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The Development of the Cerebellum of Vertebrates in Relation to the Control of Movement

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Introduction

The cerebellum is a highly specialized component of the central nervous system of vertebrates. It does not occur in invertebrates, and can first be recognized in the simplest vertebrates such as the lamprey. In Fig. 1 the brains of a selection of mammals are shown on the same scale. It can be seen that the cerebellum is a quite distinctive structure of the brain, and that in evolution it has prospered. Fig. 1 is only a partial illustration of the evolutionary story suggesting, as it does, a progressive development; whereas a survey of sub-mammalian vertebrates [14, 23] reveals that for special conditions of life there are amazing hypertrophies, while in other species there are regressions. It is remarkable that quite soon after the evolutionary beginning there arose the highly developed cerebellum of the selachian fish. In electric fish the cerebellum is so hypertrophied that it may account for up to 70% of the brain weight. On the other hand in anura the cerebellum has shrunken to only half a folium.

Can we utilize these extreme divergences in deriving some concept of the function of this organ, and so of its significance, high or low, in the life of these various vertebrates?

When we come to consider the evolutionary origin of the cerebellum, we must take account of its connections in primitive forms. These are largely from the lateral line organs and the vestibular mechanism, but also from the body in general. Evidently it is utilized in computing complex information about the relationship of the fish to the external world, as may be illustrated by the quotation from HOUSER [12] on the selachian, Mustelus, the dogfish, which is "a restless hunter of the seas, ever urged onward by an appetite which, apparently, has no bounds. Continually suspended in a fluid medium, and compelled to balance itself at every turn, the animal requires a precise mechanism of equilibration. This is to be found, in the main, in both an ear (vestibular organ) and a cerebellum developed to a degree out of all proportion to the scale occupied by the creature as a whole."

But by far the most remarkable hypertrophy occurs in electric fishes such as mormyrids, where there is an enormous development of electrical detecting organs in the lateral line system, particularly in the ampullae of Lorenzini. Evidently extremely complex computations are required in the handling of data derived by this organ and in utilizing it to evoke appropriate responses of the animal to its external environment, as signalled for example by distortion in the electric field produced by the animal's own electric discharges. Fish that do not generate electric discharges, nevertheless possess very sensitive electrical detectors in their ampullae of Lorenzini, and apparently utilize information so obtained in computing data derived from weak electric outputs by their prey, even from their muscle contractions [4]; hence the hypertrophied cerebellum.

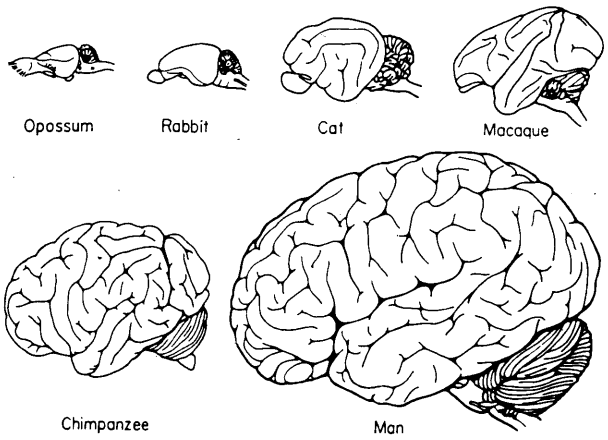

Fig. 1. Drawings on the same scale of the brains of a series of mammals. (Figure kindly provided by Prof. J. JANSEN)

The uniqueness of the cerebellum as a component of the brain is illustrated in Fig. 2, which is an assemblage of cerebella as seen from the dorsal surface. Fig. 2*A*—*G* are arranged in evolutionary sequence from the lamprey through to the bird. It will be appreciated that the existing lower forms are much modified from the primordial ancestors of higher forms. The selachian cerebellum (*B*) is relatively much larger than for standard teleosts (*C*). In electric fish the development reaches fantastic proportions, all of the brain seen from the dorsum in *D* being a special part of the cerebellum. By contrast the amphibian cerebellum is very undeveloped, being in the frog only half of a folium (*E*), and even in the reptile (*F*) it is less developed than in fish. However, there is a foliated development in the bird (*G*), and the tremendous

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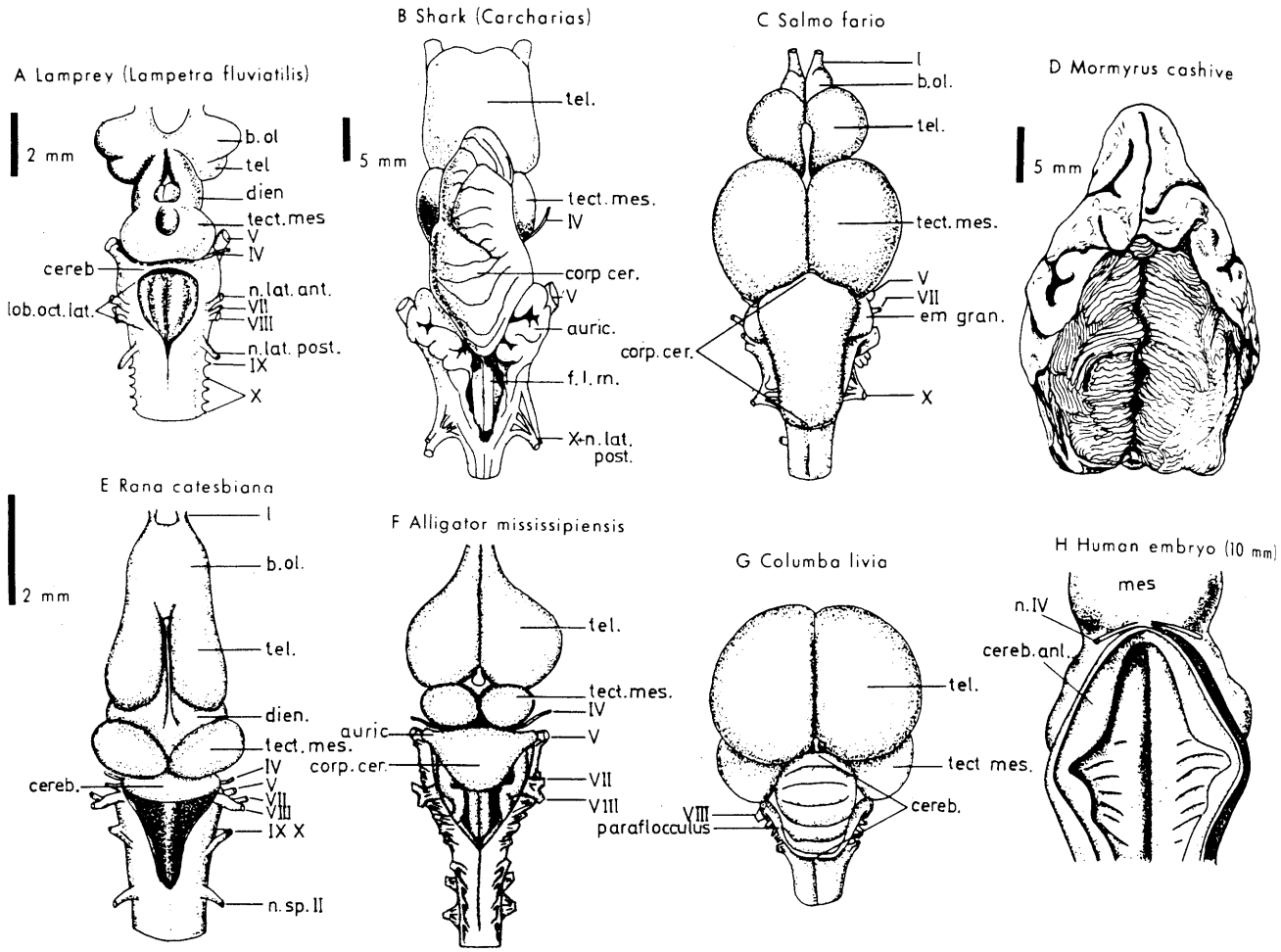

Fig. 2. Drawings of a variety of cerebella as seen from the dorsal surface. As indicated by the length scales, the drawings are not made on the same scale. *tel* telencephalon; *tect. mes.* tectum mesencephali; *cereb.* cerebellum; *lob. oct. lat.* lobus-octavo-lateralis; *corp. cer.* corpus cerebelli; *auric.* auriculae; *em. gran.* eminentia granularis; *dien.* diencephalon; *b. ol.* bulbus olfactorius; *tr. ol.* tractus olfactorius; *mes.* mesencephalon; *cereb. anl.* cerebellar anlage; cranial nerves are indicated by Roman numerals (assembled from [23])

mammalian development has already been illustrated (Fig. 1). In neurogenesis all of this wide variety of structure grows from the same small area of the dorsal lip of the rhombencephalon, where the cerebellar rudiment first can be recognized in a primitive vertebrate such as the lamprey, as may be seen by comparison of the cerebellar anlage in the 10 mm human embryo (*H*) with the cerebellum of an adult lamprey (*A*). Yet the anlage in *H* is destined to grow into the immense structure of the human cerebellum, the greater part of which is related to the cerebral hemispheres that came relatively late in development.

It is now recognized that the cerebellum functions as a very special type of neuronal machine, as witness a recent book entitled "The cerebellum as a neuronal machine" [5]. Moreover we shall see that there are certain basic neuronal elements common to all cerebella, and also certain basic patterns of neuronal connections. Evidently this "neuronal machine" gives the vertebrate brain a component of great value as a computer in processing the immense flow of data derived not only from its sense organs but also from its own internal activities. Furthermore, apparently it was not feasible in evolution to develop an equivalent neuronal machine in the neuraxis where the higher levels of the nervous system were being formed. These higher levels had to call forth the growth of immense and involved pathways to and from the greatly hypertrophied cerebellum budding out from the anlage in the dorsal lip of the rhombencephalon. As suggested by Fig. 2, an excellent example of ontogeny recapitulating phylogeny is provided by cerebellar neurogenesis.

I will give an account of cerebella of dogfish, lizards and cats, forms which are chosen both because they have been most thoroughly studied and because they illustrate a wide range of evolution. I am indebted to Doctors KITAI, SHIMONO and KENNEDY for allowing me to utilize their recent experimental work on the lizard cerebellum. The experimental investigations on the dogfish cerebellum were carried out at the Marine Biological Station, Woods Hole, in the Summer of 1968 by my colleagues, Doctors TÁBOŘÍKOVÁ, TSUKAHARA, and myself. Reference may be made to a recent book [5] for an account of the structure and function of the cat cerebellum.

It is desirable to define some of the scientific terms that will be used extensively throughout the more detailed part of this lecture. The nervous system is composed of enormous numbers of *nerve cells* or *neurones*, that can be sharply classified into various types. The enlarged nucleus containing part is called the *body* or *soma*, and from it extend branches *(the dendrites)* that serve for reception of signals from other

nerve cells, and a single branch *(the axon)* that transmits signals to other nerve cells. These signals or *nerve impulses* are brief all-or-nothing waves of potential change that travel along the surfaces of nerve fibers, which are the axons of nerve cells. As defined by the "neurone theory" nerve cells communicate with one another not by continuity but by contiguity at highly specialized regions of close contact called *synapses*. For our present purposes we can consider synapses as being of two types: the *excitatory synapses* that excite the recipient nerve cell so that, if there is summation of a sufficient number of such synaptic excitations, the nerve cell will fire an impulse down its axon and so in turn activate the synapses on other nerve cells; and the *inhibitory synapses* that counteract the excitatory synapses and prevent them from generating the discharge of impulses. There is no need to give any further account of these two quite distinct types of synapse, beyond stating that, in the cerebellum, synaptic transmission is effected by chemical substances which are quite different for excitatory and inhibitory synapses and that nerve cells are either excitatory or inhibitory, by which I mean that the axon of a nerve cell forms either excitatory or inhibitory synapses, and is never, ambivalent in its synaptic action.

Neural Structure of the Cerebellum

I will now give a brief account of the structure of the cortex of a highly developed mammalian cerebellum, which is diagrammatically shown in relation to a perspective drawing of a section of a single folium (Fig. 3; cf. [5, 11]). Many of the structural features of this diagram closely resemble those of the simplest cerebella that have been investigated. The large *Purkyně cells* (*PC*) with their enormously elaborated "antlered" dendrites are shown, one in its full array of transverse distribution (about 200 μ) and one, to the extreme right, in the very flattened configuration (about 8 μ) orthogonal thereto. This "espalier" configuration is typical of all Purkyně cells. Other Purkyně cells in Fig. 3 are merely shown in outline of their cell bodies, which form the boundary layer between the superficial molecular layer and the deeper granular layer. The Purkyně axons (*PA*) provide the only efferent path from the cerebellar cortex, and these axons end as inhibitory synapses on neurones of the deep cerebellar nuclei, and other nuclei of the brain stem [5].

There are two pathways into the cerebellar cortex: by *climbing fibers* (*CF*) and by *mossy fibers* (*MF*). Each climbing fiber makes a very extensive excitatory synaptic contact, in reality about 2000 synapses, with a single Purkyně cell, and also has a few other branches. In contrast to the unitary action of the climbing fiber, each mossy fiber branches profusely in the granular layer to make synaptic contact with some hundreds of *granule cells* (*gr*) on each of which four or five mossy fibers converge to make excitatory synaptic contact. These granule cells have characteristic claw-like dendrites, one for each convergent mossy fiber, and send up axons to the molecular layer where they bifurcate to form the parallel fibers (*pf*) that run for 2 to 3 mm along the folium, and so traverse orthogonally the "espalier" dendritic trees of the Purkyně cells. The parallel fibers make excitatory synaptic contact with spines on the dendrites of each espalier. Here is an excellent design for distributing a given mossy fiber input to some hundreds of Purkyně cells. On each of these cells as many as 200,000 spine synapses are formed by the parallel fibers. Evidently there are tremendous possibilities for summation of synaptic excitations.

The three other main cell types of the cerebellar cortex are also shown in Fig. 3: *Golgi cells* (*Go*), *basket cells* (*Ba*), and *stellate cells* (*St*). All have their main dendritic trees in the molecular layer and likewise are excited by the parallel fiber synapses on their dendritic spines, and all have been shown to be inhibitory

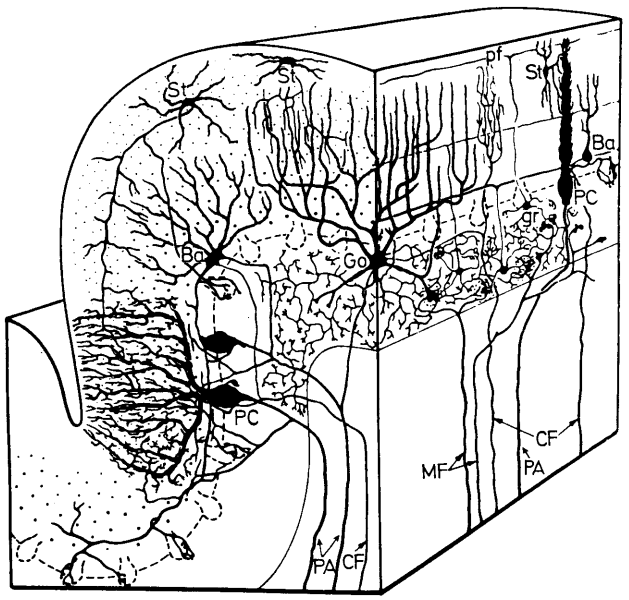

Fig. 3. Perspective drawing of a section of a folium of a mammalian cerebellum. Full description in text. Only one of the three granule cells is labelled gr. (from [5])

(Fig. 4). The Golgi cells inhibit the mossy fiber-granule cell synapses, thereby forming a negative feed-back control on the mossy fiber input. The basket and stellate cells inhibit Purkyně cells. The former have an action by synapses in a basket-like arrangement around the Purkyně somata, and have a directional action up to 1 mm transversely across the folium, as is indicated for one Ba cell. The stellate cells tend to have a much more restricted distribution.

The essential features of the synaptic connections are depicted in the longitudinal section (Fig. 4), which shows the way in which mossy fibers are related to granule cells, one such fiber giving excitatory synapses to many granule cells, and several fibers converging on one granule cell. Furthermore it shows that the axons of the granule cells, the parallel fibers, give excitatory synapses to Golgi cells that feed back inhibition to the mossy fiber-granule cell synapses. The parallel fibers excite the Purkyně cells, the stellate cells and the basket cells by means of spine synapses. Finally these two latter cells inhibit Purkyně cells. As shown in Fig. 3 this inhibition is effected for as far as 1 mm transversely from any parallel fiber "beam" of impulse that excites basket cells. Thus a parallel fiber beam excites Purkyně cells on-beam and inhibits those on either side, giving the

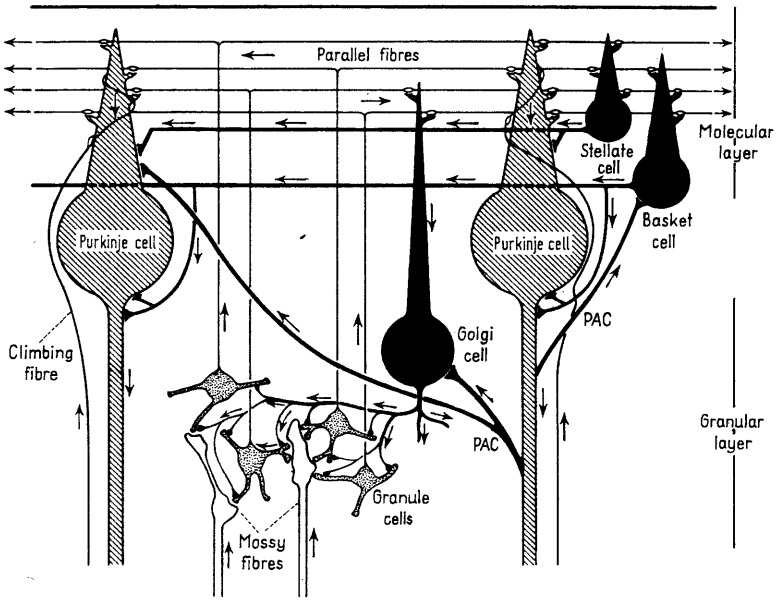

Fig. 4. Cat cerebellum. — Diagram showing the principal neuronal connections occurring in the neuronal machinery of the cerebellar cortex. The Purkyně, stellate, basket and Golgi cells are inhibitory in function and are shown by convention in black. The diagram is drawn for a section along the folium, so as to show the parallel fibers to advantage. Arrows show directions of impulse propagation. Purkyně axon collaterals are labelled PAC (from [5])

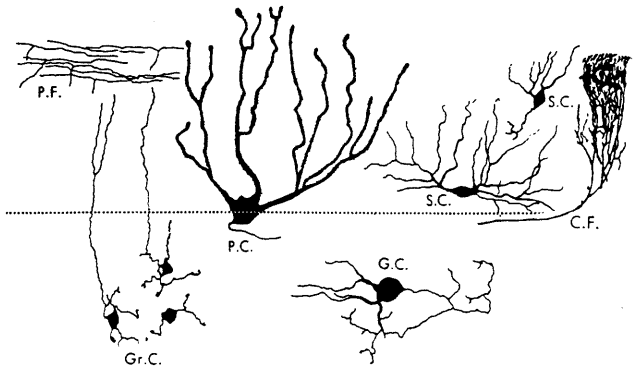

Fig. 5. Neural elements of the dogfish cerebellum. These drawings of Golgi preparations are reproduced from figures prepared by SCHAPER (1898 [25]), by HOUSER (1901 [12]) and by ARIENS KAPPERS, HUBER, and CROSBY (1936 [1]). The broken line represents the plane of the Purkyně cell bodies and separates the molecular layer from the granular layer. Full description in text

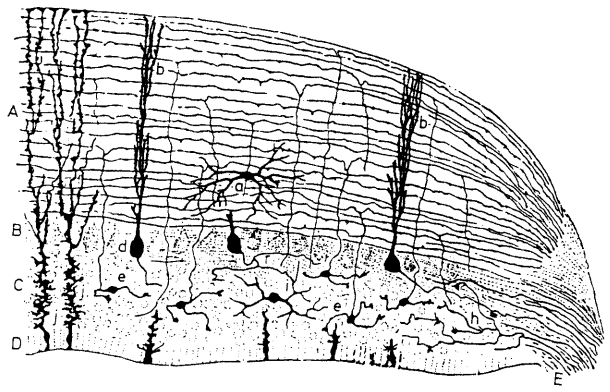

Fig. 6. Drawing of a longitudinal section along the transverse folium of a lizard cerebellum. *A*, *B*, *C* and *D* show respectively the molecular, Purkyně, granular and ependymal layers and *E* is the peduncle. Glia cells are shown in *j*. Full description in text [24]

so-called lateral inhibition. There are two additional features of design shown in Fig. 4 as well as in Fig. 3. One is the climbing fiber innervation of Purkyně cells and the other is the Purkyně axon collaterals (*PAC*) that feedback inhibition to basket cells, Golgi cells and Purkyně cells (cf. Fig. 3). Thus Figs. 3 and 4 together display the ultimate achievement of the evolutionary process in perfecting the design of the neuronal machinery of the cerebellar cortex. The neuronal machinery of more primitive cortices can now be compared in order to appreciate the changes wrought by evolution.

Fig. 5 reproduces Golgi preparations from SCHAPER (1898 [25]), HOUSER (1901 [12]) and ARIENS KAPPERS, HUBER and CROSBY (1936 [1]) of the characteristic cellular elements of the cerebellar cortex of the dogfish. In many respects there is a close resemblance to the mammalian cerebellum. For example the Purkyně cells (*PC*) have large branched dendrites covered in spines and are arranged espalier-wise in the transfolial plane, though the branching is notably less profuse. The granule cells (*Gr. C.*) with their few claw-like dendrites have a very close resemblance to their mammalian counterparts (Figs. 3, 4) and their axons ascend to the molecular layer, where by the characteristic T-shaped bifurcation they form the parallel fibers (*P. F.*) that run along the folium for an unknown distance, being orthogonal to the plane of branching of the Purkyně dendrites, just as in mammals. The stellate cells (*S. C.*) of the molecular layer also resemble the less well developed variety of stellate cells of the mammalian cerebellar cortex (Fig. 3). The absence of basket cells provides a remarkable distinguishing feature, as also is the absence of cells resembling the mammalian Golgi cells with their extensive dendritic branches in the molecular layer. The so-called Golgi cells in the granule layer (*G. C.*) have relatively stunted dendrites restricted to the granular layer. In addition to these four types of neurones and their axonal branches, a great wealth of afferent fibers enters the cerebellar cortex; nevertheless there is only a meager account of primitive climbing fibers (*C. F.* in Fig. 5) twining loosely around the Purkyně dendrites [1], and there has been no study of the numerous medullated fibers that terminate in the granular cords and that presumably correspond to the mossy fibers of higher vertebrates. A notable difference is the absence of Purkyně axon collaterals in the dogfish.

In Fig. 6 there is a drawing of the cerebellum of a longitudinal section along the folium of a lizard cerebellum [24]. Again it will be seen that there are the characteristic granule cells (*e*) with their few claw-like dendrites and their axons ascending to the molecular layer, where they bifurcate to form the parallel fibers running along the folium. As with the other cerebella (Figs. 3, 5) the Purkyně dendrites (*b*) branch profusely in espalier-form, and in transverse orientation to the long axis of the folium, being thus seen edge-on in Fig. 6. Again, as in other cerebella, the parallel fibers run orthogonally to the dendritic tree, so having maximum opportunity to form spine

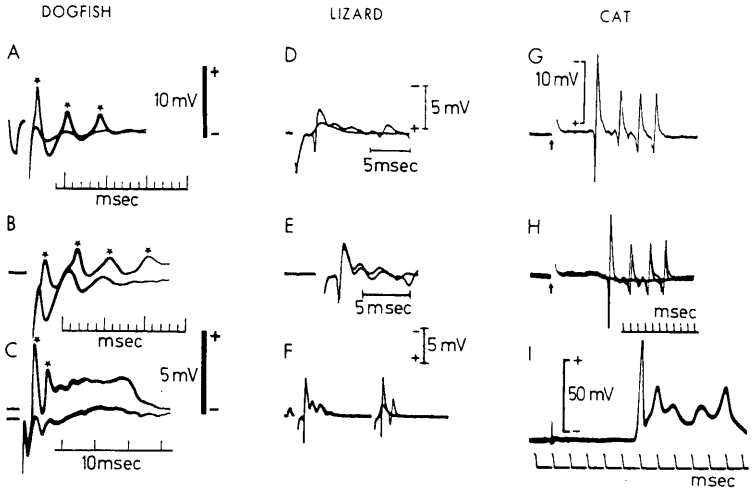

Fig. 7. Responses of Purkyně cells evoked by climbing fiber impulses. In *A*—*C* are intracellular records from a dogfish Purkyně cell showing the all-or-nothing character of the climbing fiber response when the stimulus was just straddling threshold for that fiber. *B* and *C* are recorded from another cell. In each case the spike response (indicated by stars) appears as an addition to the background potential generated by climbing fiber excitation of neighboring Purkyně cells [7, 8]. In *D* a similar response, but extracellularly, is evoked in a lizard Purkyně cell by a stimulus straddling threshold, and in *E* by stimuli above threshold, there being two superimposed traces. *F* is from the same cell as *E*, and shows the all-or-nothing character of the response to the second stimulus [13]. *G* and *H* are extracellular recordings from the same cat Purkyně cell with the stimulus to the inferir olive, *H* being formed by superposition of 4 traces, only 2 evoking the *CF* response. In *I* is an intracellular *CF* response from another Purkyně cell [5]

synapses. The axons of the Purkyně cells (*d*) travers out to the peduncle, and apparently one collateral is shown (*r*). In the molecular layer there is one large stellate cell (*a*) which resembles the stellate cells of the dogfish and the mammal, and presumably represents the cell type responsible for the observed inhibitory action upon the Purkyně cells. Mossy fibers (*h*) are shown entering from the peduncle and having the characteristic thickenings that are observed in the mammal (cf. Fig. 3). Climbing fibers have been recognized in the lizard, but are not shown in the Fig. 6. The so-called stellate cell (*i*) in the granular layer appears to correspond to the Golgi cell in Fig. 5.

In summary of the histological features disclosed in Figs. 3, 5 and 6, it can be stated that in all cerebella the Purkyně cells are outstanding because of their great branching dendrites arranged in espalier fashion orthogonal to the densely packed parallel fibers that synapse on the dendritic spines. The Purkyně cells provide the only pathway out from cerebellar cortices of all types. There is remarkable similarity in the mossy fiber, granule cell, parallel fiber structures, and the climbing fibers also are comparable with the mammal, in so far as they have been investigated in lower forms. The outstanding deficiency in the primitive cerebella lies in the absence of basket cells, in the poorly developed Golgi cell mechanism and finally in the deficiency of axon collaterals from the Purkyně cells.

The Climbing Fiber Mechanism

In Fig. 7 there are arranged the responses evoked in single Purkyně cells by climbing fiber inputs in the dogfish, the lizard and the mammal. In every case threshold discrimination shows that a single climbing fiber exerts such a powerful excitatory action that the Purkyně cell generates three or four spike potentials. These spikes are seen in recording from dogfish (*A*—*C*) and lizard (*D*—*F*) Purkyně cells. Fig. 7*G*, *H* show the repetitive spike potentials that a climbing fiber impulse evokes in a cat Purkyně cell, and I gives a good example of an intracellular record. In general it will be seen that the responses evoked in these three classes of animals are remarkably similar, all showing a repetitive spike discharge at high frequency, about 250/sec in the dogfish and 500/sec in the lizard and cat. The frequency in the dogfish corresponds to that in the cat when allowance is made for the cooler temperature — about 24 °C. Evidently, in the evolutionary story, the powerful unitary excitatory action of the climbing fiber was developed at the beginning and was very little changed thereafter. When trying to discover the reason for this unitary synaptic mechanism of such extraordinary power, it is important to recognize that it must play a key role in cerebellar function otherwise it would not have been preserved throughout all the exigencies of evolutionary remodelling.

Additional investigations on the climbing fiber responses of these three classes of animals reveal further similarities, e. g. the reduction in number of spike responses when the Purkyně cell is inhibited, and with repetitive climbing fiber stimulation. In both the dogfish [7, 8] and the cat [2, 9] it has been shown by axon-reflex tests that there is a limited branching of climbing fiber axons deep in the white matter. Apparently this is another feature in design that has been preserved in evolution. Climbing fiber responses have also been observed in the frog [3, 15, 19, 21].

The Mossy Fiber Mechanism

The mossy fiber input displays a remarkable difference from the climbing fiber input both in its structural features and in its functional performance. In Fig. 8*A* there is a diagram showing that in the dogfish the mossy fibers make synapses with the granule cells concentrated in a parasagittal cord that extends anterior-posteriorly throughout the whole cerebellum. The remainder of the cerebellar cortex has no deeplying granule cells such as occurs for the whole cerebellar cortex in the higher levels of vertebrates (cf. Fig. 3). This arrangement in the dogfish makes it particularly easy to investigate the granule cell responses by microelectrode recording.

In Fig. 8*B*—*F* there are arranged responses recorded from the dogfish granule cell cord as an Fig. 8*A*. In *B* and *C* there is an initial diphasic spike due to the incoming mossy fiber volley followed by a slow negative potential that is due to the excitatory synaptic action of this volley on granule cells. In *C* a stronger stimulation evokes a larger synaptic action with spike discharges of granule cells superimposed. *D* shows in another prepara-

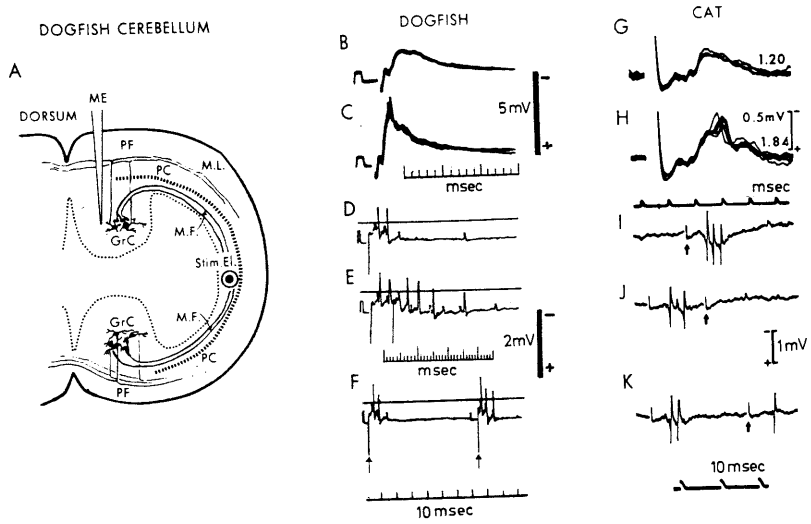

Fig. 8. Parallel fiber volleys and granule cells. — *A* Drawing of a transverse section of a dogfish cerebellum showing synapses of mossy fibers (*MF*) upon granule cells (*GrC*). The concentric stimulating electrode is shown in section (*Stim. El.*), and also the recording microelectrode *ME* in the granule cell cord. *B* and *C* are the extracellular potentials evoked by a small and a larger mossy fiber volley. *D*, *E* and *F* are from another experiment in which the spike responses of two granule cells can be recognized, *D* being evoked by a single mossy fiber stimulus and *E* and *F* by two stimuli at intervals of 7 and 70 msec respectively [8]. *G*—*K* are extracellular potentials evoked by a mossy fiber volley in cat granule cells, *G* and *H* by small and larger single volleys. *I*, *J* and *K* were similarly recorded in another preparation, *I* being in response to a single, *J* and *K* to a double mossy fiber volley [5]

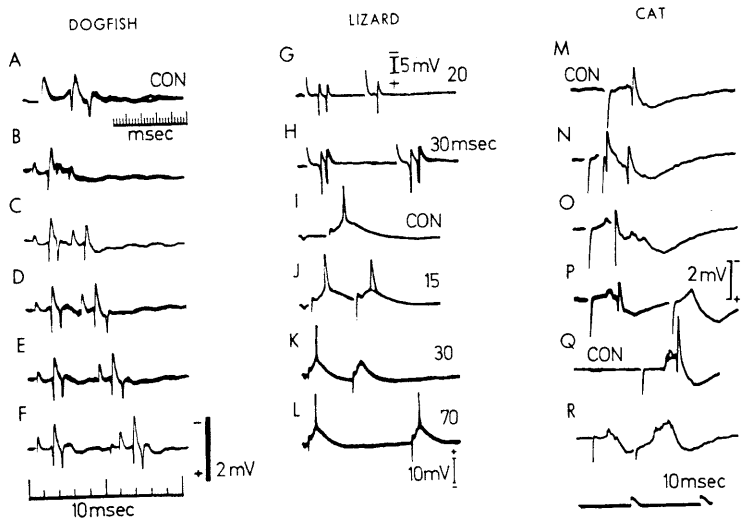

Fig. 9. Effect of parallel fiber volleys in evoking impulse discharges in Purkyně cells. All responses are recorded extracellularly except *I* to *L*. *A*—*F* Responses of a single Purkyně cell of dogfish are recorded as brief downward (positive) spikes, marked by dots. Responses of other Purkyně cells give the slower negative spikes [7]. *G*—*L* Spike responses of single Purkyně cell of lizard. In *G*, *H* unitary response is diphasic (positive-negative), and in *I*—*L* from another cell it is intracellular. Stimulus intervals in milliseconds [13]. *M*—*R* Responses of single Purkyně cell of cat appear as diphasic (positive-negative) spikes. *M* and *Q* being responses to a single volley, and *N*—*P* to a double volley, while in *R* the conditioning and testing volleys were set up by different electrodes [5]

tion the spike responses of two granule cells to such a mossy fiber input, there being two discharges of the larger unit and four of the smaller. Finally, in *E* and *F* are the responses to two volleys showing summation to produce an increased response in *E*, and even in *F* there is a larger response at a test interval of 70 msec. Unfortunately there are no observations on the granule cells of the lizard cerebellum, but Figs. 8*G* to *K* show comparable observations on the granule cells of the cat. *G* is comparable with *B* in showing the initial mossy fiber volley and the later synaptic potential of the granule cells, while *H* is comparable with *C* in showing spike potentials of a granule cell superimposed upon the stronger synaptic stimulation. *I*, *J* and *K* show test series resembling *D*, *E* and *F*, but, in contrast to the facilitation of *E* and *F*, there is inhibition in *J* and *K*, the control triple response in *I* being completely suppressed in *J* and reduced to a single spike in *K*.

Systematic investigations have indicated that the inhibition of the mossy fiber to granule cell synapses in the cat is due to the feedback inhibitory action of Golgi cells (cf. Figs. 3 and 4 [5]). We have been unsuccessful in detecting even a trace of inhibition of the dogfish granule cells [8]. The so-called Golgi cells in the granular layer (Fig. 5) differ from those in the cat (Figs. 3, 4) in that their dendrites do not extend up to the molecular layer. Moreover they may also differ in their axonal distribution, which is as yet undefined. Evidently further investigation is required before they can be assumed to be homologous with mammalian Golgi cells.

Even in the rudimentary cerebellum of the lamprey there are granule cells resembling those of higher vertebrates with their few claw-like dendrites and their axon bifurcation to form parallel fibers. Apparently this design ensures that there is a limited amount of convergence of mossy fibers [27], and such convergence results in a discharge of granule cells (cf. Fig. 8*C*, *H*), which may be repetitive when there is strong convergence (cf. Fig. 8*D*, *I*). Such limited summation of input signals gives the advantage of more selectivity of response to the mossy fiber input lines (cf. [20]), and this advantage may account for the preservation of this design feature from the most primitive to the most developed cerebella.

Excitation of Purkyně Cells

A parallel fiber volley excites the discharge of impulses from Purkyně cells by means of the extremely numerous synapses on the dendritic spines. In the dogfish this discharge may be double (Fig. 9*A*) or even triple at a fairly high frequency. Similarly in the lizard the parallel fiber volley may evoke a double discharge (Fig. 9*G*). In the anesthetized cat cerebellum the

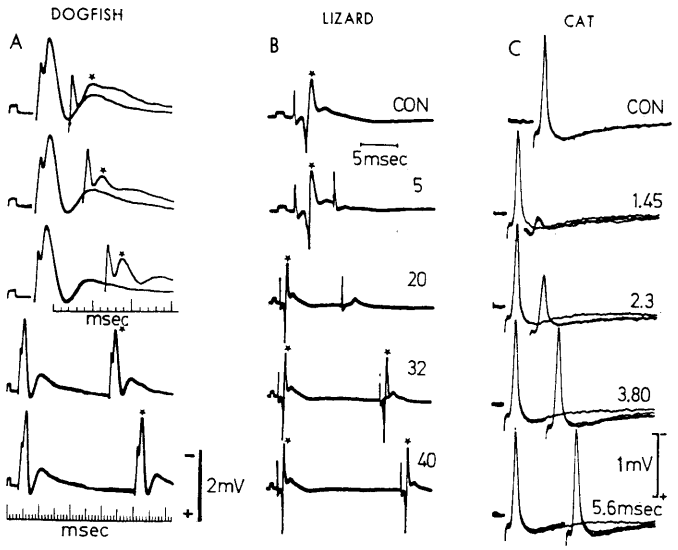

Fig. 10. Responses of Purkyně cells to single and double antidromic volleys. *A*, *B* and *C* show responses recorded extracellularly from dogfish, lizard and cat Purkyně cells respectively. At the three briefest intervals in *A* (4, 6, 9 msec) there is superposition of the double upon the conditioning response alone. The initial spike is due to the nerve volley, and the antidromic Purkyně spikes are indicated by stars. Note that the two lowest records (29 and 39 msec) are at a slower sweep speed [7]. In *B* a single Purkyně cell gives a diphasic (positive-negative) spike potential (stars), and the stimulus intervals are given in milliseconds [13]. In *C* the large negative spike is generated by a population of Purkyně cells and the stimulus interval is also given in msec [5]

discharge is usually single (Fig. 9*M*). Thus there is a general similarity in respect of the Purkyně cell discharge of impulses evoked by the parallel fiber volley. The responses to double parallel fiber stimulation are also shown in Fig. 9. In the series for the dogfish cerebellum (*B*—*F*), there is a depressed response at briefer intervals with either failure at 15 msec (*B*) or reduction to one discharge at 26 msec (*C*). At longer intervals there is recovery, and at 40 msec (*E*) the second response is double with a discharge interval comparable with the control, so recovery is then complete. In the lizard (Fig. 9*G*) the double discharge is reduced to a single at 20 msec test interval. By 30 msec (*H*) it is double, though at a lengthened interval, and by 50 msec recovery is complete (not illustrated). In another lizard Purkyně cell (*I*—*L*) a parallel fiber volley elicits only a single spike discharge (*I*). A second stimulus is effective at brief intervals (6—15 msec) (*J*), fails at 20 to 40 msec (*K*), and is fully effective at 70 msec (*L*). Fig. 9*M* shows that in the cat the parallel fiber volley evokes a Purkyně cell response resembling that in the lizard in not being depressed at a very brief test interval (*N*), then being depressed at intervals up to 11.8 msec (*O*, *P*). Recovery is complete after about 50 msec in this experimental series (not illustrated).

In general Fig. 9 illustrates the similarity of the parallel fiber Purkyně cell synapses in these three test species. Parallel fiber impulses have also been shown to evoke spike discharges in Purkyně cells in frogs [16, 21] in alligators [17, 18] and in various elasmobranchs [22].

Since in Fig. 9*A*—*P* the conditioning parallel fiber volley evoked the discharge of impulses from Purkyně cells, it is possible that the depression of the testing response arises as the aftermath of this discharge and is not indicative of an inhibitory synaptic action. Fig. 9*Q*, *R* shows that a cat Purkyně cell is depressed by a conditioning parallel fiber volley that did not discharge an impulse, so it is a genuine inhibition. Such a test has also been made with the lizard cerebellum.

In order to test the depressant action of a discharge, double test stimuli have been applied to the Purkyně cell axons and recording has been made of the field potential generated by the antidromic invasion of the Purkyně cells. In Fig. 10*A* there is a large depression of the antidromic spike response. At an interval of 4 msec it is almost completely suppressed and test intervals of 6, 9 and 29 msec show a progressive recovery, which is complete at 39 msec. With the lizard cerebellum (*B*) there is similarly a depression at the shortest test intervals and recovery is partial at 32 msec and complete at 40 msec. It should be noted that this test is done with the unitary response of a single Purkyně cell, in contrast to the population response of Fig. 10*A*. With the cat there is a much more rapid recovery, as illustrated in Fig. 10*C*, where already the second response evokes a smaller spike at 1.45 msec while at 2.3 there is already good recovery. At 5.6 msec recovery is complete and even at 3.8 msec it is almost complete. Certainly with the cat it is clear that the slow recovery following a conditioning parallel fiber volley in Fig. 9*M*—*P* is not attributable to refractoriness but to some inhibitory mechanism, as has been demonstrated in Fig. 9*Q*, *R*. In the next section it will be shown that in the dogfish and lizard a parallel fiber volley also induces a long lasting inhibition of Purkyně cells. The spike responses evoked in Purkyně cells by an antidromic invasion are similar for the three classes of animals studied, but in the cat there is a much more rapid recovery of excitability (by about 5 msec) than in the dogfish and lizard, where it takes about 40 msec. Such tests have not been performed in any other species.

Inhibition of Purkyně Cells

In order to search for the possible inhibitory function of the stellate cells in the dogfish (Fig. 5), we have tested the antidromic invasion of Purkyně cells at various intervals after a conditioning parallel fiber volley that it is hoped will excite the stellate cells [6, 7]. The specimen records of Fig. 11*A* show that the conditioning parallel fiber volley evokes a large spike response from the Purkyně cells, and, as would be expected from Fig. 10*A*, there is a depression immediately after this Purkyně spike potential with recovery therefrom complete at about 40 msec test interval. However, from about 70 msec onwards there is a later depression of the testing antidromic spike potential that continues for up to 500 msec. Evidently

there are several interacting influences upon the Purkyně cell. Firstly, there is the depression for about 40 msec that follows the spike response initiated by a conditioning parallel fiber volley. Then there is the later inhibition unrelated to the initial depression and separated from it by a phase of complete recovery. However, further tests reveal that at the longer testing intervals there are really two opposing synaptic influences, excitation by the parallel fiber volley just counteracting the inhibition at intervals of 50—70 msec with thereafter a progressive dominance of the inhibition [7].

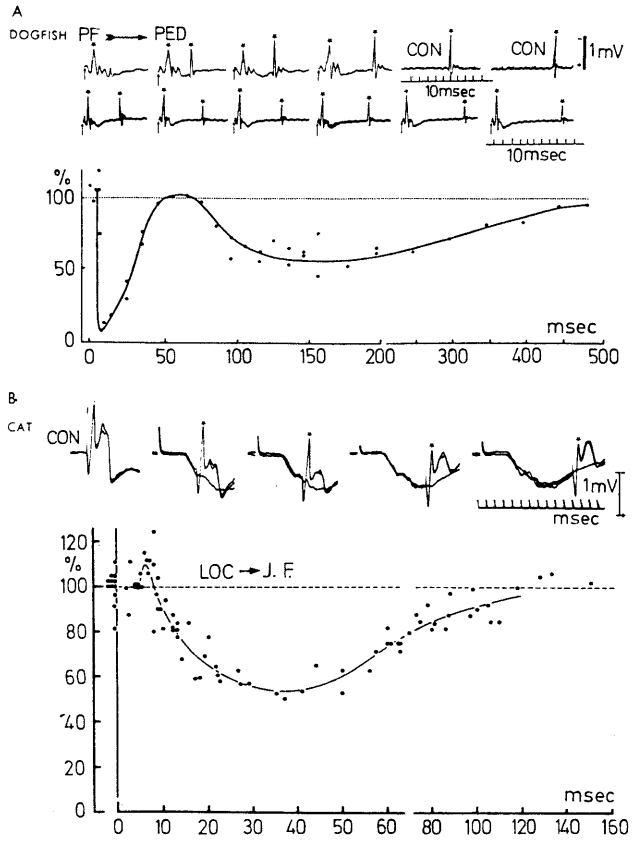

Fig. 11. Inhibition of antidromic evoked responses of Purkyně cells. *A* Dogfish. The specimen records show at the arrow the time of the stimulus to the parallel fibers. There are two controls (*CON*) of the antidromic test spike of the Purkyně cells, one at the faster speed for the four upper test responses, the other at the slower speed of the six lower test responses. Note the large Purkyně spike potential evoked by the conditioning parallel fiber volley (stars superimposed). The plotted points are the means of two or three observations, and are calculated as percentages of the mean control responses. Note the change in the time scale beyond 200 msec [7]. *B* Cat. Specimen records as in *A*, but conditioning parallel fiber volley does not evoke a Purkyně spike potential. The antidromic spike potentials are indicated by the superimposed stars. The plotted curve is constructed similarly to that of *A*. Note the change in time scale beyond 60 msec [5]

In Fig. 11 *B* the effect of a conditioning parallel fiber volley was similarly tested in the cat. The antidromic invasion is greatly depressed at intervals beyond 10 msec or so and recovery is not complete until about 120 msec test interval. Since the conditioning volley evokes no Purkyně spike potential, there is no initial depression as in Fig. 11 *A*, but the conflict between synaptic excitation and inhibition is well illustrated by the initial brief facilitation and the slowly developing inhibition. If allowance is made for the temperature difference of about 14 °C (24 and 38 °C) between the dogfish and the cat, which would effectively produce a threefold lengthening, then the inhibitory curves for dogfish and cat are seen to be comparable.

It has not been possible to perform similar experiments with the lizard [13] because the antidromic invasion was so strong that it was not depressed by a parallel fiber volley at any interval beyond that attributable to the aftermath of the spike potential evoked by the conditioning volley (Fig. 10 *B*). It may be noted in parenthesis that there were similar findings in some dogfish experiments [7]. However, in the lizard advantage has been taken of the less effective excitation

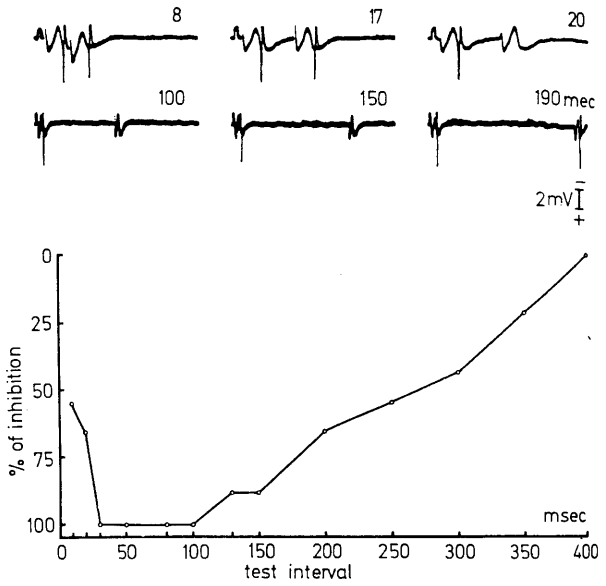

Fig. 12. Inhibition of Purkyně cells of lizard. In the specimen records there are two identical parallel fiber volleys at the intervals indicated in milliseconds, and the spike responses of a single Purkyně cell are recorded extracellularly. At each test interval there were two superimposed traces. Note the partial failure at the 8 and 17 msec tests, and the complete failure at 20 to 150 msec tests. In the plotted points the mean percentages of unitary responses are plotted against the stimulus intervals [13]

that parallel fiber impulses exert upon the Purkyně cells. In Fig. 12 the specimen records show a depression of the response evoked by the testing parallel fiber volley at intervals from 20 to 160 msec. This is a unitary testing procedure and therefore does not give the full range of the depression. In Fig. 12 the results of many such units are pooled in the plotted curve and this shows a time course of inhibition resembling that of Fig. 11 *A*, recovery not being complete until the test interval is as long as 400 msec.

In testing for a possible inhibitory action on Purkyně cells, it is mandatory to carry out control experiments in order to discover and allow for post-impulse depression, which was shown in Fig. 10 to have a duration of about 40 msec for dogfish and lizard Purkyně cells. There is no report of such essential controls in the preliminary publications of Llinás and Bloedel [15, 16] on the frog cerebellum and of Llinás, Nicholson, Freeman and Hillman [17] on the alligator cerebellum. Since the observed depressions had a duration of 40 to 50 msec at the most, the uncontrolled observations by these authors cannot be admitted as evidence for! (alligator) and against! (frog) inhibition of Purkyně cells by stellate cells. It is therefore pointless to consider the general discussion

on the evolution of cerebellar inhibition at the end of a recent paper [22] in which an inhibitory action of up to 100 msec was reported for elasmobranch Purkyně cells, again without carrying out the essential controls.

In the experiments of Fig. 11*A* and 12 on dogfish and lizard cerebella parallel fiber volleys exert an inhibitory action on Purkyně cells that is analogous to that attributed to the basket and stellate cells of the mammalian cerebellum [5]. It is postulated that this inhibition is mediated by the stellate cells of the molecular layer (Figs. 5 and 6). Furthermore, it can be postulated that in evolution some of these stellate cells grew longer axons that extended transversely across the folium and that numerous branches grew down to the somata of the Purkyně cells, there forming the baskets that so effectively inhibit these cells, as illustrated in Fig. 11*B*. In neurogenesis the basket cells of mammals can be observed to be formed in just this way from the embryonic stellate cells in the molecular layer [24], ontogeny recapitulating phylogeny. Primitive basket cells have been recognized in reptilia and are enormously developed in birds as well as in mammals [14, 23, 24].

Discussion

Fig. 13 gives a vivid display of the change wrought by evolution in the principal cell of the cerebellum. The progressive development of structure can be recognized, from the few branches in the lamprey, to the enormous elaboration in man, where it is estimated that each Purkyně cell has 200,000 spine synapses with parallel fibers [5, 10]. It is remarkable that, despite the profuse branching, the Purkyně dendrites of the mammal preserve a strict territoriality, being in leaflets of only about 8 μ thickness and not intermingling with other dendrites. This process of individualization of Purkyně cells appears to be perfected during evolutionary development ([5] Chap. XI; [27]).

Until recently there has been no explanation of the dual mechanism for evoking discharges from every Purkyně cell, by climbing fibers and by mossy fibers, both of which carry much the same information either from peripheral receptors or from the cerebral cortex. Yet it can be presumed that this dual innervation must have functional importance because it has survived in full operation from selachians (Fig. 7*A—C*) to mammals (Fig. 7*G—I*). SZENTÁGOTHAI [27] briefly mentioned the possibility that "coincidence of a climbing fiber impulse with a certain combination of simultaneously activated parallel fibers might have not only a transitory but also some 'fixation' effect on the Purkinje cell." Independently MARR [20] has arrived at the same concept and has presented it in a greatly elaborated concept as the "conjunction hypothesis of learning" by the cerebellum. If this conjunction of mossy fiber and climbing fiber inputs effects a prolonged potentiation of the parallel fiber synapses subjected to this conjunction, dual innervation achieves great functional significance in relation to learning by the cerebellum, and its evolutionary preservation is readily intelligible.

These comparative investigations on the connections and functions of the neurones of the cerebellum are of interest in relation to its evolutionary development. At the early selachian stage there are already almost all of the neuronal elements of the highly developed cerebella of mammalia. The mossy fiber-granule cell-parallel fiber-Purkyně cell and the climbing fiber-Purkyně cell pathways are fully developed, except that there probably is less convergence of parallel fibers on Purkyně cells. In contrast to these excitatory pathways, the inhibitory pathways are less developed, but there is good evidence for an inhibitory action on Purkyně cells by the mossy fiber-granule cell-parallel fiber-stellate cell pathway [6, 7]. Though there appears to be the homologue of the Golgi cell in the granular layer, no inhibitory action by it on the mossy fiber-granule cell synapses has yet been demonstrated. Another inhibitory deficiency arises from the absence of Purkyně axon collaterals.

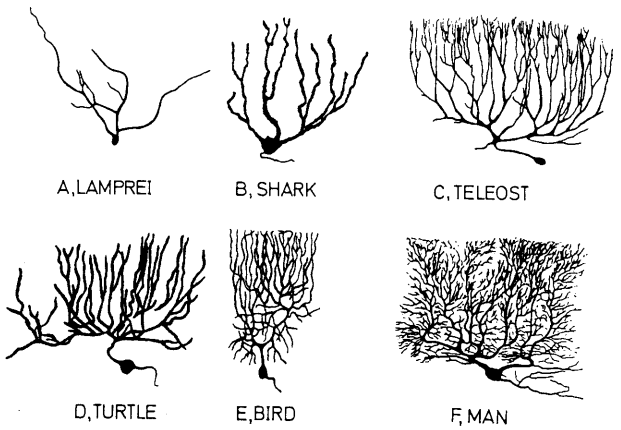

Fig. 13. Purkyně cells of vertebrates in evolutionary order [23]

It can be postulated that all the component cells of the mammalian cerebellum are represented at least primordially in the selachian cerebellum, evolution merely resulting in the development of synaptic connections, so enhancing their functional design and exploiting potentialities latent in this neuronal machine. Some of the stellate cells developed longer axons, growing transversely across the folium, and so achieving the very effective functional contact with the somata of the Purkyně cells that characterizes basket cells. The dendrites of the Golgi cells very largely grew up into the molecular layer where they were able to secure very effective synaptic excitation by the parallel fibers, and *pari passu* their axons branched extensively in the granular layer so that the Golgi cells became very effective inhibitors of the granule cells. The Purkyně cells grew axon collaterals that developed extensive inhibitory synaptic connections on several types of cell in the cerebellar cortex. We therefore postulate that the essential neuronal elements of the cerebellar cortex were present in the primordial ancestors of the Selachians, and that evolution resulted in the realization of the latent potentialities.

We can liken the evolutionary story of the cerebellum to the development of a musical composition, deriving as it does from one theme of transcendent interest and appeal which suddenly appears in the creative mind of the composer. This theme becomes the motif for all manner of evolutions in the musical composition,

yet nevertheless its identity can be recognized under all such transmutations. So in the evolutionary story of the cerebellum there appeared in the dorsal lip of the rhombencephalon of the primitive vertebrate a special neuronal structure with inherent properties of design that lent themselves to development for all the computational problems confronting the evolving organisms in the growing complexities of their struggle for survival. We can glimpse this primordial process and its history in retrospect by imaginative insight based on the cerebellar structure and function of existing vertebrates. Our present understanding is very fragmentary and inadequate for this historical task. A tremendous program of investigation for the future can be organized in relationship to the evolutionary problems here outlined.

I am moved to finish my account of this fascinating story of the evolution of cerebellar design by a quotation from SHERRINGTON in his book "Man on his Nature" [26]: "Even should mind in the cataclysm of Nature be doomed to disappear..., man will have had his compensation: to have glimpsed a coherent world and himself as item in it. To have heard for a moment a harmony wherein he is a note. And to listen to a harmony is to commune with its Composer?"

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Ultrastrukturen bei kalkschaligen Foraminiferen

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Die Gehäuse-Feinstruktur der kalkschaligen perforaten Foraminiferen spielt eine immer wichtigere Rolle bei Untersuchungen stratigraphischer, phylogenetischer und taxionomischer Art. Dünnschliff-Methoden und Untersuchungen mit dem Elektronen-Transmissionsmikroskop (ETM) werden schon seit vielen Jahren erfolgreich angewandt. Die methodische Untersuchungslücke zwischen Lichtmikroskop und ETM kann jedoch erst seit etwa fünf Jahren durch das Elektronen-Rastermikroskop (ERM) zum größten Teil überbrückt werden. Hier soll gezeigt werden, welcher Fortschritt in den Untersuchungen mit dem Elektronen-Rastermikroskop erzielt werden konnte.

Bisherige Kenntnisse

Aus den zahlreichen Publikationen über die Feinstruktur von Foraminiferen-Gehäusen seien hier nur drei Arbeiten herausgegriffen, die unsere Kenntnis wesentlich erweitert haben. REISS [5, 6] entwarf, auf den Ergebnissen von SMOUT [7] aufbauend, eine Klassifikation der perforaten Foraminiferen nach ihrer Feinstruktur im Dünnschliff-Bild. Danach ließen sich vier Typen unterscheiden: lagenid, monolamellid, bilamellid und rotaliid. Ausschlaggebend ist bei allen vier Typen die Art des Kammer-Anbaus und die Lage der Primärmembran. Ein weiteres übergeordnetes Merkmal liefert die Feinstruktur mit radialen oder/und granulären Wandstrukturen. Für diese konnten TOWE und CIFELLI [8] mit Hilfe des ETM zeigen, daß die c-Achsen der Kristalle eine bevorzugte Orientierung aufweisen. Im Gegensatz dazu zeigen die Kristallnadeln der „porzellanschaligen" Foraminiferen so gut wie keine Orientierung.

Präparationstechnik

Dank der verhältnismäßig einfachen und raschen Präparation lassen sich jeweils viele Exemplare einer Art untersuchen. Zum Teil mit Ultraschall gereinigte und künstlich angebrochene Exemplare wurden in Gruppen zu 20 auf einem zum ERM-Stereoscan der Cambridge Instrument Company passenden Aluminium-Träger aufgeklebt. Zum Festkleben wurden alle gängigen Klebestoffe probiert, wobei sich das Aufkleben auf einen doppelseitig klebenden Papierstreifen als beste Technik erwies.