THE FINER STRUCTURE OF THE SELACHIAN CEREBELLUM (MUSTELUS VULGARIS) AS SHOWN BY CHROME-SILVER PREPARATIONS.

By DR. ALFRED SCHAPER,
Harvard Medical School, Boston, Mass.

(WITH PLATES I TO IV AND ONE FIGURE IN THE TEXT.)

During my stay at the Marine Biological Laboratory at Woods Hole, Mass., in the summer of 1897, I found opportunity to extend my comparative anatomical studies on the cerebellum over the various species of Selachii to be found on that coast. I especially endeavored, among other things, to elucidate by means of the *Golgi method* the finer structure of the cerebellum of this group of vertebrates, so important from a comparative anatomical standpoint. Notwithstanding the zeal with which the various representatives of the vertebrate phylum have been investigated by means of the Golgi method during the last decade, the selachian brain has hitherto, strange to say, scarcely been included within the range of research. The first and only author so far who has given us a connected description of the histological structure of the selachian brain is, as far as I know, *E. Sauerbeck* (4). The specimens at this author's disposal were prepared by Professor Rudolf Burckhardt of Basel, under whose direction the work was prosecuted. Sauerbeck's results appeared in an article in Band XII of the *Anatomischer Anzeiger* under the title "*Beiträge zur Kenntniss vom feineren Bau des Selachierhirns.*" Although this work, as the author himself states, makes no claim to completeness, yet it is the first attempt to fill in this existing gap in our comparative histological knowledge of the central nervous system and thereby can serve as a worthy starting-point for further and more thorough studies in this field.

In all researches on the central nervous system by means of impregnations the elements of the cerebellum, as is well known, offer the greatest difficulties. A considerable number of preparations are usually required to demonstrate all the elements of this organ. This peculiarity appears to have presented itself also in the preparations at Sauerbeck's service, as, judging from his figures, all the other parts of the brain seem to have been much more completely and certainly impregnated than the cerebellum. Thus Sauerbeck was able to demonstrate only *Purkinje cells* and *ependyma elements* in the cerebellum. He has, indeed, on the ground of stained preparations confirmed the presence of a molecular and a granular layer in agreement with earlier authors (Viault (8), Sanders (3), and Rohon (2)), yet he did not succeed with Golgi preparations in learning anything of the elements constituting them. Thus there remain in reserve many important points for further investigation to determine. As I have several years since directed my special attention to the morphology of the cerebellum and contemplate subjecting this part of the brain in all the vertebrate types to a comparative anatomical and comparative embryological investigation, the present gap in our knowledge of the selachian brain, so important for my purpose, became so much the more perceptible to me. It was natural, therefore, that I should eagerly seize the opportunity, presenting itself to me in Woods Hole, of filling in, as far as lay in my power, these gaps by means of suitable investigations. My efforts toward this end were attended with some success and I take the liberty to report briefly upon them in the following.

Although I propose to publish here the results obtained from silver preparations only, yet I might for general orientation, preface this with a few words upon the morphology of the selachian brain. In so doing I confine myself to that of *Mustelus vulgaris* (*Galeus canis*) from which species my Golgi preparations were exclusively made.

As with most of the Selachii, in *Mustelus* the cerebellum is greatly developed and is traversed by numerous transverse folds. It might from this appear that the cerebellum of the

shark already approaches that of the higher vertebrates in its morphological structure. This, however, is not the case. On a closer examination we soon learn *that we have to do only with a plate thrown into transverse folds and enclosing a relatively roomy cavity, not with a solid organ like that of birds and mammals.* The essential feature in the cerebellum of the latter is the massive development of the white matter, which goes hand in hand with an extraordinarily complicated surface folding of the cortex, whence the formation of the "*arbor vitae*" so characteristic of birds and mammals. *There is none of this present in the selachian cerebellum.* There is expressed, indeed, in the folding an evident tendency to an extension of the surface, yet these folds cannot be directly (also not genetically) homologized with the "convolutions" and furrows of the cerebellum of the higher vertebrates. In the first case we have, in general, at least, to do with *actual folds*, i. e. with structures where an infolding on one side of the lamella corresponds to an outfolding on the other, while in the second case we are concerned with *solid* ridges and protuberances separated by furrows.

Concerning the finer structure, the presence of the three cerebellar layers typical for all vertebrates—the molecular, Purkinje and granular—has been already demonstrated by the earlier authors, as Viault (8), Sanders (3) and Rohon (2). I have to add, however, that the granular layer does not everywhere participate to the same extent in the structure of the cerebellar lamella. Indeed in definite and extensive portions of the latter it is entirely *absent*, in consequence of which the cortical matter in these places consists of the molecular and Purkinje layers only and the latter is separated from the ependymal layer by only a relatively thin zone of fibers. The Purkinje cells likewise are absent in certain regions of the lamella. This relation of the layers is in various respects of especial interest but I cannot enter into this here. The accompanying schema of a transverse section through the anterior portion of the cerebellum of *Mustelus* may serve to illustrate this condition.

I might however add that in the Selachii *during life*, as in younger stages in bony fishes and in certain embryonic periods of all vertebrates the *cerebellar lamella has a deep longitudinal furrow in the median line (sulcus medianus)* where the extremely thin lamella is composed solely of ependyma cells and commissural fibers.

Diagram: Cross-section through the anterior part of the cerebellum of *Mustelus vulgaris* showing the arrangement of the different layers.

I pass now to a description of my silver preparations and begin with the most characteristic cell group of the cerebellum, the

Purkinje Cells.

The presence of these cells has been already established by the older investigators (Viault, Sanders and Rohon). Sauerbeck was the first, though, to observe and describe them in *Golgi preparations*. He speaks of them as follows: "Von der Membrana limitans interna ab gerechnet im zweiten Drittel der radialen Ausdehnung (der Kleinhirnlamelle) finden sich typische Purkinje-Zellen, die nach innen, d. h. gegen die nicht versilberte

Körnerschicht hin, einen Axencylinder senden, der sehr bald in die horizontale, resp. tangentielle Richtung umbiegt; nach aussen ragen armleuchterartige Dentritenfortsätze, doch sind diese nicht so reich verzweigt wie bei höheren Vertebraten, wie auch schon bei den Teleostiern *Schaper* sie abgebildet hat." The figures given by Sauerbeck are, in consequence of their small scale, but little adapted to reproduce the characteristics of these cells. I have therefore again represented two typical forms of Purkinje cells in Plate I, Figs. 1 and 2. We see from these that the size and form of the cell-body, as well as the magnitude and arrangement of the protoplasmic processes, are subject to considerable variation. This is correlated in part with the very variable thickness of the molecular layer; we meet the more massive and coarser type (Plate I, fig. 1) mostly in the more strongly developed portions of the molecular layer, the smaller and more delicate type (Plate I, fig. 2) mostly in the thinner regions of this layer. As Sauerbeck rightly remarks, the protoplasmic processes are not so richly branched as is usually the case among the higher vertebrates and one can compare them in this respect with those of the bony fishes, as some time ago (5) described and figured by me. They are distinguished, however, even from the latter by a still more sparse arborization and their less straight, irregular course to the surface. One could say *they stand upon a still lower plane of development than those of the bony fishes and display throughout life an embryonic condition of the homologous cells of the higher vertebrates.* Like the dendrites of all Purkinje cells, these are provided with a thick covering of very fine spines and usually tend to terminate with a slight thickening.

Concerning the *axis-cylinder*, this also presents certain noteworthy peculiarities. With respect to their course, they behave differently according as they come from cells which have a granular layer beneath them or from such as, in the absence of the granular layer, lie close to the *membrana limitans interna*. (See text-figure, p. 4). In the latter case (Plate I, fig. 1) the axis-cylinder is naturally compelled, soon after its origin from the under pole of the cell, to bend laterally and proceed,

parallel with the *limitans interna* and immediately beneath the row of Purkinje cells, to its destination. In the preparation upon which figure 1 is based I succeeded in following such an axis-cylinder a considerable distance. In this way the neuraxons of those Purkinje cells situated in the cerebellar plate where a granular layer is lacking furnish the principal contingent of the often considerable mass of parallel fibers interposed between the layer of Purkinje cells and the *limitans interna*. But even where there is a granular layer, the axis-cylinder does not as a rule immediately sink into this; but first proceeds a short distance in a horizontal direction and then takes a sharp bend inward (fig. 2). It is now to be noted that I never succeeded in any of my preparations in demonstrating *collaterals* of the axis-cylinders of the Purkinje cells, although, as far as I know, their presence has been established in all other vertebrates hitherto investigated. Whether we have here a defective impregnation or whether these collaterals are actually absent in the Selachii, and their absence is perhaps the expression of a lower phylogenetic stage of development, I do not venture to decide at present. Sauerbeck likewise does not mention collaterals in his article. Among his figures of Purkinje cells, however, are some with divided axis-cylinders. May one of these branches represent a collateral?

Nerve-cells of the Molecular Layer.

These cells are for the most part uncommonly delicate and vary extraordinarily in the form and size of the cell-body as well as in the mode of branching of their protoplasmic processes. To properly illustrate this variety of form I have represented a larger number of cells in Plate I, figures 3 to 8. They lie in all levels of the molecular layer, from the layer of Purkinje cells (even pressing in between these) to close to the surface. The rule seems to obtain that the smaller the cells and the shorter their dendrites, the closer they lie to the surface and *vice versa*. The *dendrites* display in general the tendency to extend towards the surface, although in the deeper lying cells there is also a considerable extension of the same horizontally (Plate I, Fig. 3).

While the cells vary in form, yet the *neuraxons* in all the cells observed by me have essentially the same behavior. The nervous process soon loses its individuality through the giving off of very numerous lateral branches and is resolved a short distance from the cell body into its terminal arborization. Thus all the cells of the molecular layer belong to the so-called "*Golgi type*." In *course* and *mode of distribution*, however, the ramifications of these axis-cylinders display a certain variability; we find axis-cylinders which proceed horizontally a short distance and thereby give off lateral branches outward and inward (Plate I, figs. 3 and 7), others which descend and soon are lost in their terminal arborizations (Plate I, figs. 6 and 8) and others again which run outward in wide curves and then fall into more or less numerous terminal branches which mostly extend toward the surface (Plate I, figs. 4 and 5).

The question now arises: *What relation do these nervous processes bear to the Purkinje cells?* We know definitely that in the higher vertebrates, at least, a certain number of the cells of the molecular layer, the so-called "basket cells" enter into very close relation with the bodies of the Purkinje cells, embracing the latter with tassel-like terminal arborizations. Besides these, another group of cells of the molecular layer has long been known under the name of the "*star shaped*" or "*small cortical cells*" about whose axis-cylinder little was actually known. Stöhr (7) first succeeded about a year ago in demonstrating in silver-preparations of the human cerebellum well developed nervous processes on the "small cortical cells" also, whose terminal arborizations behave toward the Purkinje cells very much as the basket cells do, but without forming the typical basket of fibers. Stöhr is thereby inclined to *abandon the division of the cells of the molecular layer into basket cells and small cortical cells and to unite them all in one group with the common character that all, notwithstanding great differences of form, display the tendency to enter into close contact with the bodies of Purkinje cells by means of the ramifications of their axis-cylinders*. In this view Stöhr finds himself in agreement with Dogiel (1) and Kölliker who shortly before had similarly expressed

himself. Yet not long ago an article of Smirnow's (6) appeared, also an investigation of these elements of the cerebellum of man, as well as of the dog and hare. The results brought the author to the conclusion, in opposition to Stöhr's view, *that two distinct kinds of cells are to be distinguished in the molecular layer according to the behavior of the axis-cylinder.* The figures given in this work appear to me also to plainly show that there are cells in the molecular layer whose axis-cylinders do not bear comparison with those of the cells hitherto described as "*basket cells.*" In a number of these cells (in man also) the nervous process a short distance from its origin breaks up into numerous terminal arborizations which may be distributed in all directions. The field of distribution of many of these axis-cylinders does not extend down to the bodies of the Purkinje cells and if certain isolated branches do come into the neighborhood of the latter, yet, in view of the distribution in many directions of the remaining branches, this is to be regarded as an incidental appearance. The tassel-like arborization of the collaterals so characteristic of the basket cells can nowhere be demonstrated, at all events, with these cells. There appears, after all, to be only one characteristic applicable to all the cells of the molecular layer, viz.: *that their axis-cylinders soon break up into their terminal arborizations and generally do not leave the region of the molecular layer.* The *special* behavior of the axis-cylinders of the *individual* cells, however, that is the definite relations of *some* to the bodies of the Purkinje cells and the correlated typical adaptation of the terminal arborizations to the latter and the entirely disorderly mode of distribution of *other cells*, compels, or at least entitles us, in agreement with the conclusion of Smirnow previously given, *to distinguish two kinds of cells in the molecular layer, i. e. "basket cells" and the others accurately described by Smirnow which we still for the present may term "small cortical cells."* It is not thereby rendered necessary to create a *fundamental* distinction between these two categories of cells. It even appears probable to me that the basket cells are merely to be regarded *as a specialized form of the molecular cells.* Nevertheless the morphological differences in the behavior of the axis-cylinder

expressed in the higher vertebrates make the above division desirable from practical grounds.

I have entered into a discussion of this point more at length here to secure a basis for the elucidation of the relations which we have encountered in the above described cells of the molecular layer of the selachian cerebellum. As we have seen, the axis-cylinders of none of these cells exhibit the typical conduct of those of the basket cells; never were tassel-like terminal arborizations demonstrable. In certain cells lying in the deeper zone of the molecular layer, one sees here and there isolated terminal branches penetrate between the bodies of the Purkinje cells but there cannot be said to be the intimate and extensive contact-relation to the latter that there is in the case of the basket cells. Besides this, there are usually present on such axis-cylinders numerous other terminal branches which do not proceed towards the Purkinje cells. In the majority of the cells of the molecular layer we saw the ramifications of the axis-cylinder spread out in all directions without ever entering into any relation with the bodies of the Purkinje cells. *Thus all cells observed by me in the molecular layer of the selachian cerebellum more or less resemble—apart from a slighter complexity in the ramification of both dendrites and axis-cylinder—those cells which Smirnow has recently described in man and the higher vertebrates and distinguished from the true basket cells.* If we now, as mentioned above, will regard the basket cells merely as a particular specialization of the cells of the molecular layer, this specialization has not yet appeared in the cerebellum of the Selachii; *these cells are here still in a more primitive or phylogenetically younger stage of development.* I might use this opportunity to mention that I likewise have not hitherto succeeded in demonstrating true basket cells in the molecular layer of the cerebellum of *Teleosts* (5). Probably the state of affairs here is similar to that in the Selachii.

Nerve-cells of the Granular Layer.

The granular layer in the cerebellum of Selachii has hitherto been established as such and homologized with the cor-

responding layer of the higher vertebrates from stained preparations only. For such a homologization the proof was yet to be brought that this layer also contained similar *elements* and that these elements exhibited the same behavior as in the higher vertebrates.

I have now succeeded in my preparations in furnishing this proof, viz.: *that there are both "small granule cells" (granule cells sensu strictiori) and "large granule cells" (so-called Golgi cells of the cerebellum) in the granular layer of the Selachii.*

Regarding the *small granule cells* (Plate II, figs. 9, 10 and 11) the cell body and protoplasmic processes behave throughout like those of the higher vertebrates. The relatively small, mostly round or polygonal body sends in all directions a limited number (usually 3 or 4) of delicate protoplasmic processes of which the majority terminate in the way familiar in the other vertebrates, by means of a claw or brush-like structure.

The extremely thin *neuraxon* arises in the majority of cases from a protoplasmic process and then winds zig-zag between the other granules, usually proceeding directly to the molecular layer. When it reaches the molecular layer, *the axis-cylinder divides in a T in the manner typical for all vertebrates.* The two branches proceed in opposite directions parallel to the surface of the cerebellum and the long axis of its folds and finally (very probably a considerable distance from the point of bifurcation) break up into their terminal arborizations (Plate II, fig. 12). I have unfortunately only once succeeded (notwithstanding a most careful examination of my sections), in following one individual axis-cylinder of a granule cell without interruption from its origin to its point of division in the molecular layer. But I have repeatedly come upon the pieces of these T-shaped bifurcations, as shown in figure 12 (Plate II). There can scarcely be any doubt as to the connection of these fragments with the axis-cylinders of the granule cells and I have no hesitation in declaring, in spite of any inadequacy of observation, *that the behavior of the axis-cylinder of the small granule cells in the cerebellum of the Selachii is identical throughout with that in the other vertebrates.* I might mention here a certain peculiarity found

in the axis-cylinder of a granule cell, which I have figured in figure 9 (Plate II). We see here *the nervous process divide fork-like while still in the granular layer*. I recollect having once before happened upon a similar condition in the cerebellum of a mammal. We have here, doubtless, a *developmental anomaly* viz.: an abnormal division of the axis-cylinder.

I have above called attention to the fact that most of the nerve processes of the small granule cells appear to run in a more or less *direct* course to the molecular layer; frequently, however, one meets with cells also where the axis-cylinder first proceeds *horizontally* for a considerable stretch and then wends upward in a wide curve (Plate II, fig. 11 below). I have unfortunately not been able to follow such an axis-cylinder into the molecular layer. I conjecture however that these axis-cylinders are devoted to those parts of the cerebellar plate where a granular layer is lacking. We also find here in the molecular layer numerous axis cylinders of granule cells ascending and are thereby forced to the conclusion that these portions of the cerebellum are supplied by the granule cells of *other* regions.

It remains to be mentioned that the neuraxons of the granule cells on their entrance into the molecular layer appear to gain somewhat in caliber and are more thickly studded with varicosities than in the granule layer (Plate II, fig. 11). Shortly before their termination in the molecular layer they usually turn upwards abruptly and thus send their terminal arborizations to the upper portions of this layer (Plate II, fig. 12).

The "*large granule cells*" (Plate II, figs. 13, 14, Plate III, fig. 15) are, as in the other vertebrates, cells of the "*Golgi-type*," i. e. those whose axis-cylinders break up in their terminal arborizations soon after their origin. The very *voluminous cell-body* is usually round and in size often exceeds that of the Purkinje cell. The extremely coarse *protoplasmic processes* are small in number and exhibit no great tendency to branching. The nervous process arises in the majority of cases directly from the cell body, only seldom from a dendrite (Plate II, fig. 13). Its terminal arborization is less complex than in the higher vertebrates and is distributed in an entirely irregular manner among the small granule cells.

Nerve Fibers.

Although the course of the nervous processes of the various types of cells has already been described individually, yet it might be advisable to consider again here in particular the behavior of the neurites in their totality and mutual relations, especially as these relations are of a peculiar nature in the cerebellum of the Selachii. Besides this there are still *those axis-cylinders* to be considered *which enter the cerebellum from other parts of the central nervous system*. The presence of such nerve fibers in the cerebellum of selachians, also, is to be assumed *a priori* both from the standpoint of comparative anatomy and from the necessity that external impulses must be transmitted to the cerebellum. Notwithstanding this, the positive proof of these fibers has presented the greatest difficulty to me. After a painstaking search through my preparations, I have only been able to actually demonstrate one isolated fiber unquestionably of this kind. This one is shown in figure 16 (Plate III). We see this fiber ascend in an irregular course through the granular layer and break up in its terminal arborization in the molecular layer. This very meager demonstration of the existence of "*ascending fibers*" in the selachian cerebellum must suffice at present. In its caliber and morphological characteristics this axis-cylinder observed by me scarcely differs from that of the Purkinje cell. Nothing was observed of "*moss-like outgrowths*" at definite intervals, such as were described by Ramón y Cajal and others on the ascending fibers of Mammalia (*fibres mousseuses*).

The tangle of nerve fibers in all layers of the cerebellar plate is infinitely complicated and it is, in fact, very difficult to find one's way. As already mentioned above, the neuraxons of the Purkinje cells, before they enter the granular layer, usually proceed a longer or shorter stretch in a horizontal direction beneath and in the layer of the Purkinje cells. In this way they form a dense nervous plexus (Plate III, fig. 17, above). This plexus is especially strongly developed where there is no granular layer under the Purkinje cells, in consequence of which *all*

the axis-cylinders are obliged to pursue their destination along the *membrana limitans interna*.

If we further consider the *granular layer*, we find here in general a dense tangle of fibers proceeding in all directions (Plate III, fig. 17); in the upper part near the layer of Purkinje cells a perpendicular direction of the fibers does indeed prevail, in the deeper layers, however, they cross each other without any order. In figure 17 (Plate III), which illustrates these relations, the delicate axis-cylinders of the granule cells are omitted; the larger fibers present come in part from the *Purkinje cells*, another part, however, undoubtedly belongs to the "*ascending fibers*." As mentioned above, there do not appear to be any characteristic marks attached to these two groups of fibers, so that they can only be distinguished by following them to their origins or to their terminal arborizations. This is, naturally, only practicable in a few fibers and in the cerebellum of selachians is especially difficult from reasons mentioned below.

The course of the fibers within the granular layer is somewhat differently arranged in those parts of the cerebellar plate where the former does not border directly upon the *membrana limitans interna*, but is separated from it by a *compact layer of medullated fibers* (the first rudiment of a central white matter) and where, besides, externally the Purkinje cells are as a rule entirely lacking so that the granular layer borders immediately upon the molecular layer, the latter being usually very thin in such places (Plate III, fig. 18). Here one sees, instead of an irregular network, well marked *fiber-bundles* at definite intervals ascending in a vertical direction within the granular layer. They stand in connection below with the basal fiber layer and extend upward to the boundary between the granular and molecular layers or also somewhat into the latter. Here they end, as though cut off, usually with a small hook-like bend. The fiber-bundles are so compact that they traverse the granular layer as completely closed masses. The axis-cylinders of the granule cells do not participate in their formation but proceed upwards between the bundles in the usual manner. They are not drawn in figure 18 (Plate III). I have unfortu-

nately not been able to ascertain with perfect certainty the further course of those fibers. Only this much is certain, that they turn in a horizontal direction along the boundary between the granular and molecular layers and proceed a distance further here in the form of isolated bundles. The roundish cross-sections of these fiber-bundles which I have met with lying at certain intervals from each other between the molecular and granular layers demonstrate this sufficiently. I conjecture that these fiber-bundles now gradually lose their individuality and finally go over into the thick nervous plexus which we saw locally so greatly developed beneath the Purkinje cells in other regions of the cerebellar cortex. I further conjecture that these bundles contain both centrifugal and centripetal fibers, i. e. both the axis-cylinders of Purkinje cells and "ascending fibers," or, in other words, all those tracts which, by means of the crura cerebelli, furnish the functional communications of the cerebellum with the other portions of the central nervous system.

Concerning the fibers of the *molecular layers*, here the ascending axis-cylinders of the small granule cells and their horizontal branches are especially prominent. The former form locally a dense forest, especially in those parts of the cerebellar plate where the Purkinje cells are lacking (Plate II, fig. 11). The axis-cylinders, as mentioned before, here increase somewhat in thickness and are quite thickly beset with varicosities. The nervous processes of the "cortical cells" also participate in the tangle of fibers in the molecular layer and likewise the terminal arborizations of the "ascending fibers," of which I have brought to view but little.

I might mention here one peculiarity which all these fibers appear to possess which pass from the granular into the molecular layer and *vice versa*. This consists in the fact that all these fibers in their passage from one layer to the other usually undergo a *double (bayonet shaped) flexure* in that they bend for a shorter or longer stretch into a horizontal direction, along this intermediate zone, and then after a second bend, about at a right angle, enter the other layer. The cause of the difficulty

with which an individual fiber can be followed from one layer to the other in *sections* is now clear from this characteristic of the neuraxons, in the transition zone the fibers usually leave the plane of the section and are thereby withdrawn from further observation. It thus comes about that we frequently see in silver preparations that the majority of the fibers ascending and descending through both layers end in the intermediate zone as though cut off, of which figures 11 and 18 (Plates II and III) furnish a clear illustration.

These observations on the arrangement and course of nerve-fibers are still of a meager nature and require much amplification by means of further investigations. The study of these relations in the cerebellum of selachians is rendered considerably more difficult and complicated through the variations in the combination of the different layers and especially through the entire absence of a granular layer in extensive regions of the cerebellar plate. The selachian cerebellum presents conditions, owing to these peculiarities, very unlike, as far as I know, the cerebella of all other vertebrates.

The Neuroglia.

The neuroglia of the selachian cerebellum is in various respects, both from the morphological and phylogenetic point of view, of especial interest. The supporting substance as a whole undoubtedly stands phylogenetically on a very low scale. Sauerbeck has already succeeded in demonstrating *true ependyma cells* in the cerebellum of *Mustelus*, "*welche der membrana limitans interna ansitzen deren Fortsätze sich bis zur membrana limitans externa verfolgen lassen.*" He conjectured that they form the principal constituent of the whole supporting substance, not having succeeded in demonstrating other elements belonging to the neuroglia type. I can confirm the observation of Sauerbeck's in so far as that the *ependyma cells play a leading role in the constitution of the supporting tissue of the selachian cerebellum*; I have to add, though, that the majority of them are much modified in their morphological appearance, that they retain their connection with the *membrana limitans externa*

only in certain parts of the cerebellar plate and, furthermore, that other neuroglia elements also are present, which are probably not derived from the ependyma cells.

The *most primitive forms of ependyma cells* with an entirely embryonic habit we find along and in the immediate neighborhood of the median line of the cerebellar plate, where the median furrow nearly reaches the surface. Cells of this kind are shown in figure 19 (plate III). We see here one or two processes arise from a round-oval or triangular cell body, which processes may again divide and extend in a fairly straight course to the surface where they lie against the limitans externa with a conical expansion. *These processes are entirely smooth.* Usually a number of processes arising from different cells are united into a thick bundle and form in this way bulky column-like structures which come out very clearly even in simply stained preparations. Not infrequently there arises also from the under pole of the cell body a short frequently branched process. Purkinje cells are not present in this portion of the cerebellar plate.

All other ependyma cells are to be essentially distinguished from those here described, above all because most of them have completely lost their connection with the membrana limitans externa. The few which retain a permanent connection with the surface I have found, in my preparations, almost exclusively in the thinner regions of the cerebellar plate only and especially immediately cephalad of the transition of the latter into the velum medullare posterius. Such an ependyma fiber is shown in figure 20 (plate IV). We see here the fiber arise in the typical way from a pyramidal cell body lying close against the membrana limitans interna, pursue an irregular course through granular and molecular layers and attach itself with a conical expansion to the limitans externa. On its way the fiber gives off several lateral branches which partially fall into numerous terminal twigs.

Those supporting fibers of ependymal origin which have lost their connection with the surface of the cerebellum terminate in various planes of the granular layer or reach the Purkinje cells. These elements are impregnated with extraordinary ease

and usually in great numbers so that often a dense forest of them comes into view. Figure 21 (plate IV) furnishes us an illustration of this. It shows us a closely packed multitude of fibers proceeding from the conical cell bodies on the *membrana limitans interna* and ascending in a very irregular zig-zag course in the granular layer. They are beset with numerous richly branching lateral branches which are closely interwoven with neighboring twigs and thus form an extremely delicate and complicated supporting framework, in whose meshes are found lying the nervous elements of the granular layer. At their free ends also the fibers break up in a similar manner into delicate terminal arborizations. It is striking that the fibers, where they encounter a blood vessel, frequently are closely united to the wall of the same, as illustrated in the upper left corner of figure 21. It thus appears that these fibers here enter into similar intimate relations with the blood vessels as has already been often described regarding the processes of the "astrocytes" in other vertebrates.

Where the granular layer is absent in the cerebellar plate, the ependyma fibers have a somewhat different appearance. They proceed usually more directly, have a smoother surface and give off only scattered branches during their passage through the zone of fibers. Such a fiber is shown in figure 22 (plate IV). Towards the layer of Purkinje cells we see it break up into several slender terminal branches which are distributed between the bodies of the Purkinje cells and usually end with a knob or brush-like enlargement in the vicinity of the molecular layer.

Besides the supporting fibers hitherto described of an undoubtedly ependymal origin, we find a second very characteristic kind of neuroglia elements (fig. 23, plate IV), which are confined exclusively to the molecular layer and in my opinion are to be regarded as the homologues of Bergmann's fibers in the molecular layer of the cerebellum of higher vertebrates. These fibers arise from irregular pear-shaped cell bodies lying between the Purkinje cells and extend in a tolerably direct course to the surface where they are placed against the *limitans externa* with conical enlargements. They are invested along their whole

length with a more or less dense moss-like covering which presents an uncommonly delicate aspect. They people the molecular layer in great numbers and form, when many are impregnated, an almost impenetrable thicket. These elements have in fact a great resemblance to ependyma fibers. One can imagine that these fibers have retained their original connection with the *limitans externa* and that with the progressive thickening of the cerebellar plate the cell body, released from the *limitans interna*, is gradually withdrawn into the interior of the plate. I am more inclined, however, to the view that these fibers are genetically dissimilar to the ependyma cells, that they owe their origin to a part of the derivatives of the "*germinal cells*" and are accordingly to be regarded as secondary supporting elements or as *glia elements* in a narrower sense. The same obtains for the Bergmann's fibers in general. However, as far as I know, a positive proof for this conception is not yet brought forward. Further investigations are necessary here. Should they actually prove to be true *glia elements*, the fibers described would represent, according to my observations at all events, the only ones of their kind in the cerebellum of selachians, inasmuch as I have not succeeded in demonstrating any elements comparable to "*astrocytes*" or "*mossy cells*" in my preparations.

It emerges from the foregoing considerations that the fundamental structure of the cerebellar cortex of the selachians as a whole shows already the typical features of that of the higher vertebrates. A lower stage of development, however, can be established in regard to the individual cellular elements, which is expressed both in the less complexity of the dendrites and axis-cylinder and in the prevailing ependymal character of the supporting structure.

Harvard Medical School, Boston, Mass., Nov. 17, 1897.

LITERATURE LIST.

1. *Dogiel, A. S.* Die Nervenlemente im Kleinhirn der Vögel und Säugetiere. (Arch. mikr. Anat., XLVII, 1896).
2. *Rohon, J. V.* Das Centralorgan des Nervensystems der Selachier. (Denkschr. K. Akad. Wiss., Wien, XXXVIII, 1877).
3. *Sanders, A.* Contributions to the anatomy of the central nervous system in vertebrate animals. (Philos. Trans. R. Soc., London, CLXXVII, 1882).
4. *Sauerbeck, E.* Beiträge zur Kenntniss vom feineren Bau des Selachierhirns. (Anat. Anz., XII, 1896).
5. *Schaper, A.* Zur feineren Anatomie des Kleinhirns der Teleostier. (Anat. Anz., VIII, 1893).
6. *Smirnouw, A. E.* Ueber eine besondere Art von Nervenzellen der Molecularschicht des Kleinhirns bei erwachsenen Säugetieren und beim Menschen. (Anat. Anz., XIII, 1897).
7. *Süsskr, Ph.* Ueber die kleinen Rindenzellen des Kleinhirns des Menschen. (Anat. Anz., XII, 1896).
8. *Viault, F.* Recherches histologiques sur la structure des centres nerveux des Plagiostomes. (Arch. Zool. expériment. et générale, V, 1876).

EXPLANATION OF FIGURES.

All the figures are drawn with a magnification of about 240.

- B. L.*—Basal fiber-layer.
F. L.—Fiber-layer (underneath the Purkinje cells).
G. L.—Granule layer.
L. e.—Limitans externa.
L. i.—Limitans interna.
P. C.—Purkinje cells.
S. m.—Sulcus medianus.

PLATE I.

Fig. 1. Large Purkinje cells from a portion of the cerebellar plate where the granular layer is lacking.

Fig. 2. Small Purkinje cells.

Figs. 3-8. Various forms of nerve cells from the molecular layer.

PLATE II.

Fig. 9. Small granule cells.

Fig. 10. Small granule cells; their axis-cylinders ascending between the fiber-bundles of the granular layer.

Fig. 11. Section through a portion of the cerebellar plate, where Purkinje cells are lacking. Compactly arranged ascending axis-cylinders from small granule cells in the molecular layer. In the granular layer a number of cells, of which the under ones send their axis-cylinders sidewise; besides these, numer-

ous axis-cylinders which partly belong to the granule cells (very delicate), partly to the Purkinje cells and are partly derived from cells lying outside the cerebellum.

Fig. 12. Axis-cylinders of small granule cells proceeding horizontally and their terminal arborizations in the molecular layer. Two T-shaped divisions of the ascending main fibers.

Figs. 13-14. Large granule cells.

PLATE III.

Fig. 15. Large granule cell.

Fig. 16. Ascending nerve fiber of extraneous origin and its terminal arborization in the molecular layer.

Fig. 17. Irregular arrangement of the nerve fibers (neurites of the Purkinje cells and "ascending fibers") in the granular layer.

Fig. 18. Arrangement of the nerve fibers transversing the granular layer in distinct buudles which go over into a basal fiber-layer.

Fig. 19. Ependyma cells from the neighborhood of the median furrow of the cerebellar plate.

PLATE IV.

Fig. 20. Modified ependyma fiber traversing the whole thickness of the cerebellar plate.

Fig. 21. Modified ependyma fibers in the granular layer.

Fig. 22. Modified ependyma fiber from a region of the cerebellar plate where the granular layer is missing. The slender terminal arborizations of the fiber penetrate between the Purkinje cells.

Fig. 23. Neuroglia cells of the molecular layer (Bergmann's fibers).

Fig. 1

Fig. 2

Fig. 3