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Identification under the Electron Microscope of Climbing Fibers and their Synaptic Contacts

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Summary. An attempt is made to identify, under the electron microscope, the climbing fibers of the cerebellum (in the cat) and their synaptic contacts with Purkinje cells and other cortical neurons. — Two kinds of axonal profiles, having synaptic contacts with primary and secondary dendrites of Purkinje neurons, can be recognized: One being terminal fibers densely packed with neurofilaments, having mainly contacts "de passage" with the dendrite surface, with small accumulations of synaptic vesicles at the presynaptic side of the contact. The others are rather knob-shaped contacts filled with synaptic vesicles and poor in neurofilaments. In chronically isolated folia, in which only local neurons and their processes have survived, all filamentous profiles have disappeared while vesicular ones are not appreciably reduced in number. It is inferred from this, that the neurofilamentous profiles correspond to climbing fibers, whereas the vesicular ones could be the endings of outer stellate axons, recurrent Purkinje axon collaterals, or ascending basket axon collaterals. — Similar two kinds of axon-terminal profiles are found in synaptic contact with Golgi and basket cell bodies. As in chronically isolated folia only the vesicular profiles survive, it is inferred that the climbing fiber has axo-somatic terminals on Golgi cells and basket cells as well. Previous information of this kind, gained with the light microscope and with degeneration studies, is thus substantiated with the aid of the electron microscope. The vesicular presynaptic profiles on Golgi and basket neurons are in the first case certainly and in the second with high probability endings of recurrent Purkinje axon collaterals. — The few axosomatic synapses found on outer stellate neurons may also be terminals of climbing fibers, but degeneration evidence for this is not conclusive. — The observations are summarized and evaluated from the functional point of view in a diagram, with consideration to recent physiological information on the function of climbing fibers.

Key Words: Cerebellum — Cerebellar synaptology — Climbing fibers — synapses.

Introduction

Since their discovery by RAMÓN Y CAJAL in 1888 the climbing fibers of the cerebellar cortex have remained in the focus of interest of neurohistologists, due to their peculiar synaptic relation to the Purkinje cells. The synapse is remarkable on one hand by the clear one-to-one relation between the articulating elements,

while the long and intimate contact between the climbing fiber terminal ramification and the dendritic tree of a Purkinje cell, on the other, has served as a characteristic example for one of the main types of synaptic relations: the parallel contact. — Little if anything has been added since Cajals classical description to our knowledge of the climbing fiber until the recent Golgi study of SCHEIBEL and SCHEIBEL (1954) indicating that their synaptic relations might be more extensive than originally supposed. — In spite of repeated attempts to clarify their origin using degeneration techniques (MISKOLCZY, 1931; SNIDER, 1936; ROSIELLO, 1937; SCHIMERT, 1939, CARREA, REISSIG and METTLER, 1947) it has not been until 1959 (SZENTÁGOTHAI-RAJKOVITS) that their origin from the inferior olive was established. This was mainly due to the difficulties in the staining of axonal degeneration fragments in the molecular layer of the cerebellar cortex. Although one of the present authors (J. Sz) has seen already in 1938 that after olivary lesions degeneration fragments of axons could be traced to the primary dendrites of Purkinje cells he became confused by degenerated collaterals seen branching off from the main fibers in or somewhat below the level of Purkinje cell bodies. As this did not fit the classical description of an undivided course — in the submolecular layers — the otherwise logical assumption of the climbing fibers being of olivary origin was abandoned. Only after the description by SCHEIBEL and SCHEIBEL (1954) of such collaterals given by the climbing fibers could the signs of degeneration be interpreted correctly.

Although electron microscopy has substantiated, on the whole, the concepts of light microscopy on structure and localization of synaptic contacts, the light microscopic criteria of synaptic contacts are often equivocal and easily subject to erroneous interpretation. It is therefore advisable to reinvestigate all important synaptic systems with electron microscope techniques. This paper presents an account of our attempt to identify under the electron microscope the synaptic contacts established by the climbing fibers.

Materials and Methods

These investigations were carried out on adult cats, whose upper vermis was exposed under ether anesthesia. Cold buffered glutaraldehyde solution was dropped for some minutes on the surface of the cerebellum. Small tissue blocks were cut out from the cortex during continuous dropping of glutaraldehyde solution and immediately immersed in the same fluid. After 60 min. the blocks were transferred into buffered 1% osmic acid solution. The blocks were then embedded into epon resin and sectioned with an LKB Ultrotome. Ultrathin sections were stained with lead citrate after REYNOLDS (1963) and investigated with the "Tesla" (BRNO) BS242B table electron microscope.

Taking into consideration the difficulties met with in attempts to exploit fresh secondary degeneration on the EM level as a means to identify synaptic contacts of a certain kind, our technique of persisting elements (SZENTÁGOTHAI, 1958) in chronically isolated nervous tissue slabs has been used. Small parts of single folia — 2—4 mm in diameter — have been isolated by complete undercutting. (The technique for isolation of cortical slabs has been described in detail previously (SZENTÁGOTHAI, 1962). The animals with isolated folium islands were left to survive for two months and the isolated slabs fixed as normal material. Only slabs have been used, in which complete isolation from the remaining part of the cortex was indicated by complete loss of mossy terminals. Climbing fibers being the only extraneous elements of the molecular layer, it was assumed that they have to disappear in the chronically isolated folium, whereas all other elements ought to persist. Survival of all kinds of cortical neurons in isolated cerebellar folia has been shown in earlier light microscope observations (SZENTÁGOTHAI, 1965).

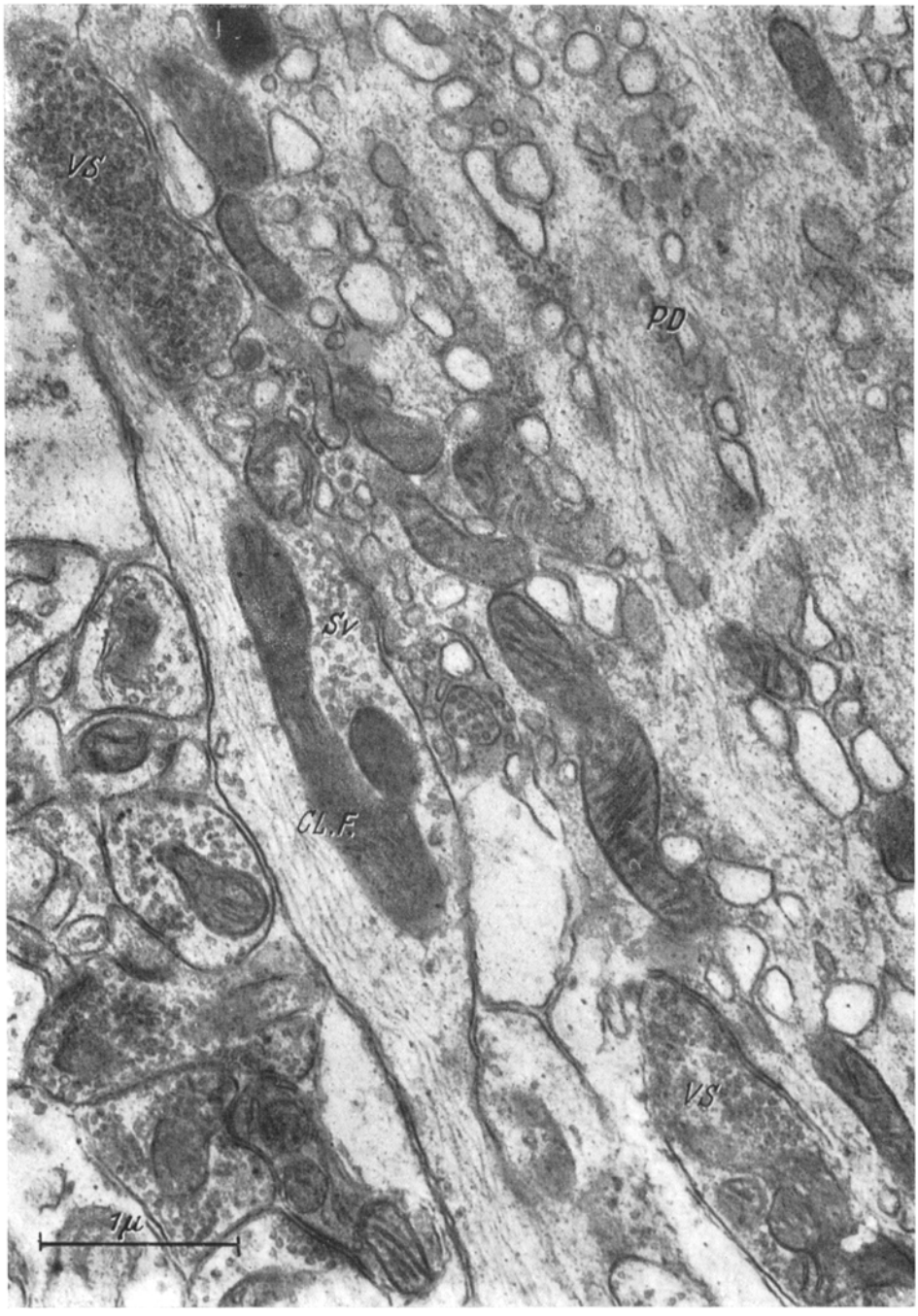

Fig. 1. *Large primary dendrite of Purkinje cell* (PD) having synaptic contact "de passage" with large terminal axon, containing coarse neurofilaments and identified as climbing fiber (Cl. F). Only few synaptic vesicles (Sv) accumulate at the sites of contact. Other vesicular synaptic profiles (VS) have more the character of terminal synaptic endings

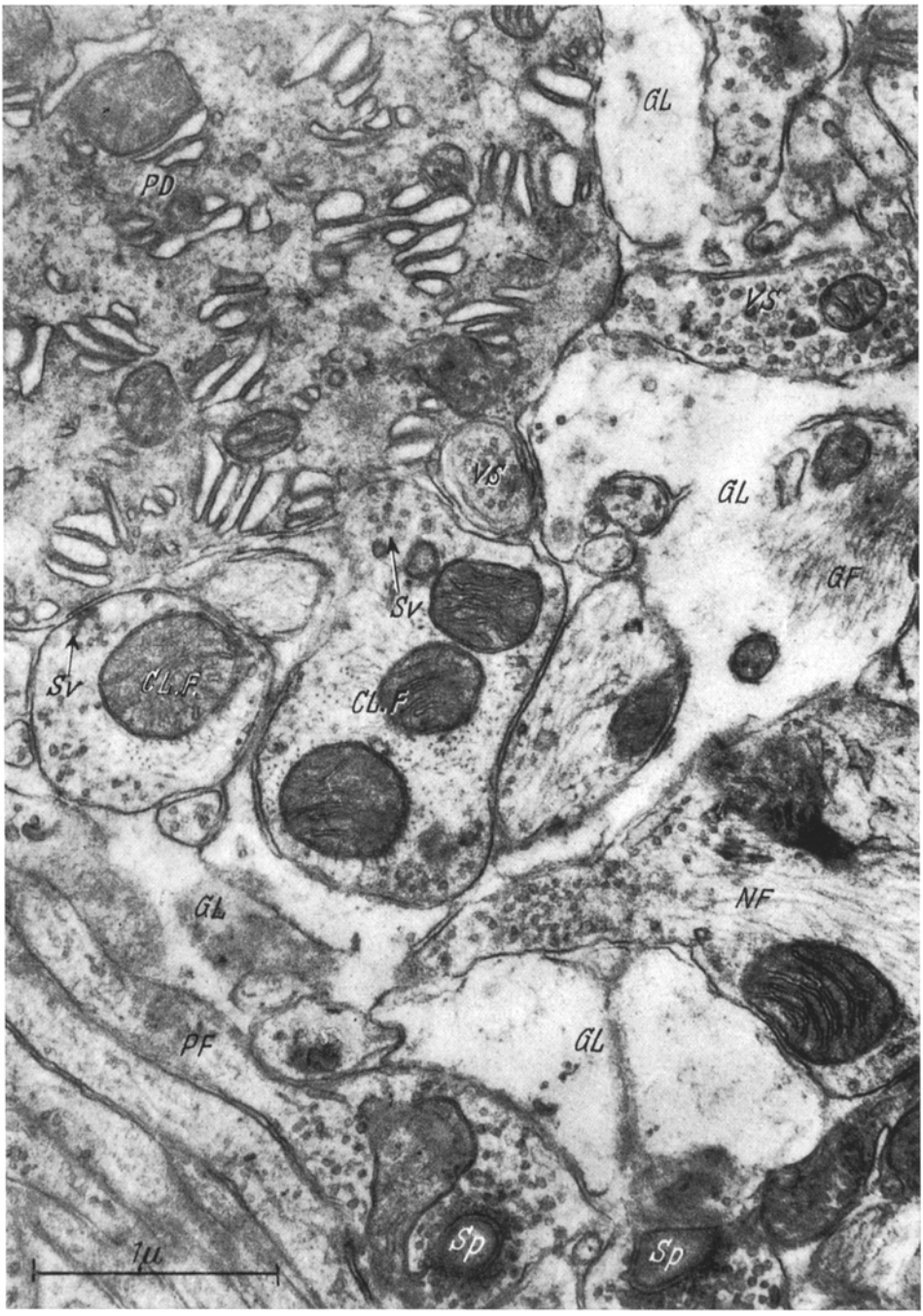

Fig. 2. *Secondary Purkinje cell dendrite* (PD) with several axonal and terminal profiles attached. Profiles packed with neurofilaments can be identified as climbing fibers (ClF), having small accumulations of synaptic vesicles (SV) mainly at sites of close attachment to the dendrite surface. Vesicular synaptic profiles (VS) are poor in neurofilaments. Most part of the dendritic surface is covered by glial processes (GL), some of them containing bundles of glial filaments (Gf). Irregular axonal profile at left containing coarse neurofilaments (Nf) is ambiguous: it could be either a climbing fiber ot a basket axon, which is also rich in neurofilaments. At bottom two dendritic spines (Sp) can be seen embedded and synapsing with thickened parts of parallel fibers (Pf)

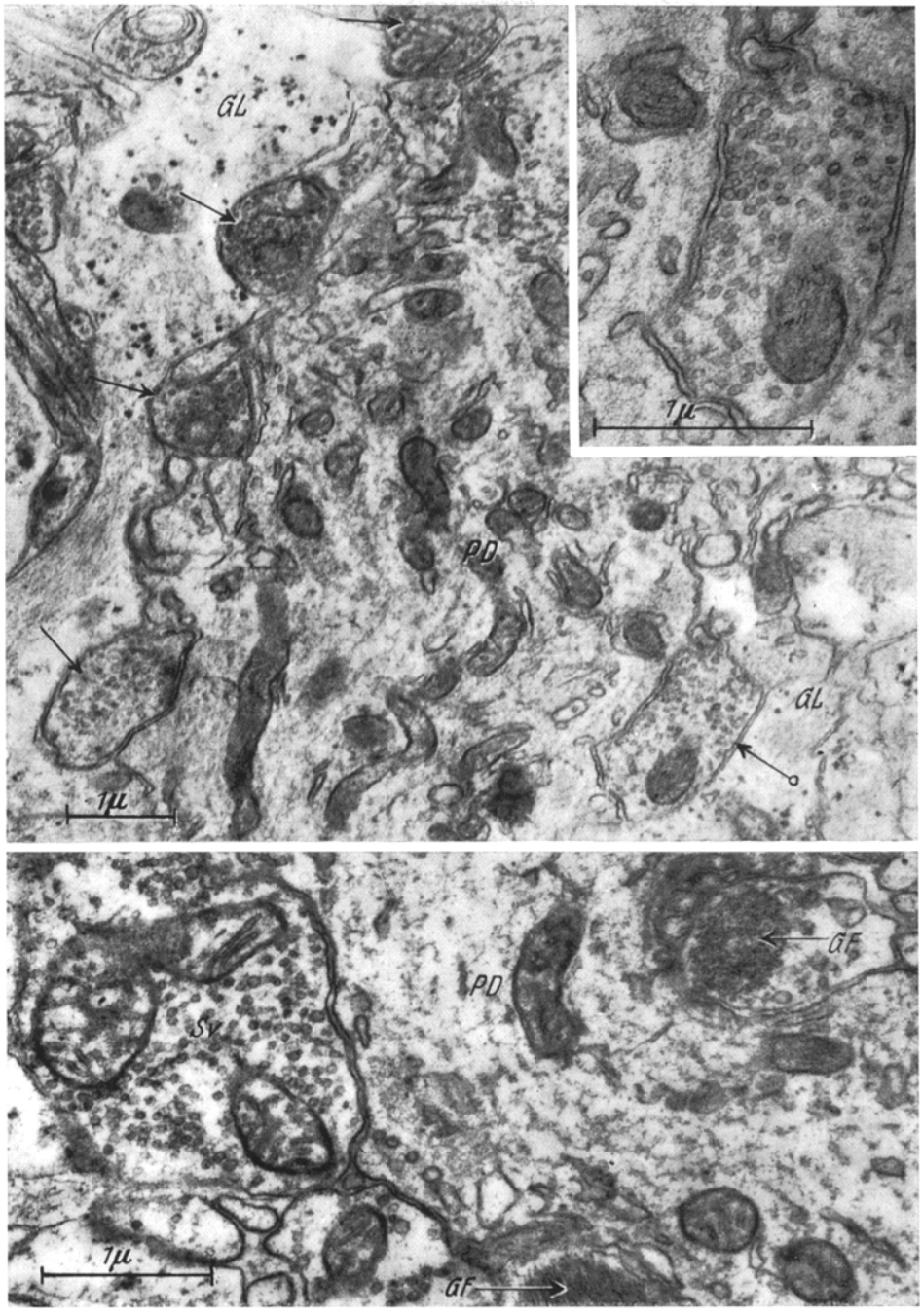

Fig. 3. *Secondary Purkinje dendrite* (PD) from chronically isolated folium with five intact vesicular synaptic terminals (indicated by arrow) but no axonal profiles containing neurofilaments. Glial profiles (Gl) somewhat hypertrophic and containing glycogen bodies. Vesicular profile indicated at left by ringed arrow is shown in upper inset at larger magnification. — Lower inset: secondary Purkinje dendrite in isolated folium with one synaptic terminal intact filled with synaptic vesicles (Sv). Hypertrophic glial profiles containing bundles of glial filaments (Gf) have presumably replaced degenerated climbing fibers

Observations

1. Climbing fiber — Purkinje dendrit contacts

The primary and secondary dendrites of Purkinje neurons can easily be identified under the electron microscope by tracing them upwards from the cell bodies in sections perpendicular to the surface and to the axis of the folium (HÁMORI-SZENTÁGOTHAI, 1964). Most part of their surface is surrounded by a glial envelope (HÁMORI-SZENTÁGOTHAI, 1965) mainly from Bergmann glia, which apparently has been mistaken by HERNDON ([1963 Fig. 5]) as climbing fibers. There are, however, characteristically one or two profiles of nerve fibers attached to each of these primary and secondary Purkinje dendrite profiles (Figs. 1 and 2). From longitudinal sections it appears (Fig. 1) that some of the preterminal nerve fibers have a predominantly longitudinal course running parallel with that of the Purkinje dendrite. The profiles are densely packed with coarse neurofilaments and have rather few synaptic vesicles at regions of immediate attachment to the Purkinje dendrites exclusively. The zones of synaptic attachment correspond in structure to the 2nd type of GRAY (1959). The relation between the Purkinje dendrites and the strangely neurofilamentous preterminal axons becomes more clear in transverse sections (Fig. 2). — Beside the neurofilamentous profiles another kind of profiles of synaptic terminals of almost purely vesicular character occur frequently at the same site (Figs. 1 and 2), but from individual sections it is impossible to decide whether these profiles are the endings of a separate kind of preterminal fibers, or merely non filamentous terminal side branches of the filamentous axons. It will be easy to answer this question on the basis of degeneration experiments in one of the next paragraphs.

As shown earlier (HÁMORI-SZENTÁGOTHAI, 1965) there are in the molecular layer another kind of axonal profiles of similar neurofilamentous character: the basket neuron axons. It would be difficult to decide in the normal picture of the molecular leyer, purely on the basis of their course and synaptic relations, wheter any given axonal profile of neurofilamentous character belongs to a basket neuron, a climbing fiber, a recurrent Purkinje axon collateral, or an outer stellate axon. In the Purkinje cell layer the basket axons are easily recognized on the basis of their characteristic relation to the bottom of the Purkinje cells and to their basal process — considered generally their axon — but for many reasons better be labelled as pre-axon (HÁMORI-SZENTÁGOTHAI, 1965).

In order to decide about the origin of the axons having synaptic relations with the primary and secondary Purkinje dendrites, we have to resort to degeneration studies. In the chronically isolated folium we have not been able to find a single axonal profile of neurofilamentous character in immediate attachment to the Purkinje dendrites. There are numerous well preserved synaptic contacts on these dendrites, however, they are exclusively of the vesicular character (Fig. 3). It appears thus that the heurofilamentous axonal profiles longitudinally attached to the Purkinje axons are the climbing fibers, whereas the intact synaptic contacts of vesicular character must be terminals of local origin: i.e. either from recurrent Purkinje axon collaterals, from outer stellate axons, or from basket axons. — A possible objection against this conclusion would be to assume that the neurofilaments become atrophic in the isolated folium in consequence of deprivation from

nervous input, relative anoxia, etc. Although, as known from light microscopy, the neurofibrils become rather hypertrophic in the undercut cortex, and especially in the isolated folium (SZENTÁGOTHAI, 1965), there is even better evidence against this objection by the well preserved neurofilaments in the basket terminals of isolated folia.

2. Climbing fiber — Golgi cell body contacts

The first collaterals of the climbing fibers are given at the level of the Purkinje cell bodies (SCHEIBEL and SCHEIBEL, 1954). They have been traced by secondary degeneration after olivary lesions to the cell bodies and proximal parts of the dendrites of the large Golgi cells, characteristically situated slightly below the level of the Purkinje somata (SZENTÁGOTHAI-RAJKOVITS, 1959). The Golgi cells can easily be recognized in this region on the basis of their characteristic plasma structure. (A detailed description of the electron microscopy of the Golgi cell body and some of their dendrites will soon be given in a forthcoming paper by one of us [J.H.]). The cell body surface of the Golgi cell (c.f. SZENTÁGOTHAI, 1965) is covered almost alternatingly, by axon terminals of very filamentous and vesicular character (Fig. 4). Little is known, on light microscope level, of the origin of axo-somatic synapses on Golgi cells, but considering the terminal ramification patterns of various axons and their collaterals there are virtually only two possibilities: one being recurrent Purkinje axon collaterals, and the Scheibel collaterals of the climbing fibers the other. Both basket axons and outer stellate cell axons can safely be discarded as highly improbably on Golgi evidence. Mossy fibers are occasionally mentioned as having synapses in — and immediately below — the Purkinje cell body layer with other elements than those of the cerebellar glomeruli (cerebellar isles), however, electron microscopy shows all such contacts being accidental and without the slightest signs of synaptic structural differentiations (HÁMORI-SZENTÁGOTHAI, 1965 [Fig. 8]). Thus the contacts, recently found by the Scheibels and treated in detail by BRODAL (1958), between mossy fibers and the Purkinje baskets as well as granule cell bodies undoubtedly exist, but they are non-functional; provided of course that our present concepts of the significance of ultrastructural synaptic differentiations are correct. We have found no EM evidence of immediate contact between Golgi cell bodies and mossy fibers. There is ample evidence both on the light and electron microscope level of participation of descending Golgi cell dendrites in the glomeruli, but this question is beyond the scope of this paper and will be dealt in detail by one of us (J.H.) in near future.

On the basis of this reasoning the chronically isolated folium ought to be a particularly favourable model for the study of the origin of the axo-somatic Golgi cell synapses. And indeed it is: as seen in the right half of Fig. 4 there is one kind of preserved synaptic contact on the surface of the Golgi cell under such circumstances. The persisting synaptic contacts are without exception of the vesicular type, all filamentous ones having disappeared and their places having been taken over by hypertrophic glial profiles, which also contain filaments but whose glial nature is obvious. Thus one may safely conclude that about half of the synaptic terminals on Golgi cell bodies, i.e. the filamentous ones belong to climbing fibers, whereas the vesicular ones are obviously endings of the infra ganglionic plexus (CAJAL) of recurrent Purkinje axon collaterals. — The possibility cannot be flatly

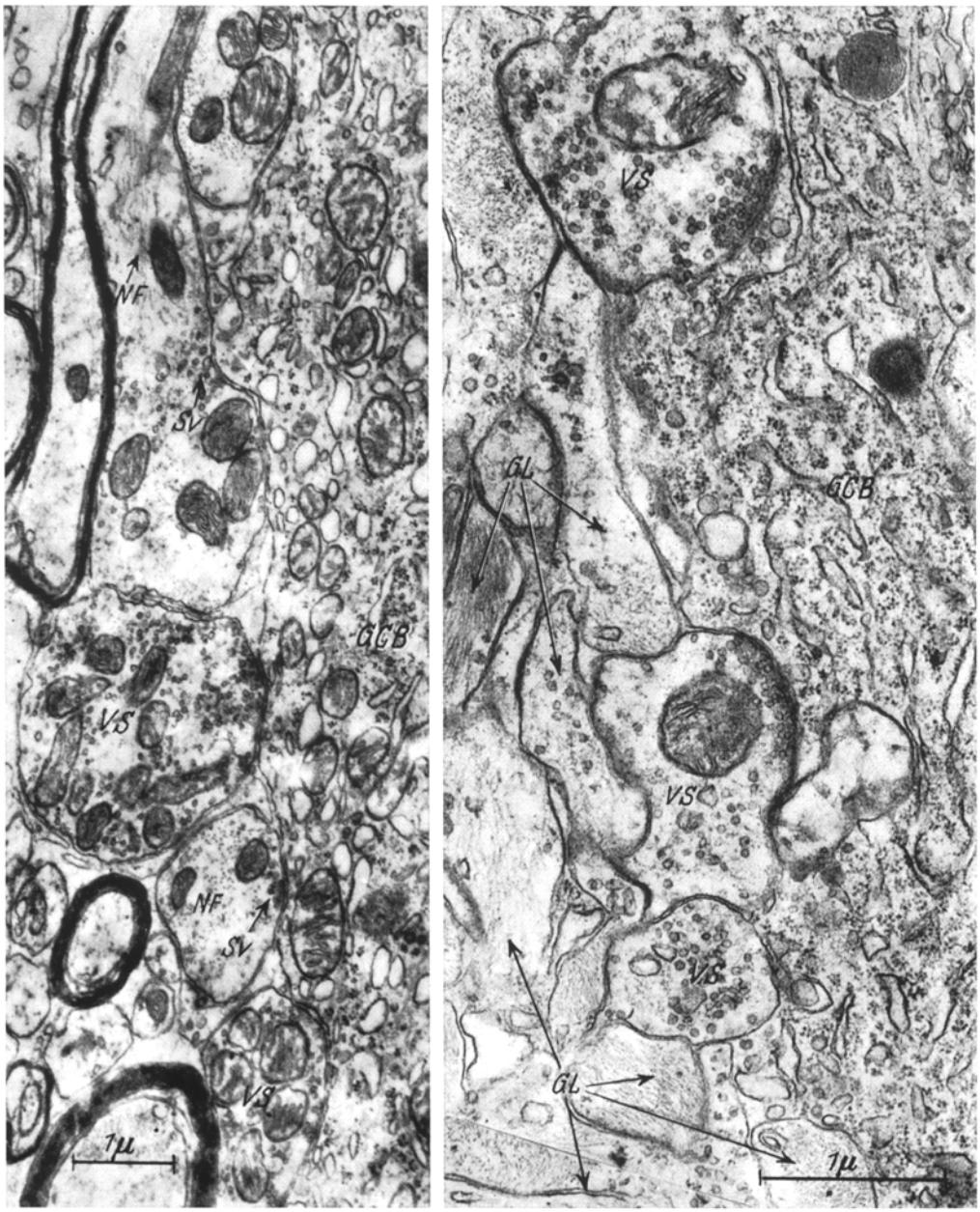

Fig. 4. *Left: Axo-somatic synaptic contact on the surface of Golgi cell body* (Gcb) in the normal cerebellar cortex. The presynaptic terminal profiles are alternatingly filled with neurofilaments (Nf), having small accumulations of synaptic vesicles only at sites of immediate contact (Sv), whereas others are vesicular in character (VS). — *Right:* same in chronically isolated folium with vesicular synaptic contacts (VS) intact, while the neurofilamentous profiles have disappeared and their places been taken over by characteristic hypertrophic glial (Gl) profiles

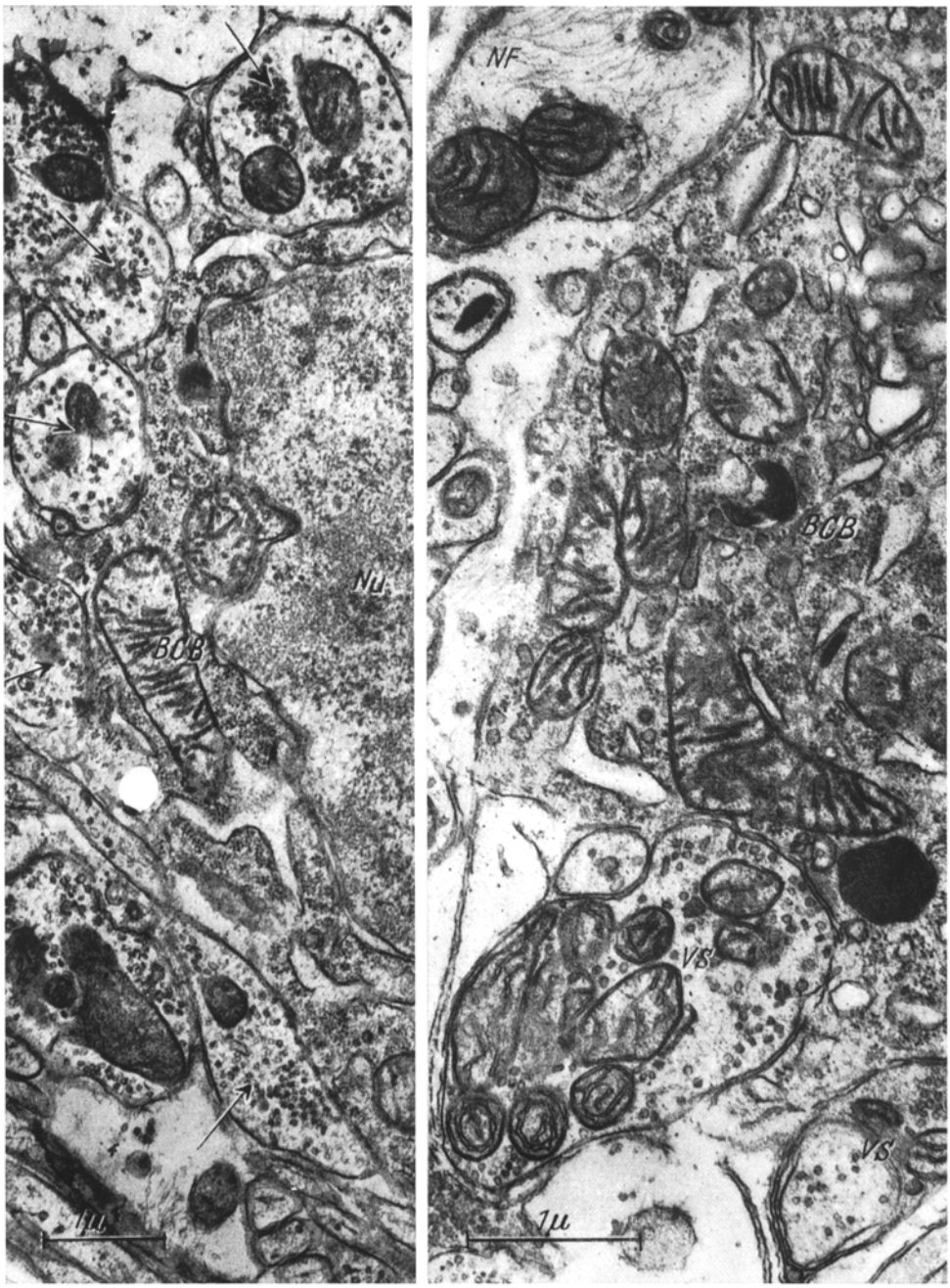

Fig. 5. *Left:* Characteristic "rugged" body profile of basket neuron (Bcb) covered with numerous synaptic profiles (arrows). Nu = basket cell nucleus. The difference is much less conspicuous than in the synaptic terminals on main Purkinje dendrites or on Golgi cell bodies, however, in large megnification (right) the neurofilamentous terminal profiles (Nf) differ clearly from vesicular terminals (VS). Normal cerebellar cortex

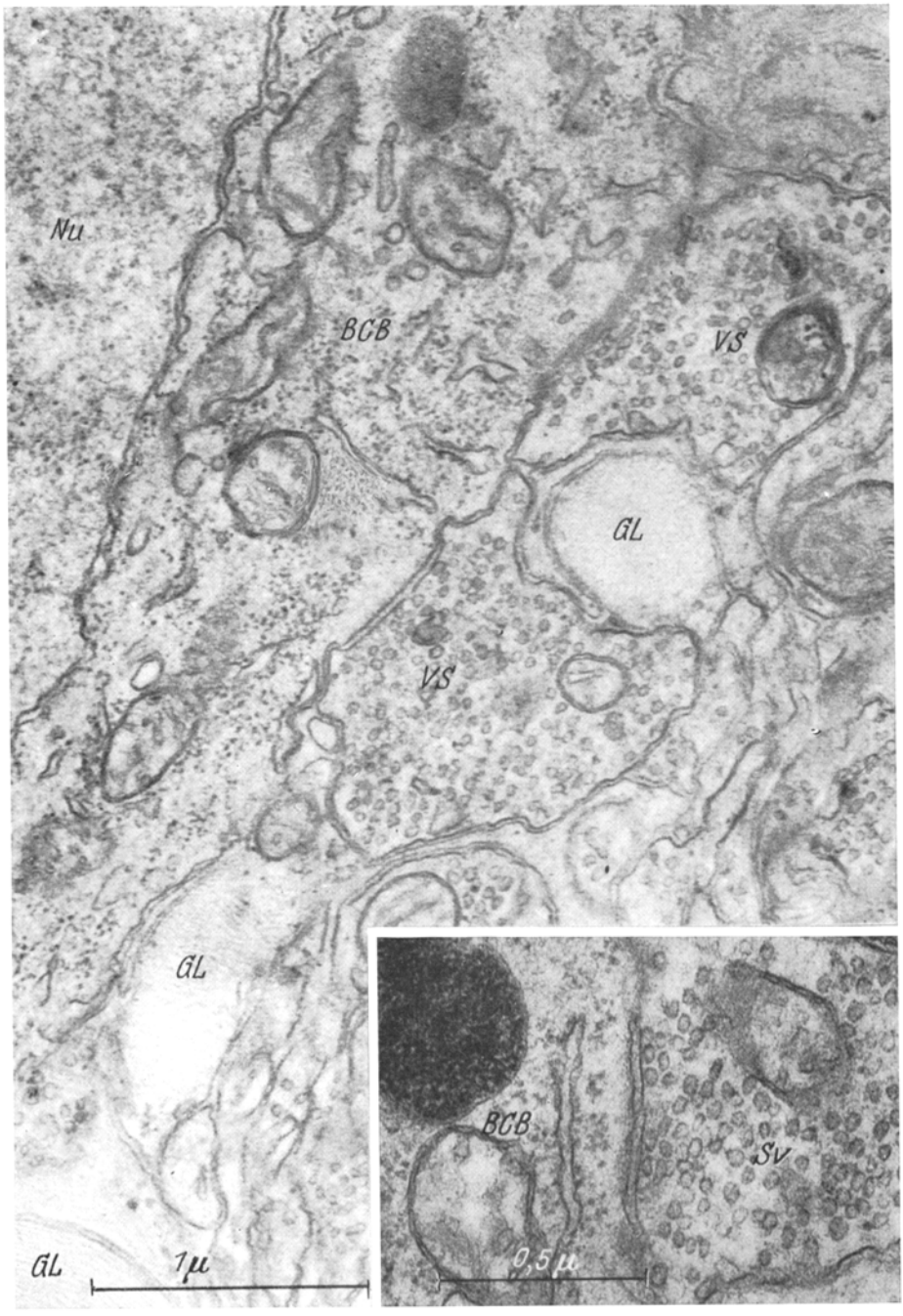

Fig. 6. *Basket cell body* (Bcb) with nucleus (Nu) in chronically isolated folium with two intact presynaptic profiles of vesicular character (VS) and hypertrophic glial process profiles (Gl) having taken the places of degenerated terminals. Inset shows similar persisting vesicular terminal at higher magnification

rejected that some of these terminals might be from Golgi cell axons themselves, however, neither Golgi evidence nor investigations of the Golgi cell axons in isolated folia both with light (SZENTÁGOTHAI, 1962, 1965) and electron microscopy (HÁMORI, 1964) would support this.

3. Climbing fiber — baskat neuron contacts

SCHEIBEL and SCHEIBEL (1954) have presented convincing evidence of axo-somatic contacts between climbing fibers and basket cell bodies. They claim to have seen axo-dendritic synapses between climbing fibers and basket cells as well as axo-axonic contacts with basket axon collaterals. The Golgi evidence of this seems to us to be far less convincing, as it is extremely difficult to judge from the overcrossing at close distance of an axon and a dendrite or two axons, whether there is any real contact, and even if so, whether the contact is merely accidental or specific. In an extensive materal of the molecular layer both in the adult cat (part of it published already: HÁMORI-SZENTÁGOTHAI, 1964) and from human biopsy material with the sectioning plane very carefully oriented in parallel and perpendicularly to the parallel fibers, we have never seen the slightest indication of specific axo-axonic contacts. Neither have we been able to see filamentous terminals on the dendrites of basket cells. So far the only axo-dendritic synapses of basket cells that we have been able to identify are axon-spine synapses with parallel fibers (HÁMORI-SZENTÁGOTHAI, 1964).

The basket cell is most characteristic both in the adult cat as well as in man on the basis of the highly irregular almost rugged surface of the cell body and its irregular nuclear surface. The contrast to the nicely spheroid smooth cell bodies of outer stellate cells and their equally spheroid nuclei is striking. The surface of the basket cell body is always densely covered with synaptic terminals (Fig. 5), some of them deeply embedded into depressions of the cell surface. Because of the irregularity of the surface it is difficult to obtain clear pictures of the synaptic membranes and clefts. The presynaptic terminals are again of two different kinds, one poor in synaptic vesicles having numerous neurofilaments, the others are filled with synaptic vesicles. Unfortunately the neurofilaments are much less coarse than in the terminal axons around Purkinje dendrites and Golgi cell bodies. Thus the difference between the two types of synapses is less obvious than in the two other cases. In the case of the basket cell bodies the synaptic terminals obviously could belong to (1) climbing fibers, (2) recurrent Purkinje axon collaterals and (3) stellate axons, if the possibility of basket axon collaterals is left unconsidered. This last possibility will be dealt with in the general discussion. Because of the three main possibilities the findings on isolated folia are not promising to be as conclusive as it was in the case of the Golgi cell.

As seen from Fig. 6 part of the axo-somatic synapses on basket cell bodies does persist in the chronically isolated folium. The persisting ones are clearly of the vesicular type. It seems thus that the degenerated filamentous terminals, the places of which have been occupied by hypertrophic glial processes, are endings of climbing fibers, whereas the persisting ones are either (1) from recurrent Purkinje axon collaterals, or (2) from stellate axons. We have no histological evidence that would enable us to choose between the two possibilities, so that this question is better discussed from the functional point of view.

4. Climbing fiber — outer stellate cell contacts

The stellate cell bodies can be identified easily under the EM on the basis of their smooth round shape and the round nucleus with a characteristic tongue-shaped invagination. There are rather few axo-somatic terminals on the cell body, generally only one or very rarely two presynaptic profiles being visible (in any given section) on the whole circumference of the cell. The synaptic terminals do not show structural details — for example neurofilaments — on the basis of which one would be able to differentiate different kinds. But this does certainly not mean that they are not originating from climbing fibers.

It could very well be that the neurofilaments are becoming gradually more delicate in the climbing fibers towards the cortical surface. Already at the level of the basket cell bodies the difference between neurofilamentous and vesicular synaptic terminals is much less conspicuous than around main and secondary Purkinje dendrites and Golgi cell bodies. This is also in agreement with the difference in length of the climbing fibers in specific "neurofibrillar" silver staining (e.g. Bielschowsky type procedures) and less "fibrillum specific" stainings (e.g. Cajal silver stains) and finally in non "fibrillum specific" procedures, (Golgi stains). In the first types of staining generally only the lower "neurofilamentous" part of the climbing fiber is visible, in the second types somewhat more, while the whole arborization is stained only in Golgi preparations.

The Golgi evidence presented by SCHEIBEL and SCHEIBEL (1954) seems to be convincing enough to consider the few axosomatic synapses of stellate cells seen in the EM as deriving from climbing fibers. — We have not observed intact terminals on stellate cells in the chronically isolated folium, however, considering the scarcity of terminals in the normal cerebellar cortex this is far from showing conclusively that only climbing fibers are approaching the stellate cell bodies.

The parallel fibers have certainly no axo-somatic synapses with stellate cells; they can be seen to curve around the cell body surface in immediate contact with it, but without the slightest indication of structural specialization.

Discussion

The climbing fiber and its synaptic terminals can be safely recognized in the EM picture on the basis of three criteria: (1) Its characteristic course running parallel along the primary and secondary dendrites of Purkinje cells, (2) its strongly "neurofilamentous" structure, and (3) disappearance in the chronically isolated folium, while synapses of the axons of local elements remain intact. It is very much to the credit of the ingenuity of RAMÓN Y CAJAL that almost all of his conclusions on the synaptic connextions among cerebellar neuronal elements have so far been substantiated by electron microscopy. M.E. SCHEIBEL and A.B. SCHEIBEL share in this by having pointed out first that Cajal and the older authors have been mistaken on their assumption that the climbing fiber forms contacts exclusively with a single Purkinje cell. The "recurrent" climbing fiber collaterals of the Scheibels have been already traced by light microscopy degeneration procedures to the Golgi cell bodies (SZENTÁGOTHAI-RAJKOVITS, 1959) and this is now substantiated by EM analysis. The existence of axosomatic contacts of climbing fibers with basket neurons claimed by the Scheibels is also well founded according to the present investigations. Although less conclusively, our investigations appear to support also their observation of axo-somatic synapses of climbing fibers on outer stellate neurons. The other suggestions of SCHEIBEL and

SCHEIBEL, particularly on axo-axonic contacts of climbing fibers with parallel fibers, basket — and stellate axons did not meet with positive findings in the EM picture. — Electron microscopy on one hand has immensely increased demand for Golgi information, however, it has restricted on the other its value in the decision of "what is connected synaptically with what else". It is only the EM picture from which one can judge with some safety, whether a contact between two nerve elements is a "synapse" or merely accidental. So far clear physiological evidence of impulse transmission has been found only where specific attachment regions are found in the EM, but final judgement, of course, has to be left for the future. For the time being it is obviously the most logical and safe course to consider as a synapse only contact regions with specific structural differentiations.

The existence of axo-dendritic synapses with other than Purkinje neurons are difficult to prove or to disprove under the EM. There is fairly good evidence (HÁMORI-SZENTÁGOTHAI, 1964), that most of the dendritic spines of Purkinje as well as basket neurons and with high probability also of stellate and Golgi neurons establish synapses exclusively with parallel fibers, i.e. "granule cell axons". The numerous "non-spine" axo-dendritic synapses especially in the outer part of the molecular layer are in great majority with local neurons, as they are preserved in the chronically isolated folium. Considering the Golgi picture they must be mainly synapses between stellate axons or the upper collaterals of basket axons and Purkinje dendrites. — For further reasoning allong these lines there is one crucial bit of information: the basket dendrites — which can easily be recognized (HÁMORI-SZENTÁGOTHAI, 1964) — have practically only axo-dendritic synapses on spines, the remaining part of their dendrite surface being completely sealed by glial elements. Thus it seems improbable that they have synaptic contacts from stellate axons. Both stellate axons and basket axon ascending collaterals are, therefore contacting probably mainly Purkinje cell dendrites.

In the deeper strata of the molecular layer the situation is somewhat different. The numerous vesicular synaptic terminals, persisting on large Purkinje dendrites in the chronically isolated folium, could be either from (1) recurrent Purkinje axon collaterals, or (2) stellate axon terminals, or (3) basket axon collaterals. From the viewpoint of Golgi light microscopy and EM the assumption (1) is highly and assumptions (2) and (3) are less, but (between each other) equally probable. The supra ganglionic plexus of Cajal — partly myelinated — fed mainly from recurrent Purkinje collaterals would have equally good access to both primary and secondary Purkinje dendrites and basket cell bodies. They could terminate at either site, or at both. The ascending basket axon collaterals more probably terminate on Purkinje dendrites.

The vesicular synaptic terminals on Golgi cell bodies are derived with very high probability from recurrent Purkinje axon collaterals, otherwise the infraganglionic plexus of Cajal would have no explanation, neither could we account for the origin of synapses deriving from local cerebellar neurons.

The stereoscopic diagram on Fig. 7 tries to explain the neuronal activities set up or influenced by a single climbing fiber. This diagram is based on histological information as derived from earlier findings on the light microscopy level and the present study, but it takes also into consideration the most recent and spectacular findings on the microphysiology of cerebellar neurons. According to ECCLES,

LLINÁS and SASAKI (1964a) the climbing fiber has an extremely powerful excitatory synaptic action on the single Purkinje cell on the primary and secondary dendrites of which it terminates. This excitation cannot be suppressed even by the most powerful basket cell inhibition (ANDERSEN, ECCLES and VOORHOEVE, 1963). — The climbing fiber has additional synapses on basket cell bodies and

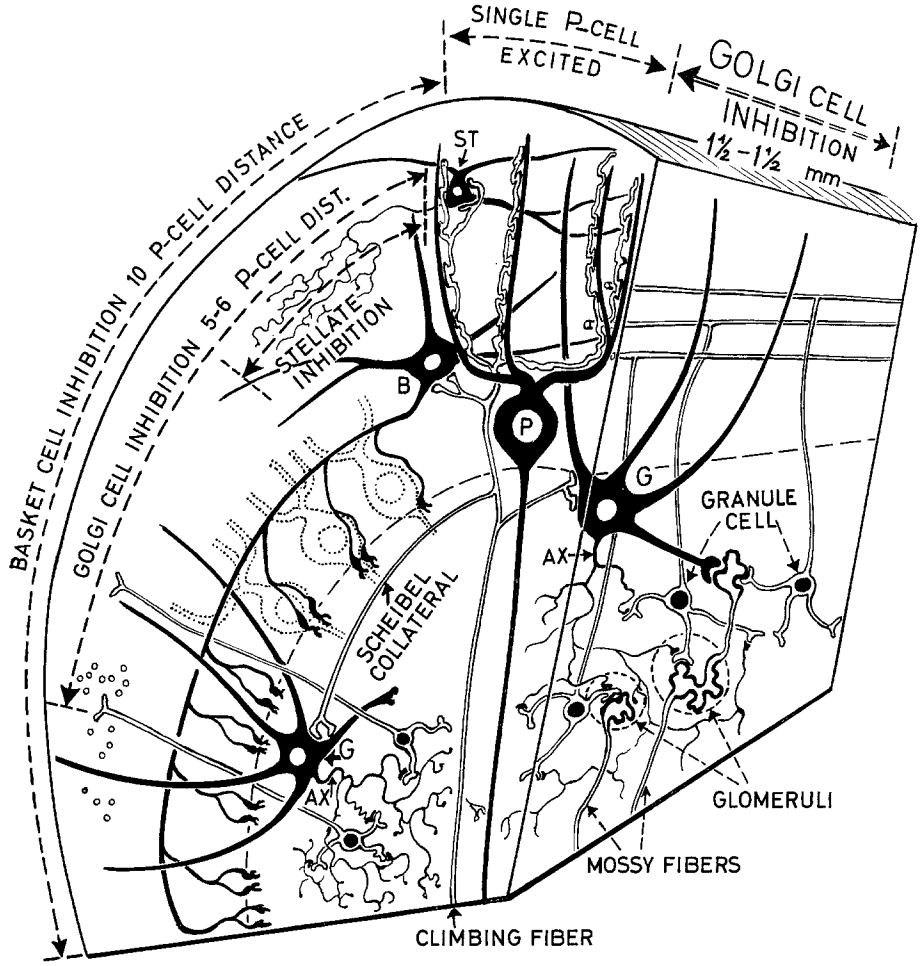

Fig. 7. *Stereoscopic diagram of contacts established by a single climbing fiber with one Purkinje cell* (P), several *Golgi cells* (G) and several *basket cells* (B only one representative cell shown here) and probably several outer stellate cells (ST). Fibers and cells known from recent physiological evidence to be excitatory are drawn in outline while inhibitory elements in full black. Potential distances (i.e. fields) of activities set up, or influenced, by the climbing fiber are indicated in the diagram, as derived from light microscopy information. (SZENTÁGOTHAI, 1965). Note that Golgi cell axons (Ax) establish synapses in the glomeruli with granule cell dendrites immediately "downstream" from their main synapse with the mossy terminal. Further explanation in the text

very probably on outer stellate cell bodies. With consideration to Dale's principle, of a single mediator being liberated from different endings of the same neuron, it is most probable that all other types of neurons are also excited by the climbing fiber. It is, of course, questionable, whether some axo-somatic boutons are as effective an activator of basket or stellate cells as the numerous repeated contacts

between the climbing fiber and the Purkinje neuron. The basket synapse having been proved by ANDERSEN, ECCLES and VOORHOEVE (1963) as inhibitory on Purkinje cells, this would mean that an inhibitory influence could be exercised on Purkinje cells on both sides across the folium as far as the tenth Purkinje cell from the site of termination of the climbing fiber (SZENTÁGOTHAI, 1965). As according to the most recent findings of ECCLES and COWORKERS (personal communication) the outer stellate cells are inhibitory on Purkinje cells, this would mean an additional stellate cell inhibition at somewhat closer distance. — According to our earlier degeneration studies the Scheibels recurrent collaterals of the climbing fiber can reach Golgi cells as far as the 5—6th Purkinje cell, again in transverse direction of the folium (SZENTÁGOTHAI-RAJKOVITS, 1959; SZENTÁGOTHAI, 1965). As this influence also ought to be excitatory, but as according to ECCLES, LLINÁS and SASAKI (1964b) the Golgi cell is postsynaptically inhibitory on impulse transmission through the mossy fiber-granule dendrite synapse, the climbing fiber could exercise a depressing influence on the main general input paths of the cerebellum almost as far across the folium as the basket cell inhibition. However, Golgi cell inhibition would be transneuronally "fed in forward" — with respect to the position in the circuit of the Purkinje cell — whereas basket cell inhibition would be postsynaptic. But there is an additional crucial difference between the two inhibitions. Although the influence of the basket axon is not rigidly confined to the transverse plane of the folium, its terminal branches being able to reach seven Purkinje cell bodies in a row longitudinal to the folium axis (SZENTÁGOTHAI, 1965), this is still only a distance of a few hundred microns at the highest. The Golgi cell on the contrary, by reaching with its axon the far majority of the glomeruli in the entire depth of the granule layer and by having an axon ramification of the width and depth roughly comparable and corresponding to its dendritic ramification in the molecular layer could heavily impair the input through mossy fibers along a longitudinal beam of parallel fibers of about 3 mm. length (FOX and BARNARD, 1957). With respect to the distance bridged by the Scheibel collateral of 5—6 Purkinje cells in transversal direction of the folium the potential influence of a single climbing fiber by way of the Golgi cells would be a field of 3 mm length in longitudinal and 10—12 Purkinje cells in transversal direction of the folium. Most probably a single climbing fiber would not have too much influence with the exception of exciting a single Purkinje cell, but a group of climbing fibers arising from a group of closely neighbouring cells in the inferior olive — which according to the sharp point-to-point projection in this pathway would terminate on closely neighbouring Purkinje cells — could very well exercise a decisive inhibition on this rather large field.

Although this study has brought forward some quite important information on the connexions of recurrent Purkinje collaterals, these have not been incorporated into the diagram, being intricate enough as it is. The problem is additionally complicated by the fundamental discovery of M. ITO (1965) of the specific inhibitory character of the Purkinje neurons. If this were general for all Purkinje cells, we would have to apply Dale's principle to the recurrent Purkinje axon collaterals as well. This would mean that the active Purkinje cell would exercise a disinhibitory influence by way of Golgi cells and basket cells. As the distances bridged by the recurrent Purkinje axon collaterals are much larger than

those bridged by the climbing fiber connexions (FREZIK, 1963), this disinhibition would have a far greater range than any other intracortical relay of the cerebellar cortex. We have seen that our evidence is conclusive only for the Golgi cell, which undoubtedly has axo-somatic contacts from recurrent Purkinje axon collaterals. The evidence is not entirely conclusive for the basket cells. The supra-ganglionic plexus of the recurrent Purkinje axons might contact either basket cells or Purkinje dendrites or both. The positive evidence of vesicular synapses persisting in chronically isolated folia could be put on the account of stellate axons in both cases and to that of basket axon collaterals, in the case of Purkinje dendrites. — Cajal thought that the recurrent Purkinje axon collaterals terminate mainly on the large dendrites of Purkinje cells, but he was probably biased by his general concept that initial axon collaterals terminate on the same kind of neurons. This concept has been proved erroneous in many places (motor axons, thalamic neurons) by recent physological as well as histological findings. Turning to pure speculation one could hardly see any point in having two conflicting influences set up at the same site by the same mechanism. If the Purkinje neuron is inhibitory, its synaptic terminals, certainly present on Golgi cells, would effect their inhibition and thus in the end disinhibit many Purkinje cells in the formers field of influence. If the recurrent axon collaterals would contact Purkinje neurons directly, they would be inhibited. A Purkinje recurrent inhibition of basket cells would bring in a further feedback disinhibition. — Similar difficulties would arise if one would assume an inhibition of basket cells by axo-somatic synapses from stellate neurons, or the reverse by basket axon collateral synapses on stellate dendrites. This would mean three inhibitory links in succession and additionally in a closed loop. The simplest and yet most meaningful concept would be to assume the following inhibitory connexions only: (1) Stellate axons terminating on Purkinje dendrites, (2) basket axons terminating in the Purkinje cell baskets, (3) ascending basket axon collaterals terminating on Purkinje dendrites, (4) Purkinje axon recurrent collaterals on Golgi cell bodies and (5) on basket cell bodies and finally (6) Golgi cell axon endings in the glomeruli of the granule layer. The termination (7) of Purkinje axon collaterals on Purkinje cell dendrites and conflicting with mechanisms (4) and (5) — from which existence of (4) is certain — might be considered as alternative to (5). — It is up to the physiologist to find out, which of the two alternative possibilities (5) or (7) is more likely to be the correct solution.

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