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The Length of Cerebellar Parallel Fibers in Chicken and Rhesus Monkey

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ABSTRACT

The cerebellar parallel fibers, which course through the molecular layer parallel to the long axes of the cortical folds known as folia, originate from ascending granule cell axons and relay the mossy fiber input to dendrites of Purkinje cells. Purkinje cell axons in the cerebellar white matter collect into sheets or zones oriented at right angles to the folia. Each of these zones, which are approximately 0.5–1 mm wide, innervates a different portion of the deep cerebellar and the vestibular nuclei. An experimental light microscopic study was carried out to determine the maximal length of parallel fibers in long folia of avian and primate cerebellar cortex. With a fine surgical knife, vermal folia were cut perpendicular to their long axes in four adult White Leghorn hens and in three adult rhesus monkeys deeply anesthetized with sodium pentobarbital. The animals were killed 3–5 days after the operation. Sections of the transected folia were stained with the Fink-Heimer or the DeOlmos-Ingram methods, which revealed the anterogradely degenerated parallel fibers as darkly stained dots. In both species, the pattern of parallel fiber degeneration in the molecular layer had a trapezoidal configuration with the shorter base bordering the Purkinje cell layer and the longer base bordering the pia mater. In both species, the length of parallel fibers averaged approximately 6 mm, although the range was 4–8 mm in chickens and 4.8–6.6 mm in monkeys. The same trapezoidal pattern of degeneration and average parallel fiber lengths of 6 mm were obtained previously from long folia of cat and rabbit cerebella. Thus, parallel fibers show only small variation in their maximal length in relation to cerebellar size. In all the species studied, parallel fibers are consistently longer than the width of single efferent cortical zones, and they modulate the activity of Purkinje cells projecting to different groups of neurons in the deep nuclei.

Key words: zones, folia, anterograde degeneration, silver methods, cerebellum

The agreement between morphological and functional studies concerning the organization of afferent and efferent fiber systems of the cerebellar cortex into sagittal zones (reviewed in Jansen and Brodal, '40; Larsell and Jansen, '72; Mugnaini, '76; Oscarsson, '79; Brodal and Kawamura, '80; Courville et al., '80; Haines et al., '82; Voogd, '80, '82) has prompted in the last few years renewed efforts to measure the length of the parallel fibers by experimental methods (Brand et al., '76; Friedrich, '78; Friedrich and Brand, '80). The parallel fibers, which arise from ascending granule cell axons and relay the mossy fiber input to dendrites of Purkinje cells, are oriented across the cortical zones. In the vermis of different species, at least six efferent zones (approximately 0.5–1 mm wide) have been identified, each projecting to a different pool of neurons in either the deep cerebellar nuclei or the vestibular nuclei. Individual parallel fibers are longer than the width of single efferent longitudinal zones. Their synaptic action, therefore, reflects on Purkinje cells projecting to more than one of the deep nuclei.

By staining sections of transected folia with a silver method for Wallerian degeneration, Brand et al. ('76) determined that parallel fibers are 6 mm long, on the average, in the long folia of the cat. The shorter parallel fibers are located at the base of the molecular layer and extend for 5 mm. They become progressively longer as they approach the pial surface, where they attain a maximum length of 7

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This study is dedicated to Dr. Jan Jansen, Professor Emeritus (Department of Anatomy, University of Oslo, Oslo, Norway), on occasion of his 85th birthday.

mm. Friedrich ('78) and Friedrich and Brand ('80) estimated the average parallel fiber length in the cat vermis with two different stereological procedures and obtained values (5.2 mm and 5.4 mm, respectively) that are in satisfactory agreement with the experimental data.

Smolyaninov ('71) concluded from stereological computations that several cerebellar parameters, including the length of parallel fibers, increase progressively from mouse, rat, cat, monkey, and man. We considered it worthwhile, therefore, to study with the experimental method of Brand et al. ('76) the vermis of chicken and the rhesus monkey, two species provided with considerably long folia, to verify a possible phylogenetic trend toward the increase of parallel fiber length that would be independent of folial length.

MATERIALS AND METHODS

Four White Leghorn hens approximately 1 year old were anesthetized with sodium pentobarbital i.v. A portion of the occipital bone, 2.5 mm from the midline, was removed with a dental drill and a bone rongeur, and a sharp cataract knife was inserted for about 5–10 mm into the corpus cerebelli in the parasagittal direction and removed along the same line. The hole in the spongy cranial bone was filled with gelatin sponge and the skin was sutured. After 3, 3, 4, and 5 days, respectively, the hens were reanesthetized and perfused through the ascending aorta with buffered saline at 37°C, followed by 10% formaline at room temperature. After fixation, the cerebellum was dissected out and the transected folia were separated and sectioned individually on a Vibratome (Oxford Instruments, Inc.) at 25 μm (Fig. 1A–D). The sections were stained with the Fink-Heimer procedure, as specified by Brand et al. ('76), or with the cupric-silver method of DeOlmos and Ingram ('71).

Three adult monkeys (*Macaca mulatta*, 7–10 kg body weight) were prepared for surgery with ketamine hydrochloride injected intramuscularly and sodium pentobarbital i.p. With the animal mounted on a stereotaxic apparatus, a portion of the left parietal bone was removed with a drill, and the occipital pole of the left hemisphere was ablated. The exposed tentorium was carefully hooked and split at 3–4 mm from the midline over the left lateral portion of the vermis to allow free passage of a knife blade. A cataract knife was then inserted ventrally into the cerebellar cortex and retracted with a smooth dorsalward semicircular movement. The cavity left by the occipital pole ablation was filled with gelatin sponge and the skin was sutured and protected with an antiseptic wound dressing solution. After 3, 4, and 4 days survival, respectively, the monkeys were reanesthetized and perfused through the ascending aorta with buffered saline at 37°C, followed by 10% formalin at room temperature. After fixation, the cerebellum was dissected out; the region of the vermis including the transected folia was blocked and stored for 2 weeks in the formalin fixative, then transferred for another week to a similar solution with 30% sucrose added, and subsequently embedded in albumin gelatin. The block was then mounted on a freezing microtome stage, cooled with dry ice, and oriented to obtain a plane of section parallel to the course of the superficially visible severed folia (Fig. 1E–G). The sections, 25 μm thick, were stained with the Fink-Heimer and DeOlmos-Ingram procedures mentioned above.

In both chicken and monkey, we chose to transect the folia laterally rather than near the midline, in case the fibers should be longer than in the cat. This facilitated measurements of the maximal length of degenerated parallel fibers toward the side of the folia opposite to the transection at the expense of the evaluation ipsilateral to the lesion. Consequently, the lateral extent of the degeneration field was measured only on the right-hand side of the transected folia. There was no change in the pattern of parallel fiber degeneration at the midline. We selected for measurement folia that appeared sectioned parallel to their course and where the "signal-to-noise" ratio of the staining permitted clear delimitation of the degeneration field. Only the highest values obtained from each animal were used for this study. Measurements were taken on the sections at ×250 with a calibrated ocular micrometer.

RESULTS

Chicken

As is already known, the avian cerebellar cortex consists mainly of the vermis and the flocculus (Larsell, '67). The homologues of the mammalian hemispheres are rudimentary and consist of bilateral, scarcely folded areas at the transition between vermis and flocculus (Fig. 1A); these areas receive a small pontocerebellar projection (Brodal et al., '50). The sheetlike avian vermal folia measure up to 10 mm in the transversal direction. Some of the folia are made of a single lamella, others are bipartite (Fig. 1B). Our lesions involved two or more of the folia numbered IV–VIII in the Larsell nomenclature (Larsell, '67). We found that the best way to obtain sections of the molecular layer parallel to the course of the severed fibers was to block and section the damaged portion of the folium, as is shown in Figure 1C,D.

Degenerated parallel fibers were stained equally well by the Fink-Heimer and DeOlmos-Ingram procedures. Both methods permitted a clear evaluation of the extent of the necrosis produced by the knife. This area varied from 0.2 mm to more than 1 mm (Fig. 2). Degenerated Purkinje cell dendrites, also stained by the silver methods in chicken (Fig. 2), as well as in monkey (Fig. 3), occur in many specimens near the lesion, but do not extend as far laterally in the folium as the degenerated parallel fibers (see Brand and Mugnaini, '76). Measurements of the degeneration field in the molecular layer were taken from folia where the lesion was discrete. The measurements involved the distance from the end of the necrotic zone at the site of the knife cut on the left-hand side of the folium to the lateralmost extent of the degeneration toward the right-hand side of the folium. The obtained values, therefore, applied to only one side of the degeneration field and excluded the width of the necrotic zone. Only maximal values from each animal were recorded.

The degeneration field in the molecular layer at the longer right-hand side of the folia forms a trapezoidal figure with the shorter base bordering the cellular layer that includes Purkinje cell bodies and Golgi epithelial cells and the longer base immediately under the pial surface (Figs. 4, 5). Furthermore, the density of the degenerated elements decreases progressively from the border of the lesion laterally (Figs 4, 5). Similar maximal lengths for the two bases, shown in Table 1, were obtained from different folia in the four animals killed from 3 to 5 days after the folial transection. The shorter and the longer bases of the trapezoidal unilateral degeneration field measured approximately 2 mm and 4 mm, respectively. The total estimated length of parallel fibers (far right in Table 1) was assumed to be double that seen on the right side.

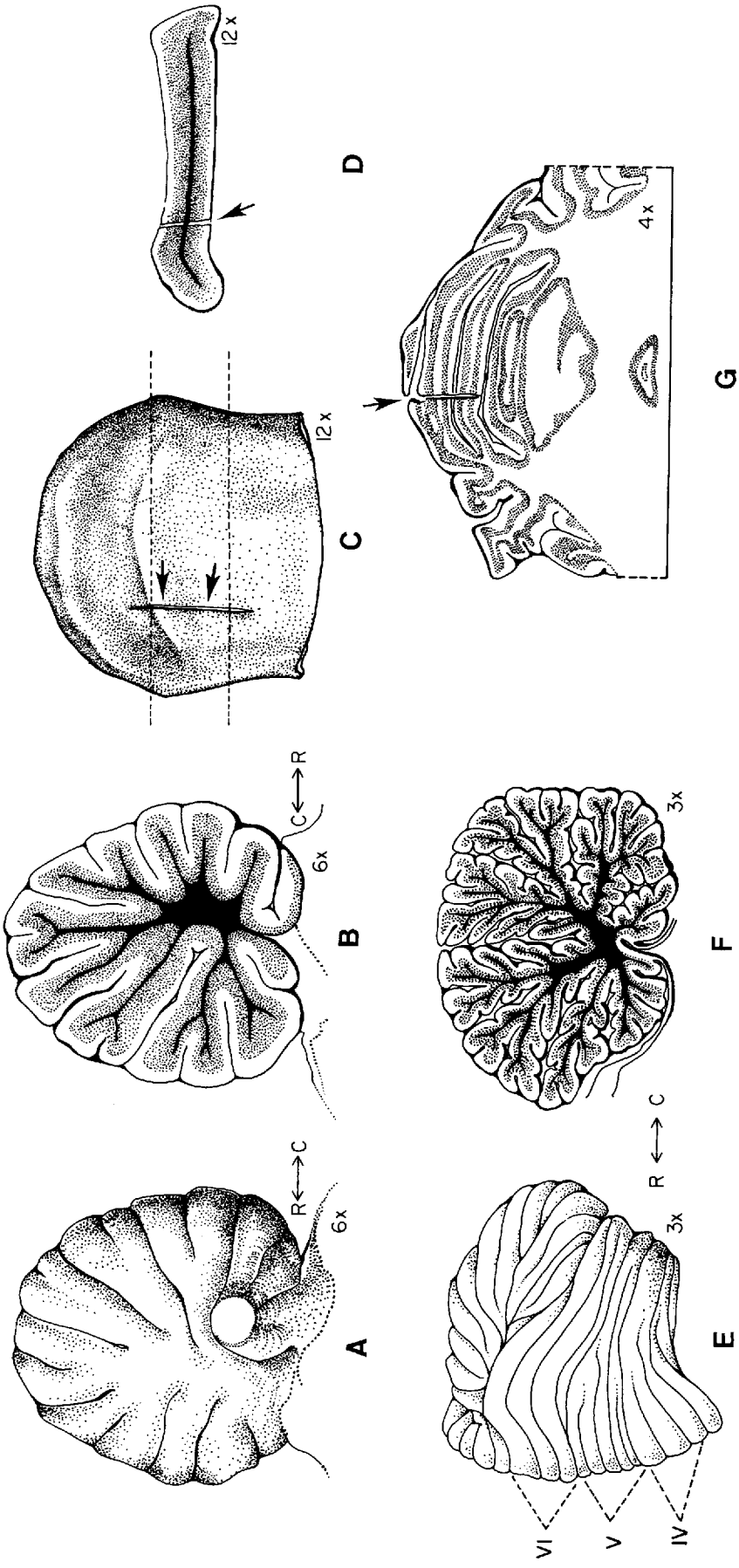

Fig. 1. Diagrams of chicken (A–D) and monkey (E–G) cerebella explaining experimental procedures. A. Left lateral view of the chicken cerebellum. B. Folial pattern of the chicken cerebellum. C. Isolated avian folium with the knife cut (arrows). The dashed lines show the borders of the folial slice from which the Vibratome section (D) was obtained. E. Anterolateral view of lobules IV–VI of the monkey cerebellum. F. Folial pattern of the monkey cerebellum. G. Microtome section of cerebellar block including transected folia (arrow). The label R-C indicates rostral and caudal directions.

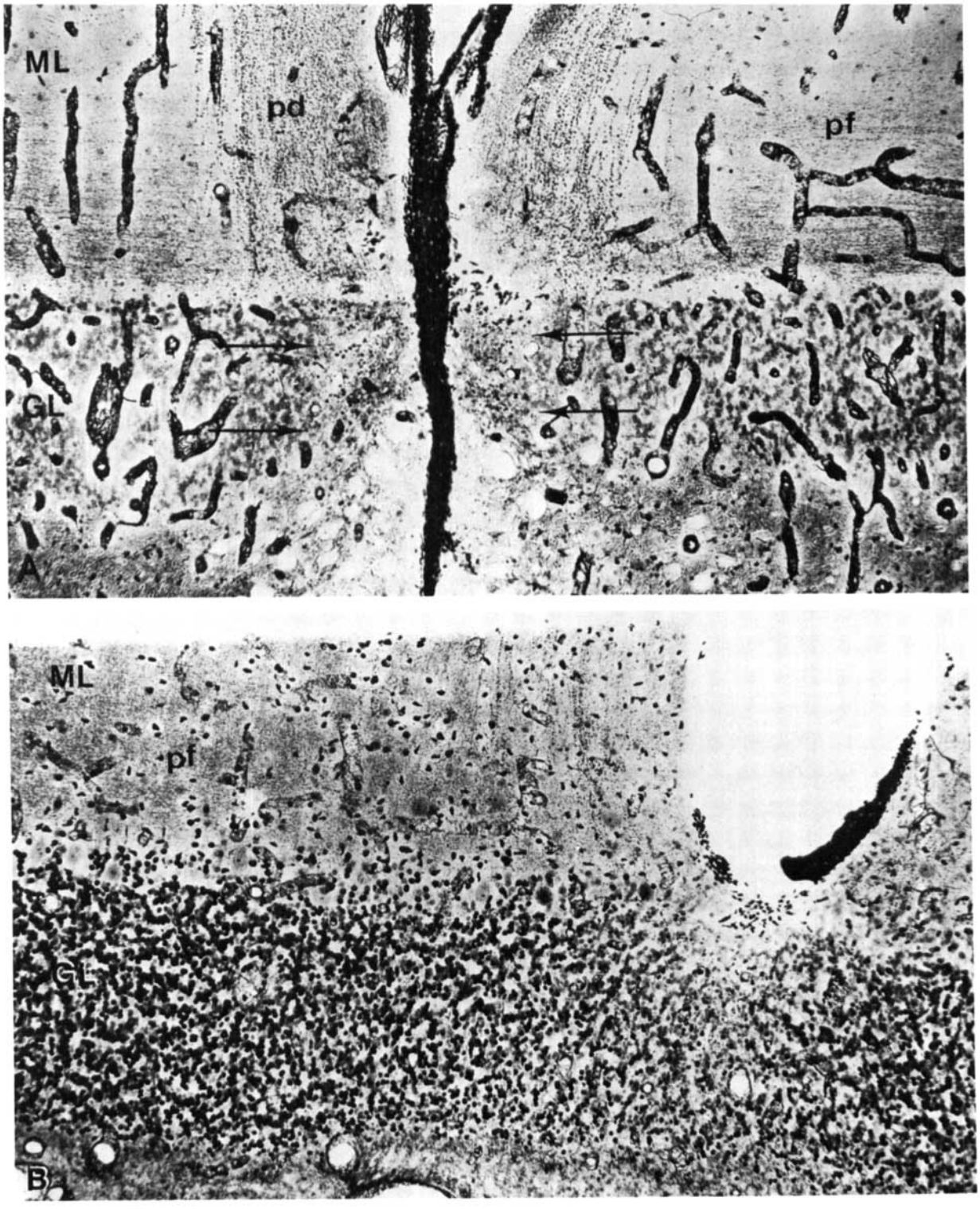

Fig. 2. Microphotographs of two transected cerebellar folia in the chicken. A. The darkly stained stripe is the blood clot marking the site of transection. The borders of the necrotic region of the granular layer are marked by arrows. The DeOlmos-Ingram-stained section is cut parallel to the course of the folium. B. In this folium, the tip of the knife has cut across the molecular layer, sparing the granule layer. The necrosis is minimal. Ascending processes of Golgi epithelial cells damaged by the lesion are not appreciably stained by either silver method (see also Fig. 3). The Fink-Heimer-stained section is cut parallel to the course of the folium. ML, molecular layer; GL, granular layer; pf, degenerated parallel fibers; pd, degenerated Purkinje cell dendrites. ×183.

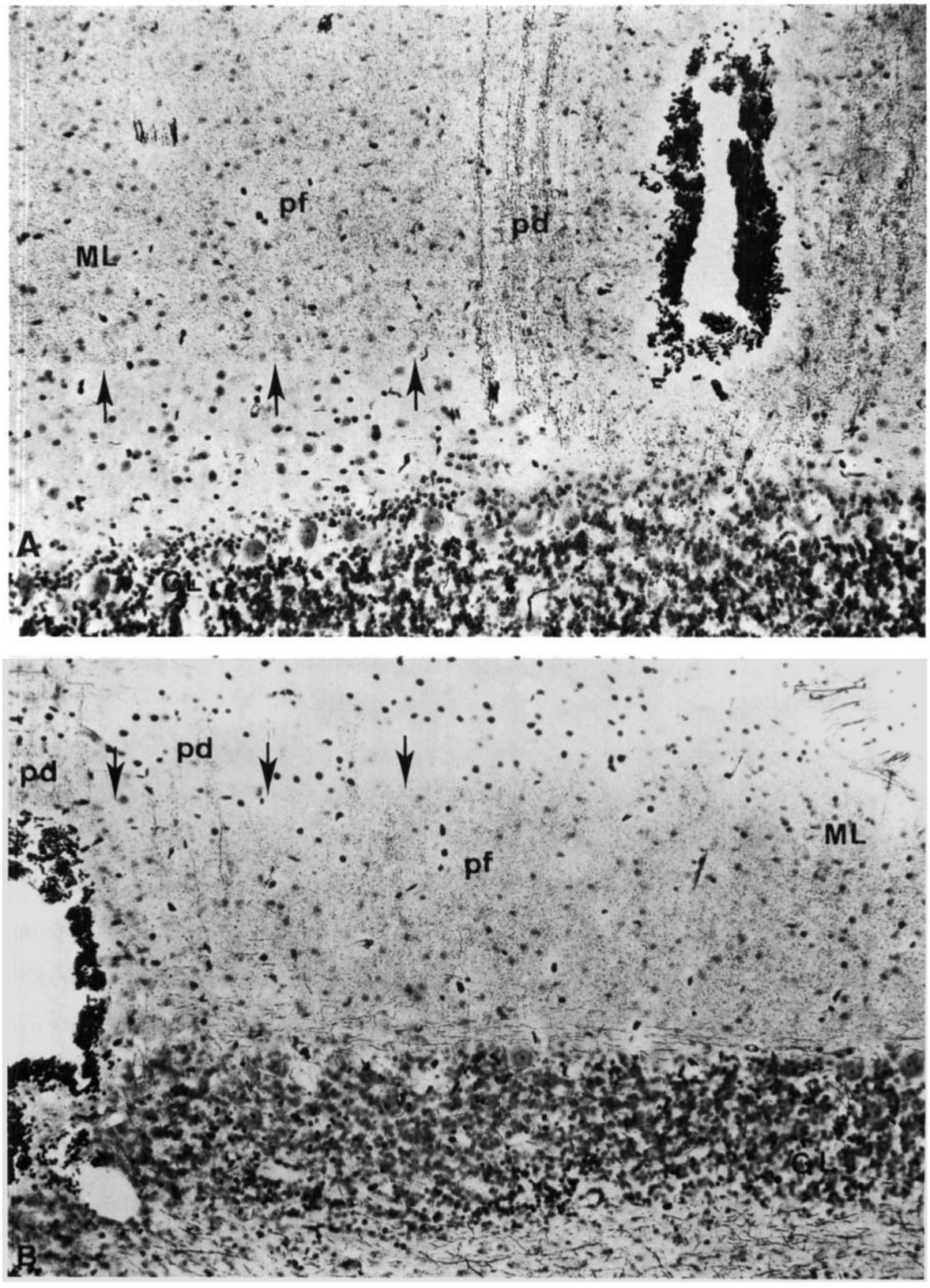

Fig. 3. Microphotographs of two transected cerebellar folia in the monkey. A. The tip of the knife has spared the granular layer and the deeper portion of the molecular layer. The border of the degeneration is sharp (arrows) and the necrosis is minimal. B. The tip of the knife has spared the upper portion of the molecular layer and the deeper portion of the granular layer. The border of the degeneration is sharp (arrows) and the necrosis is minimal. The Fink-Heimer sections are cut parallel to the course of the folium. Labels as in Figure 2. ×183.

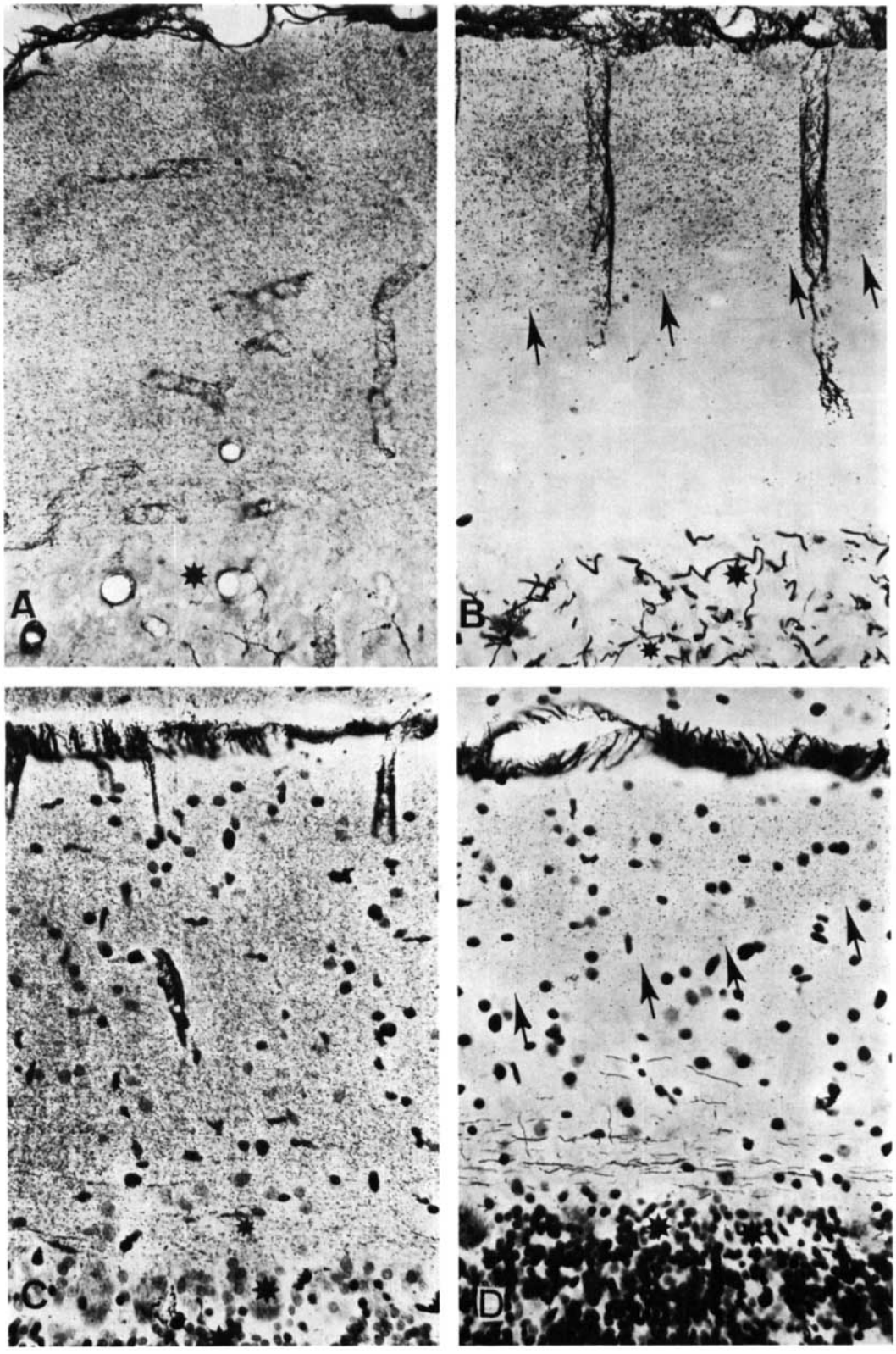

Fig. 4. Microphotographs of areas of the cerebellar molecular layer comprising degenerated parallel fibers from a DeOlmos-Ingram section in the chicken (A, B) and a Fink-Heimer section in the monkey (C, D). These microphotographs are from the areas indicated in Figure 5. Note the border of degeneration in the molecular layer (arrows). Asterisks mark the Purkinje cell layer. ×267.

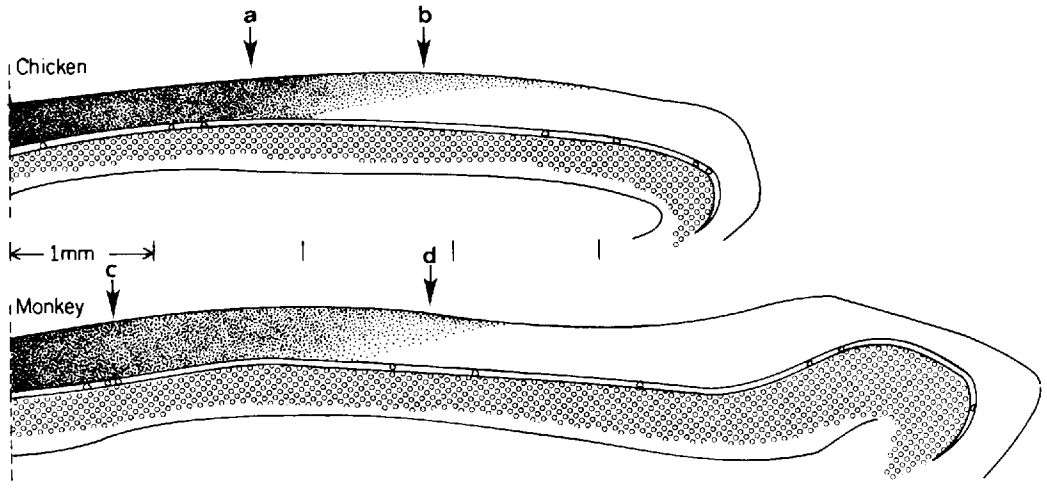

Fig. 5. Camera lucida drawings of transected cerebellar folia in chicken and monkey. The dashed line indicates the transection site. The degeneration fields in the molecular layer (dotted) have a trapezoidal configuration. Microphotographs of the areas indicated by arrows, labeled a-d, are shown in Figure 4.

TABLE 1. Measurements of Degeneration Field (Parallel Fiber (PF) Length) in Different Cerebellar Folia of Adult Chicken and Monkey

Species	Specimen no.	Survival time (days)	Length of shorter basis in mm	Length of longer basis in mm	Estimated PF length in mm	
					Deep	Superficial
Chicken	1328	5	1.85	3.93	3.70	7.86
	1328	5	2.01	3.88	4.02	7.76
	1333	4	2.15	3.81	4.30	7.62
	1335	3	2.02	4.28	4.04	8.56
	1335	3	1.93	4.24	3.86	8.48
	1335	3	2.15	4.40	4.30	8.80
	1335	3	1.95	3.93	3.90	7.86
	1335	3	2.26	3.93	4.52	7.86
	2372	5	2.02	3.90	4.04	7.80
			Mean 2.04	Mean 4.03	Mean 4.08	Mean 8.06
Rhesus monkey	2761	3	2.31	3.36	4.62	6.72
	2761	3	2.40	3.26	4.80	6.54
	2761	3	2.45	3.38	4.90	6.76
	2766	4	2.45	3.40	4.90	6.80
	2766	4	2.33	3.14	4.66	6.28
	2767	4	2.43	3.33	4.86	6.66
	2767	4	2.45	3.38	4.90	6.76
			Mean 2.40	Mean 3.32	Mean 4.80	Mean 6.64

Rhesus monkey

The longest vermal folia in the monkey cerebellum are those numbered IV-VI in Larsell's nomenclature (Larsell and Jansen, '70). Many of them measure at least 2 cm from side to side. They are oriented differently with respect to the horizontal plane in their medial and lateral portions. The folia of lobulus V run nearly in a straight line, while those of lobuli IV and VI are curved, with the concavity oriented ventrally (Fig. 1E). Each primary folium gives rise to numerous subfoldings, many of which are not visible on the surface of the cerebellum (Fig. 1F). When evaluated from the surface, our lesions involved portions of lobuli IV-VI to a varying degree in the three animals. For each case, therefore, the cerebellar lobules were blocked and oriented on the microtome in order to maximize the number of sections parallel to the course of the presumably transected folia (Fig. 1G). Also, in the monkey, the fields of degeneration in the molecular layer presented a trapezoidal configuration (Figs 4, 5). Only the hemifields on the right-hand side of the lesion were measured. The extent of the Wallerian degeneraton in the molecular layer and the necrosis were measured as done in the chicken material. The most discrete lesions were located in correspondence to the point reached by the tip of the knife (Fig. 3). The maximal values recorded from different folia in the three animals killed from 3 to 4 days after the folial transection are shown in Table 1. The shorter and the longer bases of the trapezoidal degeneration hemifield measured 2.4 mm and 3.3 mm, respectively. The total estimated length of parallel fibers (far right in Table 1) was assumed to be double that seen on the right side.

DISCUSSION

The present experimental study confirms the validity of the approach used by Brand et al. ('76) to measure parallel fiber lengths in the cat cerebellar cortex. In the transected folia of both the chicken and monkey, the degeneration field in the molecular layer lateral to the lesion has a trapezoidal configuration as in the cat. Since the staining was shown by Brand and co-workers to mark degenerated parallel fibers, we conclude that in the avian and the primate cerebellum the longer parallel fibers are also situated in the subpial region, and they become progressively shorter toward the Purkinje cell layer. This conclusion is opposite to the one reported in the literature preceding the paper of Brand et al. ('76; cf., Fox and Barnard, '57; Fox et al., '67; Eccles et al., '67; Palkovits et al., '71; Smolyaninov, '71).

The fact that the density of degeneration decreases progressively from the border of the lesion laterally is explained by the observation that parallel fibers sectioned at a distance from the bifurcation point undergo degeneration only in the segment severed from the parent cell. The lateral extent of the degeneration field, therefore, demon-

strates the maximal length of the parallel fibers which presumably arise from granule cell axons directly in the path of the lesion (Brand et al., '76: their Fig. 8).

Since we intended to determine the maximal length of parallel fibers, we did not record all the measurements obtained from each animal. Shorter measurements may indicate that (1) parallel fibers are shorter in certain folia; (2) the folia providing the lower values had been sectioned at angles to the parallel fibers; or (3) the staining was less than optimal. In order to determine between these alternatives, careful study of serial sections through the same folium would be necessary, and this was considered outside the scope of this investigation. Furthermore, the results of stereological measurements in the cat (Friedrich, '78; Friedrich and Brand, '80) indicate that the maximal and average length of parallel fibers are nearly the same.

In folia that are shorter than the potential length of the parallel fibers and in certain mammalian folia where the cortical sheet is discontinuous, asymmetry of the two branches of the parallel fibers takes place (Brand et al., '76).

In the long folia of the feline vermis and hemispheres, most of the parallel fibers reach equal lengths on both sides of their T-branching point. Assuming that the same holds true in our material, the total length of parallel fibers was expressed by multiplying our measurements at one side of the lesion by two (Table 1, right column). The maximal parallel fiber length in the chicken is 6 mm, with a range of 4–8 mm from deeper to superficial molecular layer. The corresponding values in the monkey are 5.7 mm (range 4.8–6.6 mm) and in the cat, 6 mm (range 5–7 mm). Preliminary measurements in the rabbit indicate values similar to those in the monkey (our unpublished observations). Thus, the mean length of the parallel fiber does not differ conspicuously in the chicken, rabbit, cat, and monkey. This cerebellar module, therefore, appears relatively invariant in phylogeny and may also be unchanged in man.

Our study, furthermore, indicates that the ratio of length of deep and superficial parallel fibers varies in the different animal species, being larger in the chicken (two) than in the cat (1.4) and the rabbit and monkey (1.35). The significance of this variation is unclear. It may be correlated to a different rate of growth of the molecular layer in the various species or to other parameters that are at present poorly understood. It has been emphasized elsewhere (Mugnaini, '72; Palay and Chan-Palay, '74) that axons of granule cells form synapses every 3–5 μm along their complete course (i.e., on both their ascending and parallel fiber portions). Recent anatomical studies with the Golgi method and electron microscopy, as well as microelectrode investigations in incubated cerebellar slices (Llinás, '82; unpublished observations of Llinás, Mugnaini, and Sugimori) indicate that individual Purkinje cells receive substantial input from ascending granule cell axons, and this may explain the spatial distribution of mossy fiber-related Purkinje cell activity described by Shambes et al. ('78) and Oscarsson ('79). This concept, however, does not diminish the value of understanding the modulatory role of the parallel fibers.

In conclusion, measurements in different species demonstrate that parallel fibers can reach a length of approximately 6 mm. They are, therefore, consistently longer than the width of single efferent cortical strips, and they modulate the activity of Purkinje cells projecting within several of these zones. Since the width of the cortical efferent zones may vary among species and on different folia of the same animal (Haines et al., '82; Voogd, '82), accurate maps of afferent and efferent connections of individual folia may be fruitful to study the effect on the same Purkinje cell of two mossy fiber inputs terminating in the cortex at a distance shorter or longer than one-half the parallel fiber length.

Since the size of the vermis varies in different species, in animals such as the chicken the parallel fibers span a comparatively larger region of this cerebellar portion than they do in the monkey. One may ask, therefore, whether there is a special relationship between the numbers of Purkinje cells and of their target neurons in the deep nuclei of different species, and what the effect is of the overall size of the cerebellum on this relationship.

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LITERATURE CITED

Brand, S., A.L. Dahl, and E. Mugnaini (1976) The length of parallel fibers in the cat cerebellar cortex. An experimental light and electron microscopic study. Exp. Brain Res. *26*:29–58.

Brand, S., and E. Mugnaini (1976) Fulminant Purkinje cell death following axotomy and its use for analysis of the dendritic arborization. Exp. Brain Res. *26*:105–119.

Brodal, A., K. Kristiansen, and J. Jansen (1950) Experimental demonstration of a pontine homologue in birds. J. Comp. Neurol. *92*:23–69.

Brodal, A., and K. Kawamura (1980) Olivocerebellar projection. A review. Advances in Anatomy, Embryology, and Cell Biology, Vol. 64. Berlin: Springer-Verlag, pp. 1–140.

Courville, J., C. de Montigny, and Y. Lamarre (1980) The Inferior Olivary Nucleus: Anatomy and Physiology. New York: Raven Press.

DeOlmos, J.S., and W.R. Ingram (1971) An improved cupric silver method for impregnation of axonal and terminal degeneration. Brain Res. *33*: 523–529.

Eccles, J.C., M. Ito, and J. Szentágothai (1967) The Cerebellum as a Neuronal Machine. Heidelberg: Springer-Verlag.

Fox, C., and J.W. Barnard (1957) A quantitative study of the Purkinje cell dendritic branchlets and their relationship to afferent fibers. J. Anat. *91*:299–313.

Fox, C.A., D.E. Hillman, K.A. Siegesmund, and C.R. Dutta (1967) The primate cerebellar cortex: A Golgi and electron microscopic study. In C.A. Fox and R.S. Snider (eds): The Cerebellum. New York: Elsevier, pp. 174–225.

Friedrich, V.L., Jr. (1978) Length of parallel fibers in cat cerebellar cortex: Stereological determination. Soc. Neurosci. Abstr. *4*:65.

Friedrich, V.L., Jr., and S. Brand (1980) Density and relative number of Purkinje cells in cerebellar cortex of cat. Neuroscience *5*:349–356.

Haines, D.E., G.W. Patrick, and P. Satrulee (1982) Organization of cerebellar corticonuclear fiber systems. In S.L. Palay and V. Chan-Palay (eds): The Cerebellum – New Vistas. Heidelberg: Springer-Verlag, pp. 320–367.

Jansen, J., and A. Brodal (1940) Experimental studies on the intrinsic fibers of the cerebellum. II. The corticonuclear projection. J. Comp. Neurol. *73*:267–321.

Larsell, O. (1967) J. Jansen (ed): The Comparative Anatomy and Histology of the Cerebellum: From Myxinoids Through Birds. Minneapolis: The University of Minnesota Press.

Larsell, O. (1970) J. Jansen (ed): The Comparative Anatomy and Histology of the Cerebellum: From Monotremes Through Apes. Minneapolis: The University of Minnesota Press.

Larsell, O., and J. Jansen (eds) (1972) The Comparative Anatomy and Histology of the Cerebellum: The Human Cerebellum, Cerebellar Connections, and Cerebellar Cortex. Minneapolis: The University of Minnesota Press.

Llinás, R. (1982) General discussion: Radial connectivity in the cerebellar cortex: A novel view regarding the functional organization of the molecular layer. In S.L. Palay and V. Chan-Palay (eds): The Cerebellum – New Vistas. Heidelberg: Springer-Verlag, pp. 189–194.

Mugnaini, E. (1972) The histology and cytology of the cerebellar cortex. In O. Larsell and J. Jansen (eds): The Comparative Anatomy and Histology of the Cerebellum. Minneapolis: The University of Minnesota Press, pp. 200–298.

Mugnaini, E. (1976) Organization of cerebellar cortex. Exp. Brain Res. [Suppl.] *1*:8–19.

Oscarsson, O. (1979) Functional units of the cerebellum – Sagittal zones and microzones. Trends Neurosci. *2*:143–145.

Palay, S.L., and V. Chan-Palay (1974) Cerebellar Cortex: Cytology and Organization. New York: Springer-Verlag, pp. 1–348.

Palkovits, M., P. Magyar, and J. Szentágothai (1971) Quantitative histological analysis of the cerebellar cortex in the cat. III. Structural organization of the molecular layer. Brain Res. *34*:1–18.

Shambes, G.M., J.M. Gibson, and W. Welker (1978) Fractured somatotopy in granule cell tactile areas of rat cerebellar hemispheres revealed by micromapping. Brain Behav. Evol. *15*:94–140.

Smolyaninov, V.V. (1971) Some special features of organization of the cerebellar cortex. In I.M. Gelfand, U.S. Gurfinkel, S.V. Fomin and M.L. Tsetlin (eds): Models of the Structural-Functional Organization of Certain Biological Systems. Cambridge: MIT Press, pp. 250–423.

Voogd, J. (1980) Topographical distribution of olivary and corticonuclear fibers in the cerebellum: A review. In J. Courville, C. de Montigny, and Y. Lamarre (eds): The Superior Olivary Nucleus: Anatomy and Physiology. New York: Raven Press, pp. 207–234.

Voogd, J. (1982) The olivocerebellar projection in the cat. In S.L. Palay and V. Chan-Palay (eds): The Cerebellum – New Vistas. Heidelberg: Springer-Verlag, pp. 134–160.