and 18). Both, type (2) terminals may have accumulations of synaptic vesicles opposite a membrane thickening of a type (3) ending and the reverse. In chronic cases distinction of the two types, in consequence of their enlargement, becomes insecure.

After cortical ablations in the occipital lobe — but outside the striate region proper — signs of degeneration are similar, but they occur mainly on small darker axon terminals. The majority of degenerated boutons can be seen outside the

glomeruli in contact with the dendrites. Or even, if they are incorporated into the periphery of a glomerulus (Fig. 15) they had contacts with a larger dendrite. — Persistence after bilateral optic nerve transection of the small boutons lined up along the dendrites and termed cuff synapses have been reported already earlier (SZENTÁGOTHAI 1965a).

Discussion

These experiments essentially confirm the earlier description of geniculate glomeruli (SZENTÁGOTHAI 1962, 1963, 1965a, b; PETERS and PALAY 1965) of the cat, and with some minor differences — obviously caused by species differences — that given by COLONNIER and GUILLERY (1964) of the monkey and by KARLSSON (1966) of the rat LGB. — The principal synaptic sites — i. e. those containing optic terminals — are densely packed glomerular complexes of various types of axon terminals and mostly not the dendrites themselves, but either spheroid bulges of dendrites or the "drumstick-heads" of the short "spine-like" side branches of dendrites. They are, however, not true spines as they always contain one or several large mitochondria. For distinction from spines these processes have been called dendritic protrusions (SZENTÁGOTHAI 1962, 1963, 1965b). — From the three kinds of axon terminals that can now be distinguished in the cat glomeruli, type (1) can be recognized with certainty as of retinal origin. It is characteristically localized in the center of the glomerulus, however, it is most easy to recognize it on the basis of its characteristic irregular mitochondria having unusually sparse cristae. This agrees with the original identification by SZENTÁGOTHAI (1962, 1963) and PETERS and PALAY (1965). There is a slight discrepancy with the description by COLONNIER and GUILLERY (1964) in the monkey, where the optic terminal was found by degeneration to be a "large pale" terminal. Comparing the figures of COLONNIER and GUILLERY (particularly Fig. 4) with those of this study, it can be recognized — mainly from the mitochondria — that the terminals found to be the optic ones are essentially identic. It may be a species difference that in the cat the optic terminals are darker. Axonal components "A" of the rat LGB (KARLSSON 1966) can be identified very probably with the optic terminals. Lacking the crucial light microscopy information on Golgi 2nd type neurons the significance of the sometimes large or more often medium sized, very light boutons of the glomeruli was not understood. The numerous grape-like boutons shown in Fig. 1 are obviously localized mainly in glomeruli. Thus according to light microscopic evidence a very considerable fraction of the axon terminals in the glomeruli ought to belong to Golgi 2nd type neurons. This is in fair agreement with the fact that the very light axon terminals do not degenerate after optic nerve transection or/and cortical ablations in the occipital lobe. It is therefore with considerable confidence that we can identify the type (2) endings as Golgi axon terminals.

The small dark axonal profiles (type 3), first described by COLONNIER and GUILLERY (1964), can be observed to degenerate after cortical ablations in the occipital lobe. The fact that even in extended ablations only few degenerated profiles are met with in glomeruli, could be explained either by assuming that recurrent fibers from other cortical regions also contribute terminals to the geniculate glomeruli, or by subcortical non optic afferents of the LGB, which have been postulated by many authors. Both assumptions could be tested only by extensive

Nauta degeneration studies on systems afferent to the LGB, both from non occipital cortical and subcortical regions. So far our material of lesions in various parts of the brain stem reticular formation has brought forward little positive evidence of such systems. However, any final conclusion could be drawn only on the basis of systematic investigations. — The majority of cortical recurrent pathways appear to terminate not in the glomeruli, but rather directly on the dendrites. The "cuff-synapses" of the dendrites remain intact after optic deafferention. As described earlier (SZENTÁGOTHAI 1963, 1965a) they are small boutons: and it can be said now in addition that they are considerably darker than the Golgi neuron terminals in the glomeruli. — It remains a question, whether the small boutons of the "cuff-synapses" are of the same origin as the small dark boutons in the glomeruli, i. e. of the same function, or specifically different. There would be nothing against the assumption that there are two specifically different cortico-geniculate systems from which one would terminate in the glomeruli and the other in the dendritic cuffs. As to be mentioned in one of the following paragraphs, there is indeed a further kind of specific synapse of local origin that has to be considered: the synapse of the recurrent initial collaterals of GCR axons.

The LGB is a favourable material for the study of the elementary signs of secondary degeneration of synapses. This is due to the fact that the optic terminals can be recognized already on the basis of the structure and that they can be brought to degeneration quantitatively. According to this study the discrepancies between the description of secondary degeneration of synaptic terminals as given by GRAY (1964) on one side and by WALBERG (1964, 1965) on the other, are due to two different stages of degeneration, which may occur side by side in consequence of different speed of the degeneration process in various fibers. — Undoubtedly the most reliable signs of secondary degeneration of axon terminals are those descripbed by WALBERG. The high density and irregular outlines of the terminals become apparent in the LGB on the fifth postoperative day (Figs. 11, 12). As the optic terminals can be recognized — in retrospect — already in the normal material on the basis of their mitochondria, earlier stages of degeneration can be identified here with fair security. Already on the second day the synaptic vesicles become more irregular in size and begin to accumulate on the periphery of the terminal. The appearance of dense granula — probably glycogen — cannot

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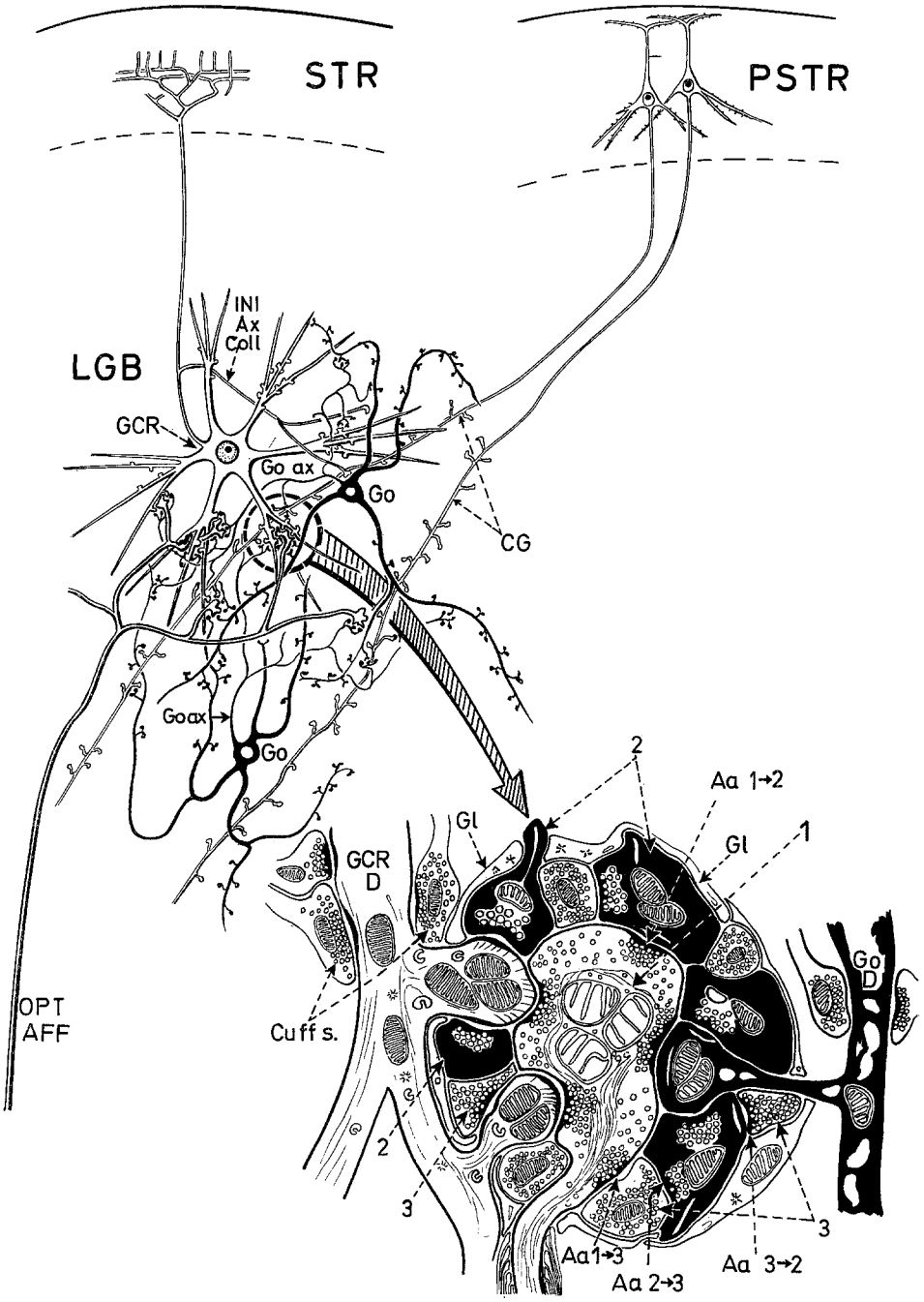
Fig. 19. *Elementary neuronal circuit diagram of the LGB (upper left) and structure diagram (lower right) of the LGB glomerulus.* Only one geniculo-cortical relay (GCR) neuron is indicated projecting to the striate (STR) region. Optic afferents terminate at the GCR neurons with endings resembling the mossy fiber rosettes of the cerebellum. Synaptic sites are the so called geniculate glomeruli. — Two Golgi 2nd type neurons (Go) are incorporated in the diagram with their axon (Go ax) ramifications. As they can be assumed to be inhibitory, they are drawn in full black. The Golgi axons participate mainly in the glomeruli. — An initial axon collateral (INI. Ax. Coll.) of the GCR neuron is assumed to terminate on Golgi 2nd type neuron, but the site of this synapse has not been identified as yet. — Cortico-geniculate descending (CG) fibers originating in abundance from parastriate regions (PSTR) are giving rise to straight preterminal fibers and are contacting various neurons at probably various sites rather indiscriminatively. — In the glomerulus (lower right), surrounded by a thin glial capsule (Gl), three different types of axon endings can be distinguished: type (1) large and dense with sparsely cristated mitochondria can be identified by degeneration as the optic afferent; type (2) is very pale, — here drawn in black as they are assumed to belong to inhibitory Golgi 2nd type neurons — having very few vesicles; type (3) terminals are small, dense with densely cristated mitochondria. — Protrusions of both GCR dendrites (GCR D) and of Golgi 2nd type neuron dendrites (Go D) participate in the glomeruli; the latter are drawn in black as they are supposed to belong to inhibitory neurons. Synaptic contacts resembling type (3) are found on larger dendrites outside the glomeruli, which have been termed earlier "cuff synapses" (Cuff s.). — Axo-axonic contacts (Aa) are very frequent in the glomeruli: they are either 1→2 and 1→3 or 2→3 and 3→2, but never 2 or 3→1

be considered as a reliable sign of degeneration as they may occur in synaptic terminals of normal animals, although not in the LGB of the cat. On the fourth day most optic terminals are in the filament hypertrophy stage (Figs. 9, 10) with dense accumulations of vesicles and mitochondria in the rugged periphery. It is



conceivable that this stage of filament hypertrophy occurs only in "filamentous" types of endings, to which the optic terminals certainly belong (COLONNIER and GUILLERY 1964). — What was unexpected is the extremely quick removal of the degeneration fragments on the 5th and 6th postoperative day. The glia becomes active already on the 2nd and 3rd day and most degenerating terminals are surrounded by glial processes intruding into the glomeruli by the 4th day. Large fragments mainly of preterminal fibers are seen much later too, however, then the site of any fragment does not indicate any more the original site of the synapse. It is, therefore, advisable to investigate the earliest reliable signs of bouton degeneration in order to be sure that they are still at their original sites. — The numerous glomeruli devoid of optic terminals, but otherwise fairly well reconstituted — seen from the 5th day onwards — show that it is easy to miss the crucial period of degeneration. The slight hypertrophy of the non optic axon terminals occurring after degeneration of the optic endings matches the similar observation on cerebellar glomeruli deprived of their mossy fibers, where the persisting Golgi axon terminals (HÁMORI 1964; FOX *et al.* 1966; HÁMORI and SZENTÁGOTHAI 1966) are also enlarged.

It remains, finally, to consider the significance of these observations for neuron circuitry of the LGB. Topographic and other aspects of neuron connectivity — for example: divergence of optic and other afferents and convergence of optic afferents on various types of cells, as well as overlaps between different arborizations — are largely beyond the scope of this report. They will be treated in more detail in separate papers presently under preparation. Only a few general questions of neuron coupling will be, therefore, discussed here with the aid of a diagram (Fig. 19). This diagram is an improved version of SZENTÁGOTHAI's (1962) first approach (see l. c. Fig. 3), that needs now correction in two major respects: [1st] that the Golgi 2nd type neurons and particularly their dendritic and axonal arborization patterns were ill known at that time and [2nd] that erroneously all large endings of the glomeruli were considered as optic.

The left center of Fig. 19 shows the two main cell types of the LGB as seen in the adult cat with the perfusion Kopsch technique. Only one geniculo-cortical relay (GCR) cell is shown with its characteristic radiate dendrites and their arborization tufts. Small bulges or spheroid protrusions of the dendrites are most abundant immediately around and distally from the dendritic tufts. The axon of these cells runs to the striate cortex, but quite often an initial collateral is seen to branch off. This collateral most probably terminates within the LGB, although its further course has not been observed clearly. It can be assumed, however, that they terminate on the Golgi 2nd type cells, which could be the anatomical basis of the recurrent inhibition described first in the ventrobasal nucleus by ANDERSEN *et al.* (1962) and found more recently by SEFTON and BURKE (1965) to occur in the LGB of the rat. — Two representative Golgi 2nd type cells are shown to indicate the fact that besides synaptic contacts established on GCR neurons, Go cell axons have also synaptic contacts on the other Go cells. This is obvious also from Fig. 1. — The relatively coarse optic afferents have synaptic sites that resemble the mossy fiber rosettes in the cerebellar cortex. They establish synapses exclusively with dendrit protrusions in the so called glomeruli. As far as it is possible to judge from the light microscopical pictures, optic afferent establish direct synapses with both

GCR and with Go neurons. But this cannot be proved beyond doubt on the EM level. Thus the diagram (Fig. 19) is hypothetical in the one respect that it assumes dendrites of both types of cells being engaged in the same glomerulus. An alternative hypothesis could be based on the assumption that there are tow kinds of glomeruli, one having optic afferents and established with GCR cells and others having only Go axon and recurrent cortical axon terminals and may be other non optic afferents and being engaged exclusively with Golgi cell dendrites. That Golgi dendrites are angaged in glomeruli is obvious from their light microscopy (Fig. 1). So far there is no clear EM evidence of glomeruli having no optic afferents, although this is difficult to decide from simply looking at ultrathin sections.

The structure of the synaptic glomerulus is shown in the lower right part of Fig. 19. The optic terminal (1) generally of central position, is characterised by its large poorly cristated mitochondria and its filamentous character (COLONNIER and GUILLERY 1964). — As discussed in the previous paragraph the most probable — although not exclusively possible — interpretation of the relatively small dendritic profiles within the glomeruli is that they are spheroid bulges or the heads of the "drum-stick" side branches of dendrits of both main cell types. The bulges would belong mainly to GCR neurons, they are strongly filamentous and have in general very short stout connexions to the main dendrites. The Golgi 2nd type neurons have numerous "spine-like" side branches, however, as there are no true spines visible in the EM picture*, and the drum-sticks can be clearly seen in Golgi pictures to enter glomeruli (Fig. 1) they are probably not spines, but dendrite protrusions containing at least one mitochondrium in their ends. In order to conform with the recent usage to indicate inhibitory elements in full black and excitatory ones in outlines, the drum-stick side branch of the Golgi cell dendrite is shown here in black. For the same reason the terminals type (2) of Golgi axons, which are of very light structure in reality, are shown in black too. They are of medium size generally, but can be quite large so that the large size is no reliable criterion of the optic nature of any terminal. (This has not been understood earlier by the authors studying the glomeruli, including ourselves). — The third type (3) of axon terminals is small and of dense structure, with densely cristated mitochondria. This type of axon-terminal does not differ appreciably from other non glomerular synapses seen in the neuropil, particularly those lined up along the dendrites "downstream" from their contacts in the glomeruli. These contacts have been called "cuff synapses" referring to the fact that they are arranged as "cuffs" around the dendrites approaching the glomeruli. They are certainly non optic, as they do not degenerate after optic nerve transection. Some of them, both in the glomeruli and in the "cuffs" show clear degenerative changes after occipital cortical ablations.

Very little can be said of non optic (distant) afferents of the LGB, with the exception that they originate in considerable abundance from cortical areas surrounding the striate region (WIDÉN-MARSAN 1961; SZENTÁGOTHAI 1963). All over the thalamus and the geniculate bodies the preterminal branches of cortical descending axons have a straight course with short terminal side branches that end up in one or two terminal knobs (TÖMBÖL 1967). As the preterminal axon branches are giving off their terminal rami along stretches of considerable lenght, but without concentration of boutons at any specific site, these connexions ought to be rather non selective, giving "indiscriminatively" one synaptic contact to a neuron here, another to another neuron there etc. These characteristic features of the descending cortical afferents of the LGB, have been indicated in the diagram (Fig. 19). There is good evidence from degeneration studies that a considerable fraction of the non glomerular — particularly "cuff" — synapses are of cortical

* Dendritic micro-spines protruding into invaginations of axonal endings have been shown to occur in the rat LGB (KARLSSON 1966); they are very rare in the cat.

origin, but some of the type (3) endings in the glomeruli are too. It is uncertain, whether (i) there exist other non optic distant afferents and unknown how they might terminate; (ii) it is also unknown, whether the dendritic "cuff synapses" and the type (3) glomerular synapses of cortical origin are similar functionally or conversely specifically different.

Finally the existence in the LGB glomeruli of axo-axonic (presynaptic-to-presynaptic) contacts has to be discussed with consideration of recent reports on presynaptic inhibition of optic terminals (ANGEL *et al.* 1965; IWAMA *et al.* 1965; SUZUKI and KATO 1965). The observation of such contacts has given rise to speculations about the possibility of a presynaptic cortical inhibition of optic terminals (SZENTÁGOTHAI 1962). On more careful observation it appeared, however, that the accumulation in such contacts of synaptic vesicles was generally found in the large central axon terminal. Thus it was assumed that — at least by structural standards — the optic terminal ought to be "presynaptic" to non optic ones (COLONNIER and GUILLERY 1964; PETERS and PALAY 1965). This assumption is verified by the present observations. It has to be added, however, that many axo-axonic contacts persist after optic nerve transection; in chronic cases with additional cortical ablations their number even appears to be increased. Thus many of the axo-axonic contacts are obviously established between (2) and (3) type endings in which either type can be "presynaptic". The anatomical explanation of the depolarization of optic terminals (ANGEL *et al.* 1965) after stimulation of the reticular formation meets several difficulties. First there is no direct connexion of any significance known from the reticular formation to the LGB. The effect could be conveyed — of course — indirectly over the occipital cortex or some unknown subcortical relay. Secondly the structural polarity of axo-axonic contacts of optic terminals is the reverse of what would be required by the physiological observations. — One wonders, whether the ultrastructural polarity, that seems to fit so nicely with present notions of transmission functions in the ordinary synapses, would necessarily have the same significance in axo-axonic involvements. Several kinds of axons are very closely packed together in the glomeruli and the whole complex is very neatly surrounded sometimes by several folds of glial processes. It is difficult to understand on the basis of information presently available how the excitation of some of the axon terminals might influence the functional state of others. It is not inconcievable even that the optic endings are depolarized without specific ultrastructural devices just by being immediately surrounded at by far the larger part of their surface by other axon endings. There are also some other lines along which one might start speculating, but it is probably more advisable to wait until more physiological information will be available.

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