THE RELATIONSHIP OF BODY SIZE, NERVE CELL SIZE, AXON LENGTH, AND GLIAL DENSITY IN THE CEREBELLUM*

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It is generally recognized that the nerve cells of large animals are somewhat larger than the nerve cells of small animals. A quantitative comparison of cell size to body size is difficult to establish because of the variations of shape and size of nerve cells, even within one nucleus. Also, measurements of perikarya only are not an adequate determination of total cell volume because of variations in the extent of dendrite ramification.

A comparative study of cell size is facilitated in the cerebellar cortex which, in its anatomical architecture and physiological function, persists remarkably constant throughout phylogenetic development. The diameter of Purkinje cells can be measured with ease. Since all the dendrites extend toward the surface of the molecular layer, the thickness of this layer can be regarded as a parameter of maximal dendrite length. The width of the area covered by dendrite branchings can be estimated by the distance between Purkinje cells if the sections are cut in the plane of dendrite branching, that is, perpendicular to the axis of the gyri. Such measurements of nerve cells, in addition, can be compared with relative changes of glial density. The following provides a short investigation in eighteen species with the aim of indicating trends among species rather than to establish norms for each individual species.

Material and Methods.—The vermis of the cerebellum was investigated in each of the following species: horse, cow, elk, man, deer, lion, sheep, pig, dog, rhesus monkey, cat, rabbit, rat, gerbil, canary, parakeet, mouse, and frog. The tissues were fixed in 10 per cent neutral formalin. Frozen sections, 15 μ and 30 μ thick, were cut exactly perpendicular to the axis of the gyri and were stained with chromalum gallocyanin. The sections were mounted in glycerin-gel, thus eliminating shrinkage from dehydration. Measurement in many brains of rat and man[8] had shown that the individual variations of such measurements were quite small; this was in agreement with Ellis'[3] observation.

The following methods were used for measurements:

A. *The thickness of the molecular layer* was measured between its surface and an imaginary line through the center of the majority of Purkinje cells; from 27–90 measurements were made for each species; the fewer measurements were made if there was little scatter of the data.

B. *The area ratio of molecular to granular layer* was determined by planimetric measurements of drawings of sections projected on cardboard. In these drawings, large pieces of cortex, including several convolutions, were separated from the rest of the cortex by lines which cut each layer at 90°. The area of granular and molecular layer then was measured planimetrically.[7, 8]

C. *The maximal diameter of Purkinje cells* was measured in a plane parallel to the cortical surface; about 50 measurements were made per species. Hyperchromic shrunken cells were disregarded. The volume of the cells was calculated, assuming

spherical shape. This represented only an approximation, and the true volume was assumed to be somewhat larger. This difference, however, evidently did not affect the comparison of species.

D. *The distance between Purkinje cells* in a 15 μ section was determined by projecting and drawing the cerebellar cortex on cardboard. The actual length of a given portion of Purkinje cell layer between two easily recognizable landmarks was calculated from comparison with a millimeter scale projected on the same drawing. Then the tissue section was studied microscopically and Purkinje cells distinguishable between the above-mentioned landmarks were counted.

There is an error inherent in counting particles of different sizes in tissue sections of equal thickness; thus, the counts were corrected by using Abercrombie's formula[1] to eliminate a distortion of the data due to the species difference of Purkinje cell size. From these data, the number of "nuclear points" of Purkinje cells per mm of Purkinje layer in a 15 μ section was calculated. Since the distance between Purkinje cells is different in the gyri than in the sulci,[7, 2] equal portions of both were included.

E. *The glial density (Bergmann cells) in the Purkinje layer* was also determined. An arbitrary definition of the thickness of the Purkinje layer had to be used because of the gradual transition between the Purkinje layer and the molecular layer. This was done using two parallel lines of an object micrometer as delineation of the Purkinje layer; in the tissue, these lines represented a distance of 31.9 μ. The deeper line touched tangentially the deep circumference of the majority of Purkinje cells. All glial cells between the two lines were counted through several continuous millimeters of cortical length. Approximately 800 cells were counted per species. Care was taken not to count granular cell nuclei displaced into the Purkinje layer. These were distinguished from Bergmann glial cells by their spherical shape, smaller size, and darker staining. The data from *D* and *E* permitted one to calculate the number of glial cells per one Purkinje cell. The data in Table 1 represent counts corrected for both size of Purkinje cells and size of glial cells. As stated in paragraph *D* above, Abercrombie's formula[1] was used for the corrections.

F. *The glial density per volume of molecular layer* was counted with an ocular net micrometer. No attempt was made to distinguish between types of glial cells, but endothelial nuclei were counted separately. The latter were identified by their attachment to the basal membrane of vessels which were easily distinguishable in glycerin-gel mounted sections with the condenser in a lowered position. The counts in Table 1 represent the total number of all nuclei minus the number of nuclei unquestionably identified as endothelial or vascular cells. The data in Table 1 represent an average of 50 counts each.

G. Determination of the true *average axonal length of Purkinje cells* is practically impossible. The majority of these axons terminate in the cerebellar nuclei; others bypass them and terminate in the vestibular nuclei.[11] The radius of the vermis should provide a rough estimate for the probable differences of axonal length among species. Since the diameter of the vermis varies greatly in different directions, the following method was developed: the area of a median-sagittal section of the vermis was measured planimetrically. This area was considered a circle. The radius of this circle represented a factor which characterized differences, among species, in average axon lengths of the Purkinje cells of the vermis. It is logical that the axons in a

TABLE 1*

	Cow	Man	Horse	Sheep	Elk	Pig	Dog	Deer	Lion
Average radius of vermis in mm (factor indicating "axon length").	37	36	27	27	23	23	22	18	17
Glial cells per one Purkinje cell.	53	44	50	43	46	21	25	33	43
Body weight in grams.	500,000	160,000	650,000	50,000	386,000	250,000	13,000	65,000	125,000
Diameter of Purkinje cells in μ; (vol. of Purkinje cells 1000μ³ in parentheses).	32 ± 2 (17)	27 ± 1 (10)	31 ± 2 (16)	31 ± 2 (16)	27 ± 1 (10)	29 ± 2 (13)	30 ± 1 (14)	29 ± 2 (13)	29 ± 2 (13)
Thickness of molecular layer in μ (factor indicating "dendrite length").	409 ± 48	398 ± 18	416 ± 56	383 ± 25	380 ± 19	375 ± 27	378 ± 19	364 ± 23	318 ± 12
Spacing of Purkinje cells ("dendrite spread").	43	61	42	43	50	37	37	49	43
Glial cells per volume in the molecular layer.	22 ± 4	29 ± 4	28 ± 4	23 ± 3	21 ± 3	12 ± 2	22 ± 3	18 ± 3	24 ± 4
Basal metabolic rate calories/kilogram/day.	16	24	?	26	?	14	36	?	?
Ratio of molecular layer to granular layer.	1.52	1.58	1.64	1.50	1.52	1.50	1.69	1.58	1.41

	Monkey	Cat	Rabbit	Gerbil	Rat	Parakeet	Canary	Mouse	Frog
Average radius of vermis in mm (factor indicating "axon length").	17	11.5	9.5	5.5	5	5	5	3	0.1
Glial cells per one Purkinje cell.	17	18	15	9	9	9	6	5	1
Body weight in grams.	3,200	3,000	3,500	100	200	30	16	20	50
Diameter of Purkinje cells in μ; (vol. of Purkinje cells 1000μ³ in parentheses).	26 ± 1 (9)	30 ± 1 (14)	22 ± 1 (6)	18 ± 1 (3)	16 ± 1 (2)	20 ± 1 (4)	18 ± 1 (3)	19 ± 1 (4)	7 ± 2 (1.5)
Thickness of molecular layer in μ (factor indicating "dendrite length").	328 ± 16	402 ± 14	292 ± 10	216 ± 27	199 ± 16	234 ± 23	207 ± 21	156 ± 12	240
Spacing of Purkinje cells ("dendrite spread").	27	31	34	14	22	9	9	10	8
Glial cells per volume in the molecular layer.	35 ± 5	22 ± 4	17 ± 3	58 ± 11	50 ± 8	49 ± 6	60 ± 8	50 ± 4	24 ± 8
Basal metabolic rate calories/kilogram/day.	48	50	47	?	130	225	310	170	?
Ratio of molecular layer to granular layer.	1.64	1.63	1.50	1.31	1.61	1.37	1.40	1.22	0.73?

* Since all measurements were done in the vermis, the species were listed in order of the average radium of the vermis (factor indicating "axon length"); this arrangement was considered more proper than either body weight (which can be quite misleading; e.g., in the pig) or brain weight (which depends largely on the development of the hemispheres). The order used in Table 1 places deer and lion, for some unknown reason, lower than one would place them according to their body size.

4-mm cerebellum are about 1/10 of those in a 4-cm cerebellum, even if the true length of any given axon is not known. The lengths of projections to the vestibular nuclei will vary proportionally and will, therefore, not affect the usefulness of this factor.

Results.—The results of the measurements are collected in Table 1. This table is self-explanatory, but a few comments may be in order to interpret the observations.

1. *The ratio of molecular layer-granular layer* was surprisingly constant among the species, except for a slight decrease in the smaller species. This observation agreed with previous investigations,[7, 2] which indicated that the ratio of molecular layer to granular layer in various mammals represents a constant and biologically important parameter. Large shifts of this ratio in human brain are pathological and represent a sensitive indicator of edema.[8]

2. *The size of the Purkinje cells* increased with body size, but the extent of increase did not nearly keep pace with that of body size. The diameter of the Purkinje cells of the cow, for example, was only about twice that of the mouse. A comparison of cell volume and body weight evidenced a crude logarithmic relationship of nerve cell size and body size (Fig. 1). There was considerable scatter of the measurements, but one cannot anticipate that a more precise prediction of the size of a nerve cell could be made from body weight.

Since all the dendrites of the Purkinje cells extend toward the surface of the molecular layer, its thickness provided an estimate for average dendrite length.
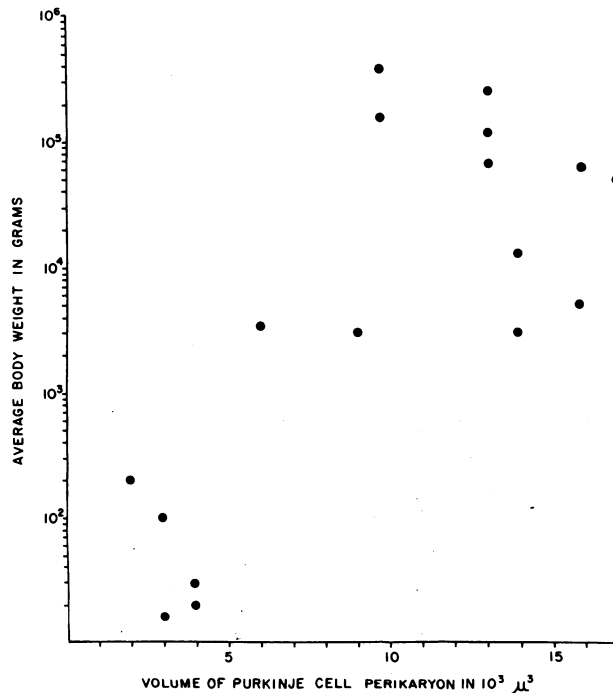

Fig. 1.—Data from Table 1: Scatter graph showing the logarithmic relationship of body weight and volume of the perikarya of Purkinje cells.

The thickness of the molecular layer increased proportionally with the increase of the diameter of Purkinje cells. Therefore, the ratio of cell size to dendritic ramification could be assumed to be approximately constant for Purkinje cells. In the developing rat brain, Ellis[3] observed an increase of the thickness of the molecular layer with body weight.

Since the dendrites of Purkinje cells branch within a two-dimensional plane, the distance between Purkinje cells can provide a parameter of the lateral extent of dendrite spread. The distance between Purkinje cells increased somewhat more than did either the diameter of the Purkinje cells or thickness of the molecular layer. If one assumes that there was also a change of spacing in depth, the disproportion between thickness of the molecular layer and the spacing of Purkinje cells is understandable. It would seem, therefore, that the increases of the size of perikarya, of the dendrite length, and of the dendrite spread were in proportion with each other.

Tentative measurements of the nuclei of granular cells were made. The diameter of these nuclei likewise increased with body size: the diameter increased from 4 μ in the canary to 7 μ in the horse, the rest of the species being graded accordingly.

3. *The ratio of glia cells to Purkinje cells* (glia index) in the Purkinje layer increased with body size and cell size, but showed the best correlation with average axon length (Fig. 2). A simple calculation showed that not only the glia-Purkinje cells ratio, but also the absolute glial density in the Purkinje layer, increased with brain size. Measurements of the diameter of Bergmann-glia nuclei showed a scatter between 6 and 8 μ, without a consistent trend of change.

4. One would expect that the *glial cell density of the molecular layer* would be fairly constant, that is, the same number of glial cells available for a given volume of molecular layer or length of dendrites. It was surprising, therefore, to observe striking differences of the glial density of the molecular layer among species. These data did not show evident correlation with any other measurement. Since it is known that brain respiration[4, 12, 13] and respiratory enzyme activity,[5, 15] as well as basal metabolic rate, vary inversely with body size, we added, in Table 1, some data on the basal metabolic rate from Spector's handbook.[14] This section was incomplete, as no such comparison had been intended at the beginning of the investigation.
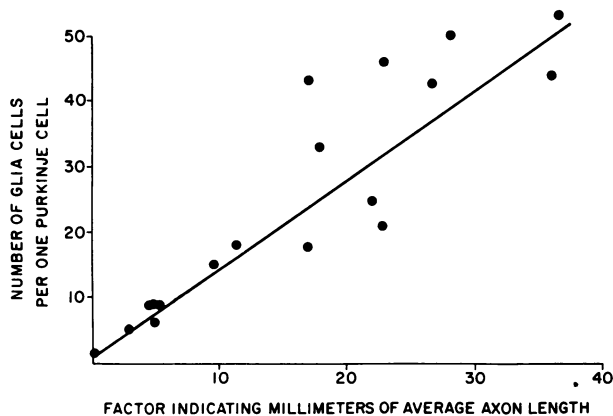

FIG. 2.—Data from Table 1: Scatter graph showing the linear relationship between average axon cells per one Purkinje cell.

It was suggested, however, that high glial density in the molecular layer might have some relationship with high basal metabolic rate.

Discussion.—The increase of the size of Purkinje cells shows a *logarithmic* relationship to the increase of body size. Axon length, on the other hand, must inherently increase in a *linear* relationship with brain size. That implies that nerve cells in large brains have to maintain a relatively much larger volume of axoplasm than nerve cells in small brains. This difference is even more evident if one considers that all of the increase of the perikarya of nerve cells could be accounted for by the increased length and ramification of their dendrites; thus, there would be virtually no increase of the perikaryon to account for the longer axon. Our data suggest that the difference in proportion between the perikarya and their processes, particularly the axon length, is compensated by a numerical increase of attached glia cells. The glial cells themselves are not markedly larger in larger species. The number of glial cells per Purkinje cell was in a surprisingly good linear relation with the average axon length. This observation is in agreement with previous findings in Clarke's column,[9] where the number of perineuronal glial cells showed a linear relation to axon length. (Clarke's column facilitated the investigation of this relationship because the approximate lengths of the axons of large cells depended on the segment in which the cell bodies were residing.) Likewise, nuclei with short intra-nuclear connections have very few glial cells per nerve cell, while nuclei with long projections showed more glial cells per nerve cell.[9] The number of glial cells per nerve cell generally increased with brain size.[6] Hawkins and Olszewski[10] showed very high glia-indices in the whale cortex.

All these observations support the theory that glial cells serve as "auxiliary metabolic units"[9] which are attached to the nerve cells when there is an increased metabolic demand on the nerve cells. The addition of glial cells could be homologous to the hypertrophy of cells in other organs.

An increased demand may be conditioned *anatomically* by the proportion of perikaryon to axon. Increased demand, on the other hand, may be conditioned *physiologically* by the metabolic rate of the nerve cell; no anatomical factor, for example, could be invoked to explain the marked variations of glial cell density per volume of molecular layer. Physiological factors, such as the metabolic rate, might be considered. The actual glial density of the nucleus, therefore, probably represents the result of a complex interaction of several anatomical and physiological factors.

Summary.—Measurements of the thickness of layers, cell size, and glial density were made in the cerebellar cortex of 15 species. The ratio of molecular layer per granular layer was remarkably constant. The size of the perikarya of the Purkinje cells increased in a roughly logarithmic relation with body size. Dendrite length and dendrite spread increased in proportion with the perikarya. The number of glial cells per Purkinje cell showed a linear relationship to average axon length. The glial density per volume of molecular layer, in contrast, was not related to any anatomical factor; tentative comparison suggested a crude correlation with the basal metabolic rate of the species.

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[1] Abercrombie, M., *Anat. Record*, **94**, 239 (1946). Abercrombie's formula: $P = A \times M/(L + M)$ (P = average number of nuclear points per section; A = crude count; M = thickness of section; L = particle size. Explanation: "Ideal points" would be found in only one tissue section each. Particles of finite size will be cut once or more depending on both their dimensions and the thickness of the sections. This represents a source of error in counting particles in tissue sections, which can be corrected with the above formula.

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FINE STRUCTURAL CHANGES IN HEART MUSCLE IN RELATION TO THE LENGTH-TENSION CURVE*

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It is the purpose of this communication to relate the ultrastructure of heart muscle to its physiological performance as precisely defined by increasing muscle length along the length-tension curve. The ascending limb of the active length-tension curve has been explored with special interest since it is the only physiologically functional portion of the curve. These studies provide support for the view that cardiac muscle shortening occurs by a change of configuration of thin (presumably actin) filaments relative to fixed thicker filaments (presumably myosin). This is consistent with a "folding" model for contraction[1] but cannot be reconciled with a "sliding" model as proposed for skeletal muscle.[2]

Methods.—Papillary muscles were obtained from the right ventricles of cats (1.0–1.5 kg) anaesthetized with intraperitoneal sodium pentobarbital (25 mg/kg). Details of this preparation have been described elsewhere.[3] Papillary muscles measured 5 to 11 mm unstretched with a cross-sectional area (calculated on the basis of weight, assuming the muscle to be a cylinder) of 0.9 to 1.3 mm.[2] The muscle was placed in Krebs solution bubbled with a mixture of 95% O_2 and 5% CO_2. The base of the muscle was firmly fixed while its upper free end was tied by wire to a tension transducer (Statham G1–1000). The tension transducer could be moved with a micrometer stage allowing the length of the muscle to be changed at will. The muscle was stimulated with a Grass Pulse Generator (Model S4). Resting (or passive) and active isometric tension were continuously recorded on a Sanborn multichannel oscillograph.

The initial length (L_0) of the papillary muscle was ascertained at zero resting tension. Muscle