A QUANTITATIVE STUDY OF THE PURKINJE CELL DENDRITIC BRANCHLETS AND THEIR RELATIONSHIP TO AFFERENT FIBRES

By CLEMENT A. FOX and JOHN W. BARNARD

Anatomy Department, Marquette University School of Medicine, Milwaukee, Wisconsin

INTRODUCTION

Purkinje cell dendrites have two distinct, morphologically different, receptive regions each with its own specific afferents: the smooth branches, contacted by the climbing fibres, and the spiny branchlets, contacted by the parallel fibres (Cajal, 1895). The former are 'longitudinal axo-dendritic connexions' (Cajal, 1934, 1954), a type of synapse allowing an axon many contacts on the dendrites of a single neuron; the latter are 'cruciform axo-dendritic connexions of great length', a type of synapse permitting the dendrites of each neuron contact with a great number of axons and each axon contact with the dendrites of many neurons.

The recognition of two receptive regions in the Purkinje cell dendritic system resulted from Cajal's (1891) discovery of the peridendritic spines, which form a 'nap-like' covering on portions of the dendrites of certain neurons. Although their significance is unknown, Cajal considered the spines to be dendritic devices for increasing surface area and rendering the synapse more intimate. In view of his opinion, it is interesting that the spines are most abundant on the Purkinje cell branchlets, a perfect site of 'cruciform axo-dendritic connexions'.

Golgi (1894) noted that a Purkinje cell is easier to draw than to describe. The richness of its ramifications matches the richness of the related parallel fibre plexus (Pl. 1, figs. 1, 2, here only partially impregnated). Reflecting on the dendritic arborizations of the Purkinje cells, flattened in a plane at right angles to the dense stream of parallel fibres, and the extensive dendritic overlap of the Purkinje cells, Fox & Massopust (1953) concluded that this anatomical arrangement allows maximal convergence and maximal divergence in minimal space.

In the present study, an attempt was made to obtain some quantitative notion of this convergence and divergence by determining the density of the granule cells in the granular layer, the lengths of the parallel fibres, the spacing of the Purkinje cells, and the surface area of their spiny branchlets.

MATERIALS AND METHODS

The morphological observations reported here are based on a study of the extensive collection of Golgi preparations of the cerebellar cortex of the adult monkey (*Maccaca mulatta*), prepared by the modification of Fox, Ubeda-Purkiss, Ihrig & Biagioli (1951), available in this laboratory. The total length of a Purkinje cell's spiny branchlets was determined by the photographic cut-out method and the Chalkley

(1943) method. The latter gives the percentage of volume a particular element occupies in a histological section. The diameters of the branchlets and the spines were measured in sections under oil immersion with the ocular micrometer and on enlargements of oil-immersion photomicrographs.

Cell counts were made in formalin-fixed, cresyl-violet (B.D.H.) sections and the data obtained were corrected by the formula 'section thickness divided by section thickness plus nucleolar diameter' (Abercrombie, 1946; see also Agduhr, 1941; and Floderus, 1944). The actual thickness of the sections was determined under oil immersion by a series of measurements with the micrometer scale of the fine adjustment on a Zeiss Opton Microscope.

To ascertain the number of Purkinje cells per mm.^{2} of Purkinje cell layer, chamber and direct counts were employed. In the former the ocular micrometer was lined along the Purkinje cell layer and the number of cells with visible nucleoli within the length of the micrometer scale was noted. In the latter the cells with visible nucleoli were enumerated in predetermined lengths of the Purkinje cell layer, which were accurately determined with a map-measuring wheel on photographic enlargements of the same sections.

The number of granule cells per mm.^{3} of granular layer was determined by chamber and Chalkley counts. The average volume of a granule cell nucleus was calculated by direct measurements in sections and on photographic enlargements, and this value was used to convert the Chalkley ratio into absolute numbers.

The Golgi and Nissl materials used were fixed by perfusion of 10% formalin. The Golgi material was prepared far in advance of the present study; hence, the volume changes due to processing are unknown. No corrections for volume changes were made in the Nissl preparations, since the data obtained from both types of preparations were to be employed in common calculations. Thus, our results for the number of granule cells per mm.^{3} of granular layer and the Purkinje cells per mm.^{2} of Purkinje layer hold only for our fixed preparations.

OBSERVATIONS AND RESULTS

Purkinje cells in Golgi material. Cajal's (1911) description of the Purkinje cell dendritic pattern can easily be confirmed in the present Golgi material. Primary, secondary and tertiary smooth branches, successively slimming as they spread in a single plane and gradually ascend, form the basic framework and they give off (Pl. 2, fig. 8) the spine-laden, terminal branchlets. The branchlets, moderately branched and roughly the same in length, arise mostly from tertiary branches, but some of them, particularly the lowermost, take origin from secondary and primary branches.

In sharp contrast to the smooth branches, which never descend and never reach the pial surface, the inferior branchlets descend to a line running through the summits of the Purkinje cell bodies. The superior branchlets ascend to the pial surface, where some of them recurve inferiorly. Thus the branchlets and the parallel fibres are coextensive in distribution. Transversely, the branchlets extend beyond their smooth branches of origin and in intermediate regions span the spaces between the smooth branches, converting the dendritic system of each Purkinje cell into a flat,

sieve-like plate, which lies, without exception, broadside in the transverse direction of the folia. This orientation of the dendrites was known by the older anatomists, Stieda (1864), Obersteiner (1869) and Henle (1879), who revealed the smooth branches by the old carmine method. Strictly speaking, the smooth branches do form a fundamental plane, but Golgi preparations show clearly that this is not rigidly true for the branchlets. The latter are in planes two or three deep and some of them leave at slight angles to these planes. Yet owing to their thinness this does not noticeably increase the thickness of the dendritic formation. Cajal (1911) considered the staggered arrangement of the branchlets to be a further means of assuring contact with as many parallel fibres as possible.

Conspicuous oval spaces (Pl. 1, fig. 1, *s*) within the dendritic arborizations lodge blood vessels and stellate cell bodies (Cajal, 1911). We have never seen vessels here, but in some of our preparations cell bodies of unimpregnated stellate cells, tinged a chrome-yellow, are recognizable. We have also observed that a smooth branch always forms one boundary of these spaces. This is interesting in view of the recent disclosure of Scheibel & Scheibel (1954) that the climbing fibres, which run outward on the smooth branches, have processes contacting stellate cell bodies.

To appreciate the size relationships of Purkinje cell peridendritic spines, it is well to compare them with other structures and with the spines on other dendrites. For this reason all the photomicrographs on Pls. 2 and 3 were taken under oil immersion (obj. 100 × ; N.A. 132) and reproduced at the same magnification, except Pl. 2, fig. 7, which was taken under high power (obj. × 40; N.A. 0·75).

The spines emerge from the surface of the dendrites as straight, thread-like, protoplasmic processes, terminating in a spherule or knob. This is typical of their form on pyramidal cells of the cerebral cortex. They are shown on a branch of an apical dendrite (Pl. 2, fig. 3) and the dendritic shaft (Pl. 2, fig. 4) of a small pyramidal cell. On the dendrites of cells in the caudate nucleus (Pl. 2, fig. 5) they are more robust. The details of these processes can readily be observed under the high power of the microscope, but this resolution is inadequate for the proper study of the spines on the Purkinje cell branchlets. There they are too numerous and their thread-like processes are so short that the knob-ends appear to be on the surface of the branchlets. A careful study under oil immersion, however (Pl. 2, figs. 6, 8), reveals that these knob-ends are attached to the branchlets by a delicate process.

The knob-ends of the Purkinje cell dendritic spines are rather uniform in size, though this may not seem to be the case in Pl. 2, figs. 6 and 8, where the depth of field is extremely short and the spines are not all at the same level. Occasionally, in certain preparations, portions of the impregnated dendritic mass, for reasons unknown to us, have a crushed appearance and the knob-ends are dispersed like gunshot. In such fields (Pl. 2, fig. 7, high power), where the knob-ends are spread out in the same plane, their uniformity in size is striking.

Measurements of the spiny branchlets and the spines; density of the spines. In enlargements of oil-immersion photomicrographs of a number of different Purkinje cell dendrites a search was made for portions of spiny branchlets with spines in perfect focus, in order to measure the distance from the tip of one spine to the tip of the spine on the opposite side of the branchlet. Thirty such measurements reveal that this distance averages 2·9μ. This was confirmed by direct ocular micrometer

measurements. Thus it is safe to assume that a cylindrical sheath 3μ in diameter will enclose a spiny branchlet.

The diameters of the spiny branchlets, without their spines, are 1μ, and the diameters of the knob-ends of the spines, although they are not perfect spheres, are approximately 0·7μ.

From direct ocular micrometer measurements, under oil immersion, it was concluded there are from 14 to 16 spines per 10μ length of branchlet, or an average of 15 spines per 10μ length of branchlet.

Estimation of the total length of the spiny branchlets on a Purkinje cell. In estimating the length of the spiny branchlets by the photographic cut-out method a well-impregnated Purkinje cell (Pl. 1, fig. 1), with dendrites nearly parallel to the thick Golgi section, was photographed at four different focal planes, equidistant from each other, with the lens combination: Zeiss objective (25×; N.A. 0·63); ocular 12·5×, the highest power that would include the entire dendritic spread in a single field. The profile depth of the dendritic plate, at any one place, is approximately 10μ, but since all parts of this cell are not perfectly parallel to the plane of focus, the distance from the plane of the first photograph to the plane of the last photograph is 16μ. A few branchlets in the lower right-hand corner are in focus in the uppermost plane, and a few branchlets in the upper left-hand corner are in focus in the lowermost plane. Enlargements (32 × 40 in.) were made and the branchlets sharply in focus cut out. The weight of the cut-outs, compared with the weight of a known area of similarly treated photographic paper, indicated that the excised spiny branchlets represent an area of 47,000μ^2, or a total length of 15,600μ of branchlets 3μ in diameter. Another factor considered was the depth of field in the photographs. According to standard formulae for deriving depth of field (see Beadle, 1940; Brattgard, 1954) the lens combination used gives a depth of field of approximately 1μ. Allowing that the excisions may not always have been at the exact limits of sharp focus, calculations were made on the assumption that the cut-outs in each photograph represent 1·5μ of the total depth of 16μ. Therefore, in the four photographs the spiny branchlets were cut out for a depth of 6μ, or 37·5% of the total depth. The total length of the branchlets is then 41,700μ, a figure which correlates well with two Chalkley counts made on the spiny branchlets of the same cell by two different observers, working independently.

The first Chalkley count, referred to here as a random Chalkley count, was made under oil immersion with five pointers in the ocular. Arbitrarily the tip of one pointer was focused on a spiny branchlet and counts were made only at the tips of the other pointers, throughout all regions of the dendritic spread. The counts were periodically plotted on a graph, and when the curve levelled off, the hits were: 33% spiny branchlets, 7% smooth branches, and 60% unimpregnated space. The latter represents the space for parallel fibres, stellate cell bodies, and possibly portions of some of the elements sandwiched between the Purkinje cells: namely, the stellate cell dendrites, their axons, the dendrites of Golgi cells, and the Bergmann fibres. Since the Chalkley method gives only the percentage of volume a particular structure occupies, to compute the total length of spiny branchlets, it is necessary to know the volume of space in which the dendrites are spread. This was found to be 280 × 310 × 10μ, or 868,000μ^3; therefore, the volume of the spiny branchlets is 289,000μ^3.

This volume divided by the cross-sectional area of a tubule 3μ in diameter makes the length of the branchlets $40{,}900\mu$.

Since an accurate estimation of volume is important in calculations involving the Chalkley method, the second count was made in a predetermined volume ($300 \times 400 \times 10\mu$, or $1{,}200{,}000\mu^3$), which included and exceeded the volume in which the dendrites are disposed. To obtain equal samplings throughout this volume, the movements through all sectors were carefully controlled by the micrometers on the mechanical stage and the fine adjustment. These movements were systematic and supplied the necessary arbitrary element in this procedure, so that it was possible to make counts at the tips of all five pointers. The results were: spiny branchlets 23%, smooth branches 6·2%, and unimpregnated space 70·8%. The volume of the branchlets then is $276{,}000\mu^3$ and their length is $39{,}400\mu$.

The average of these three determinations is $40{,}700\mu$; thus in subsequent calculations all the branchlets were considered as one branchlet of this length. The problem of branching was disregarded because the surface area consumed by branching is more than regained by the free-ends of the branchlets; moreover, we knew of no way of gathering data to make accurate allowances for this factor.

Such a branchlet, cylindrical in shape and 1μ in diameter without spines, has a surface area of $128{,}000\mu^2$, and if there are 15 spines per 10μ length of branchlet, it has a total of 61,000 spines. If the knob-end of each spine is a spherule $0{\cdot}7\mu$ in diameter, each knob-end has a surface area of $1{\cdot}5\mu^2$ and the spines contribute a surface area of $93{,}900\mu^2$, making the combined surface area of the branchlets and the spines $222{,}000\mu^2$. The delicate filament attaching each knob-end of a spine to a branchlet was not considered; its extreme fineness renders measurements impractical, and any loss of surface area due to its attachment at the branchlet and at the knob-end of the spine must be more than compensated for by the surface of the filament itself.

The density of the Purkinje cell bodies in the Purkinje layer

Henle (1879) directed attention to the spacing of the Purkinje cell bodies and observed in many places that they are separated by less than the diameter of a single cell, and that elsewhere the separation may be three or four times this distance. In the present study we could find no significant difference between Purkinje cell counts made in mid-line sagittal sections and in sagittal sections through the mid-lateral extent of the cerebellum, but we did find, in agreement with Henle (1879) and Obersteiner (1888, 1890), that the cells are usually closer together at the summits of the folia than they are at the depths of the sulci. Obersteiner attributed this to the proportional amount of free surface exposed, '...since each cell has to provide for an equal segment of the cortical surface..., on the convexity, the superficial area is greater than it is in the concavity, the number of Purkinje cells varies accordingly'. In sections of the cerebellum the folia differ in shape and size and it is difficult to decide where to start and stop counting at the convexities and concavities of the folia. But in counts made it was estimated that the Purkinje cell ratio in the two regions is approximately four to three, respectively.

The Purkinje cell bodies form a single sheet of cells at the molecular-granular layer junction. To determine their density per mm.^{2} of this cellular sheet, counts

were made covering all portions of the Purkinje cell layer, i.e. in the fundi, banks and summits of the folia. The results were: chamber counts, 530 Purkinje cells per mm.^{2} (59 cells counted); direct count, 510 Purkinje cells per mm.^{2} (263 cells counted). Weighing these results in proportion to the cells counted and rounding the figure there are 510 Purkinje cells per mm.^{2} of Purkinje layer.

The granule cells in Nissl and Golgi preparations. The granule cells are prodigious in numbers. Unlike other neurons, their nuclei (Pl. 3, fig. 14) contain deeply staining clumps of chromatin, and their cytoplasm is devoid of recognizable Nissl substance. But comparing an impregnated cell (Pl. 3, fig. 13) with the nuclei (Pl. 3, fig. 14) gives some indication of the perikaryon's extent.

Three to six slender dendrites stem from the nearly spherical cell body and terminate in short digit-like branchlets, which sprout small protoplasmic processes (Pl. 3, fig. 13) comparable in size and structure to the dendritic spines on the cells of the caudate nucleus (Pl. 2, fig. 5). Occasionally a dendrite divides; then each resulting branch bears a set of digitiform endings.

The digitiform endings come into relationship with the mossy fibre rosettes; several or more of them from as many different granule cells converge on and contact a single rosette. We have observed a rosette articulating with the digits from three granule cells, but undoubtedly larger rosettes accommodate the digits of even more granule cells.

The sites of these complex synapses are the cerebellar islands, the clear spaces (Pl. 3, fig. 14, *i*) in Nissl preparations; here Golgi cell axons, as well as mossy fibre rosettes and granule cell digits, are involved in an intricate relationship. The granule cell bodies, clustering close together, are aggregated around the cerebellar islands. Each island, therefore, is in position to have granule cell dendrites converging on it from all directions, and each cluster of granule cell bodies, with dendrites protruding in all directions, is in position to radiate dendrites to several or more nearby cerebellar islands. The lengths of the dendrites supply some clue to the spacing of the cerebellar islands, since the length of a dendrite is dependent on the position of the cell body in the cluster and the distance it must travel to reach a rosette. We have reason to believe that a granule cell may send one of its dendrites to a more distant island, for we have observed on a few occasions granule cell dendrites of unusual length, six to seven times as long as the dendrite shown in Pl. 3, fig. 13.

The axons of the granule cells emerge either from the cell body or from a dendrite. If the latter is the origin, it is usually an ascending dendrite and not a horizontal or descending dendrite, which illustrates one of Cajal's (1909) generalities on the nervous system, the 'law of economy of matter'. The axons ascend to the molecular layer where they bifurcate T-shape (Pl. 3, figs. 10, 12) and run parallel to the long axis of the folium; hence, the designation (Cajal, 1911) 'parallel fibres'. In general, cells low in the granular layer give rise to inferior parallel fibres, and cells high in the granular layer give rise to superior parallel fibres (Cajal, 1911). This can easily be confirmed in the present material.

According to Cajal (1911) the parallel fibres vary in diameter between 0·5 and 0·2 μ, and he observed (Cajal, 1903) that only the parallel fibres in the lower third of the molecular layer stain in reduced silver preparations. The refractoriness of the superior parallel fibres to reduced silver suggested to Cajal (1926) that they were

thinner than the inferior fibres. Fox, Ubeda-Purkiss & Massopust (1950) demonstrated that the calibre of the parallel fibres gradually decreases from below upwards in the molecular layer. Pl. 3, fig. 12, shows an inferior parallel fibre slightly more than 1μ in diameter, and Pl. 3, fig. 10, shows a superior parallel fibre slightly less than $0{\cdot}2\mu$ in diameter. Both fibres are from the same field and, in the interval between them (not shown here), there are two other bifurcating granule cell axons, intermediate in size.

The parallel fibres maintain the plane adopted at the bifurcation, and their general direction is not altered by the short flexuosities imposed upon them by the innumerable dendritic processes through which they thread their way. These flexuosities are more pronounced in sections parallel to the molecular surface (Pl. 2, fig. 9) than they are in sections perpendicular to the molecular layer (Pl. 3, figs. 10–12).

The existence of synaptic endings, described by Estable (1923), on the parallel fibres in the form of boutons and warty rosettes can be confirmed in our preparations (Pl. 2, fig. 9; Pl. 3, figs. 10, 11). In addition, we have observed hook-like endings (Pl. 2, fig. 9, left side). Since they are apparent only in sections cut parallel to the surface of the molecular layer, we are inclined to believe that the warty rosettes are nothing more than hook-like endings cut in such a fashion as not to reveal their true form. For example, if the parallel fibre (Pl. 3, fig. 10) were viewed in a section parallel to the surface of the molecular layer, the warty endings seen on the left side of this figure might well have the appearance of the hook-like endings seen on the left side (Pl. 2, fig. 9).

We have never observed synaptic endings at or near the parallel fibres' T-shape bifurcation. Note the absence of endings on the fibre (Pl. 3, fig. 12). The first synaptic ending seen on the direct continuation of this fibre (Pl. 3, fig. 11) is 90μ from the point of bifurcation. However, we hesitate to draw conclusions concerning the distribution of the endings because one never knows how complete impregnations are in Golgi material.

The farthest we have traced a parallel fibre from its point of bifurcation is 1 mm. in each direction, but we were uncertain whether the actual terminations had been reached. Another observation has convinced us that even the heaviest, and presumably the longest, parallel fibres do not go much beyond this in the adult monkey. Carefully following, under oil immersion, one arm of a heavy-calibre inferior parallel fibre, similar to the fibre in Pl. 3, figs. 11, 12, we were able to observe that it maintained its usual thickness and displayed warty rosettes and sessile boutons for a stretch 90–950μ from the bifurcation point; then it thinned appreciably and 1500μ from the bifurcation point it was barely perceptible, leading us to believe its termination had been reached. Assuming the opposite arm of this fibre, cut off in section, is equally extensive, the combined length of the arms of this fibre is around 3 mm.

We feel rather certain that the maximal length of the parallel fibres is approximately 3 mm., and that some fibres are at least 2 mm., but we are not so certain of the minimal length. It is not unusual to follow one arm of a superor, thin, parallel fibre from 400 to 500μ, which would indicate the range of the two arms is approximately 1 mm. or slightly less.

To determine the average length of the parallel fibres from the data obtained with

the Golgi method is impossible. For this reason calculations were made on the basis that the average length is 2 mm. or 1·5 mm. However, we believe the average length is nearer 1·5 mm. than 2 mm., because there are many more of the shorter superior parallel fibres.

Ratio of the granular layer to the molecular layer; average thickness of the granular layer; density of the granule cells. The ratio of the cross-sectional area of the granular layer to the cross-sectional area of the molecular layer was determined by Chalkley counts in a series of sagittal sections. Representative regions were surveyed from the mid-line to the lateral extent of the cerebellum and the ratio found was: molecular layer, 1·5; granular layer, 1.

The granular layer is thicker at the summits than at the troughs of the folia; undoubtedly this reflects the fact that there are more Purkinje cells at the convexities of the folia. Its average thickness, 0·2 mm. in cell-stained sections, was calculated by measuring its area on photographic enlargements and dividing by the length of the Purkinje cell layer. Direct measurements along the straight part of the folia indicate this figure to be of the proper magnitude.

The number of granule cells per mm.^{3} of granular layer is: chamber counts—2·35 million granule cells per mm.^{3} (approximately 2000 cells counted); Chalkley counts—2·44 million granule cells per mm.^{3} (approximately 2000 cells counted), giving a weighted average of 2·4 million granule cells per mm.^{3} of granular layer.

Beneath each mm.^{2} of the Purkinje cell layer (beneath every 510 Purkinje cells) there are (2·4 million × 0·2 mm.) 480,000 granule cells, or 960 granule cells for every Purkinje cell.

The convergence and divergence of the parallel fibres. How many parallel fibres come within the dendritic spread of a Purkinje cell such as shown (Pl. 1, fig. 1)? There are on an average 480,000 granule cells below each mm.^{2} of Purkinje cell layer, and the cell in question has a transverse dendritic spread of 0·31 mm. The number of fibres that could occupy the same area as these dendrites should then be the product of 480,000 × 0·31 × the average length of the parallel fibres. If the latter is 1·5 mm., the number of fibres is 223,000 and if it is 2 mm., the number is 297,000.

Calculations can also be made from another point of view. The ratio of the granular layer to the molecular layer is 1 to 1·5. Assuming the elements of the two layers are related to each other in the same ratio, the area occupied by the dendrites (Pl. 1, fig. 1) (310 × 280 μ or 86,800 μ^2) reduced by two-thirds and multiplied by the average length of the parallel fibres should give the volume of the granular layer with granule cells related to this area of dendrites. Calculated this way, if the average length is 1·5 mm., there are 208,000 fibres, and if it is 2 mm., there are 278,000 fibres.

Since there are 510 Purkinje cells in a mm.^{2} of the Purkinje cell layer, and since the transverse spread of a Purkinje cell's dendrites is at least 0·3 mm., a single parallel fibre coursing through the molecular layer can contact the branchlets of Purkinje cells in a patch 0·3 mm. in width. Parallel fibres 3, 2 and 1·5 mm. in length can diverge to 460, to 310 and to 230 Purkinje cells respectively.

DISCUSSION

The spines, also known as thorns or gemmules, have been neglected in recent times. They have been considered artefacts produced by the Golgi method (Kölliker, 1896; Meyer, 1895, 1896), endfeet implanted on the dendrites (Held, 1897; Bodian, 1940), and dendritic collaterals (Cajal, 1909). Hill (1897) concluded that they belong both to the dendrites and to the afferent fibres and logically added: 'If my interpretation of the thorns is the right one, we are brought back to the theory of Gerlach....'

The spines have been stained by methods other than the Golgi technique. Cajal (1896) and Turner (1901) revealed them by modifications of the Ehrlich method, and Hill (1896) observed that they are distinct in sections, deeply stained with haematoxylin and then broken up with needles in glycerine. Hatai (1903) devised a procedure in which small pieces of nerve tissue, simultaneously fixed and stained in a mixture of acid fuchsin, formalin, acetic acid and picric acid, renders the ordinarily achromatic portions of axons and dendrites visible. The gemmules, according to him, are merely local extensions of the dendritic ground substance, and internal staining differences distinguish them from the axonic terminals.

Cajal (1909) contended that the constancy of the spines in the same locations on the same neurons proves their pre-existence, and he argued that the impossibility of staining them by neurofibrillar methods, which stain the endfeet, indicates that the spines and the endfeet are not identical structures. In his last monograph Cajal (1934, 1954) reiterated his strong conviction that the spines are dendritic collaterals and emphasized that though always thin proximally, they are enlarged distally into a bulb or knob, while the reverse is true of the endfeet.

Although there is no unanimity as to what the spines are, we believe they are dendritic collaterals occurring in regions where the dendrites meet oblique or cruciform afferent fibres. If, on the other hand, they are synaptic endings, it is possible to determine the number of parallel fibres on a Purkinje cell's branchlets by more or less direct methods. Our estimate, then, of the number of spines, 61,000, would be the number of parallel fibres converging on a Purkinje cell. But actually the structure of the parallel fibre endings destined for the branchlets is not known. The spines (Pl. 2, figs. 6, 8) are uniform in size and smaller than the hook-like, sessile bouton and warty rosette, parallel fibre endings (Pl. 2, fig. 9; Pl. 3, figs. 10, 11), and this might be considered good evidence that the spines are not synaptic terminals. But lest this be construed as conclusive proof, it is well to remember that parallel fibres synapse also on the dendrites of Golgi cells and the dendrites of the various stellate cells. Therefore, the possibility exists that the endings specifically for the Purkinje cell branchlets may not be revealed in our preparations.

In Golgi preparations (Pl. 1, figs. 1, 2) the number of parallel fibres converging on a Purkinje cell's branchlets is enormous. To count these fibres directly in a segment through the width of the molecular layer, however, is impossible; reduced silver techniques stain only the large inferior parallel fibres, and Golgi preparations, with their sporadic staining, are not good for quantitative analysis. Thus it was necessary to take recourse to indirect methods in arriving at some numerical notion of the convergence on a Purkinje cell's branchlets.

Incidentally, when two different types of calculations were made on the basis that

the parallel fibres' average length is 1·5 mm., the number of fibres (223,000 and 208,000) passing through the dendritic spread of the Purkinje cell approximates the 222,000 μ^2 estimated for the surface areas of the same cell's branchlets and spines. How many of these fibres contact spiny branchlets is impossible to say, but the staggering of the branchlets suggests they are set to synapse with as many fibres as possible. The calculated total length of the branchlets (40,700 μ) emphasizes that the branchlets are arranged in several planes, for if these same branchlets, 3 μ in diameter, were in lengths 280 μ (i.e. height of the dendritic system) and arranged side by side in a single plane, they would extend for 430 μ, a distance considerably greater than the dendritic spread of the cell. Not many fibres, it would seem, could work their way through this system, and miss the branchlets. Yet (considering the smallest number calculated, 208,000) if only half or one-third make contact, there would be in the first instance 104,000 and in the second instance 69,000 parallel fibres converging on a single Purkinje cell. The latter figure is close to the number estimated for the spines. Incidentally, Lorente de Nó (1934), who was undecided whether the spines are part of the dendrites or endings impregnated without their fibres, had no doubt each spine is a synapse. Certainly if the spines are dendritic collaterals for increasing receptive surface and rendering the synapse more intimate, there must be at least one synapse for each spine and the number of spines represents the least possible number of endings on the spiny branchlet.

Why thousands of fibres are in touch with a Purkinje cell's terminal spiny branchlets is unknown, but it may be meaningful in the light of the recent disclosure that dendrites have properties different from those of the cell bodies and axons. Clare & Bishop (1955*a*, *b*), studying intercortical paths activating only the apical dendrites of the cortical pyramids, have obtained evidence indicating that dendrites in the cerebral cortex do not behave as all-or-nothing conductors. Repetitive stimulation maintains them in a persistent state of negativity. They have no absolutely refractory period, and the duration of their response is much longer than that of the cell bodies and axons. They modulate the activity produced in cell bodies by afferent stimulation of cell bodies and they are capable of sustaining local activity at a stimulated region without propagating impulses to the cell body or axon. Interestingly, these disclosures were made in paths synapsing like the parallel fibres on Purkinje cell branchlets—both are exclusively axodendritic and both end on terminal spiny branchlets.

Is the fantastic number of parallel fibres on Purkinje cell branchlets responsible for the fast activity of the cerebellar cortex? Adrian (1935) and Dow (1938) have shown that the electrical discharge frequency of the cerebellar cortex is one of the fastest in the central nervous system. Snider & Eldred (1948), by isolation experiments, demonstrated that this rapid activity results from intrinsic rather than extrinsic mechanisms. Brookhart, Moruzzi & Snider (1951), with microelectrode recordings of unit activity, have found this activity to originate from structures in the Purkinje cell and/or granule cell layers. If, for the moment, this activity is speculatively assigned to the Purkinje cells, say as a result of their rich connexions with parallel fibres, the silence Brookhart *el al.* encountered in the molecular layer, while probing with microelectrodes for unit spike activity, may be explained by the dendritic properties Clare & Bishop disclosed.

The estimates that parallel fibres 3 mm. in length, 2 mm. in length and 1·5 mm. in length diverge to 460, to 310 and to 230 Purkinje cells respectively, presume that a fibre synapses with all the branchlets protruding in its pathway from the greatest number of cells possible. Regarding the maximal lengths of the parallel fibres, it is interesting that Dow (1949), recording potentials along the axis of the parallel fibres, was unable to obtain records when the stimulating and pick-up electrodes were separated by a distance greater than 5 mm. There is, perhaps, no great discrepancy between our results and those of Dow, if the technical difficulty of having stimulating and recording electrodes so close together and the possibility of stimulus spread are taken into consideration.

Convergence and divergence are responsible for central overlap in the nervous system. Sherrington (1929) used central overlap to explain the phenomena of occlusion in motor reflexes and, undoubtedly, much remains to be explained by central overlap. Lorente de Nó (1934), observing what he called the 'partially shifted' overlap in all centres studied, concluded it must be highly significant and, speculating on its possible physiological role, elaborated on its participation in the projection to and the parcellation of functional fields.

It is difficult to conceive of an arrangement better suited for convergence and divergence than the overlapping, sieve-like plates of the Purkinje cell dendrites flattened in the dense stream of the parallel fibres. It is an architecture ideal for central overlap. In the folia this overlap is fixed transversely by the width of the Purkinje cell dendrites and longitudinally by the lengths of the parallel fibres. Transversely, cell bodies separated from each other by a distance of 300μ easily share in common some fraction of their synaptic pools, while longitudinally, with the composition of the parallel fibre plexus ever changing by the addition and termination of fibres of varying lengths, there is from cell to cell an exquisite gradation in the fractionation of synaptic pools. Cells separated from each other by a distance of 3 mm. in the longitudinal direction share some fringe of fibres in common.

The axons of Golgi cells and mossy fibres converge on granule cell dendrites. Cajal (1911) and Scheibel & Scheibel (1954) have shown that a single mossy fibre sends branches to adjacent folia. Within the granular layer these branches ramify and each ramification has a series of rosettes. Since there are three or more granule cells contacting each rosette, it is clear that a single mossy fibre diverges to a large number of granule cells. But, on the other hand, each granule cell, having three to six dendrites, is in position to have converging on it as many different mossy fibres as it has digitiform endings. Thus there is convergence on a neuron as small as a granule cell. Single unit recordings in the brain stem reticular formation (Scheibel, Scheibel, Mollica & Moruzzi, 1955), in all the nuclei of the amygdaloid complex (Machne & Segundo, 1956) and in the lenticular nucleus and the claustrum (Segundo & Machne, 1956) have demonstrated that there is widespread convergence of impulses on individual neurons, even from the opposite ends of the central nervous system. To record from a single granule cell would certainly be a technical feat, but it would be interesting to know, for example, in the anterior lobe of the cerebellum, where there is some possibility of spino-cerebellar and cortico-pontocerebellar convergence, if individual granule cells may be stimulated from such widely separated sources.

SUMMARY

The primary, secondary and tertiary smooth dendrites of the Purkinje cells sprout terminal spiny branchlets, which are commensurate and coextensive with the parallel fibre plexus generated by the granule cell axons. The parallel fibres form 'cruciform axo-dendritic connexions' with the Purkinje cell spiny branchlets. The overlapping of the Purkinje cell dendrites and the rigid orientation of the Purkinje cells and the parallel fibres results in an arrangement in which there is maximal convergence and maximal divergence in minimal space. In the present study of the cerebellar cortex in the adult monkey (*Maccaca mulatta*) an attempt was made to derive some quantitative concepts of this divergence and convergence from observations and measurements in Golgi preparations and from cell counts and measurements in cresyl-violet preparations.

In our fixed preparations there are an average of 510 Purkinje cells per mm.^{2} of Purkinje cell layer and 2·4 million granule cells per mm.^{3} of granular layer. The ratio of the cross-sectional area of the molecular layer to the cross-sectional area of the granular layer is 1·5 to 1 respectively. The average thickness of the granular layer is 0·2 mm.

The parallel fibres display hook-like, sessile bouton and warty rosette endings, which are larger than the spines on the Purkinje cell branchlets. The parallel fibres decrease in calibre and in length from below upwards in the molecular layer. The inferior parallel fibres are approximately 1μ in diameter and 3 mm. in length and the superior parallel fibres are approximately 0·2μ in diameter and 1 mm. or less in length. The thin-calibre parallel fibres are more numerous than the thick-calibre parallel fibres.

The average of three determinations for the total length of the spiny branchlets on a Purkinje cell is 40,700μ. This calculation emphasizes that the branchlets are staggered in several or more planes. These branchlets have a total of 61,000 spines. The combined surface area of the branchlets and the spines is 222,000μ^2.

It is not known for certain whether the spines are synaptic endings on the dendrites or dendritic devices for increasing synaptic surface and rendering the synapse more intimate. In either case, however, their number is a good index of the number of fibres converging on the Purkinje cell branchlets.

There are more than enough fibres passing through the area of the dendritic spread of a Purkinje cell to account for the number of spines. It was estimated (based on the average thickness of the granular layer) that if the average length of the parallel fibres is 1·5 mm. or 2 mm., there are 223,000 fibres or 297,000 fibres, respectively, passing through the area of dendritic spread. It was also estimated (based on the ratio of the molecular layer to the granular layer) that if the average length of the parallel fibres is 1·5 mm. or 2 mm., there are 208,000 fibres or 278,000 fibres, respectively, passing through the dendritic spread of the Purkinje cell.

The enormous convergence on a Purkinje cell's spiny branchlets was discussed in the light of the recent disclosure that dendrites may not behave all-or-nothing.

It was estimated that parallel fibres 3, 2 and 1·5 mm. in length can diverge to 460, to 310 and to 230 Purkinje cells, respectively.

The central overlap in this system is limited by the transverse spread of the

Purkinje cell dendrites and by the lengths of the parallel fibres. Purkinje cells separated from each other by 300 μ in the transverse direction of the folia and by 3 mm. in the longitudinal direction of the folia can share in common some fringe of parallel fibres. The closer Purkinje cells are to each other in both directions, the more parallel fibres they share in common.

There is convergence on a neuron even as small as a granule cell, for each granule cell contacts as many mossy fibres as it has digitiform endings.

This investigation was supported by research grants B-642 (C5) and B-711 (C2) from the Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.

The authors wish to thank the following students: Miss Janice Eichman, Mr John MacRae and Mr William Mullins for their invaluable assistance. We are also indebted to Dr R. P. Loveland of the Eastman Kodak Company Research Laboratories for the aid and directions he gave us in the literature on 'depth of field' in microscopy.

REFERENCES

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239–247.

ADRIAN, E. D. (1935). Discharge frequencies in the cerebral and cerebellar cortex. *J. Physiol.* **83**, 32–33*P*.

AGDUHR, E. (1941). A contribution to the technique of determining the number of nerve cells per volume unit of tissue. *Anat. Rec.* **80**, 191–202.

BEADLE, D. G. (1940). Depth of focus of microscope objectives. *Nature, Lond.*, **145**, 1018–1019.

BODIAN, D. (1940). Further notes on the vertebrate synapse. *J. comp. Neurol.* **73**, 323–343.

BRATTGARD, S. (1954). Microscopical determinations of the thickness of histological sections. *J. R. micr. Soc.* **74**, 113–122.

BROOKHART, J. M., MORUZZI, G. & SNIDER, R. S. (1951). Origin of cerebellar waves. *J. Neurophysiol.* **14**, 181–190.

CHALKLEY, H. W. (1943). Method for the quantitative morphologic analysis of tissues. *J. nat. Cancer Inst.* **4**, 47–53.

CLARE, M. H. & BISHOP, G. H. (1955*a*). Dendritic circuits: the properties of cortical paths involving dendrites. *Amer. J. Psychiat.* **111**, 818–825.

CLARE, M. H. & BISHOP, G. H. (1955*b*). Properties of dendrites: apical dendrites of the cat cortex. *Electroenceph. clin. Neurophysiol.* **7**, 85–98.

DOW, R. S. (1938). The electrical activity of the cerebellum and its functional significance. *J. Physiol.* **94**, 67–86.

DOW, R. S. (1949). Action potentials of cerebellar cortex in response to local electrical stimulation. *J. Neurophysiol.* **12**, 245–256.

ESTABLE, C. (1923). Notes sur la structure comparative de l'écorce cérébelleuse, et dérivées physiologiques possibles. *Trab. Lab. Invest. biol. Univ. Madr.* **21**, 169–256.

FLODERUS, S. (1944). Untersuchungen über den Bau der menschlichen Hypophyse mit besonderer Berücksichtigung der quantitativen mikromorphologischen Verhältnisse. *Acta path. microbiol. scand.* **53**, 1–276.

FOX, C. A. & MASSOPUST, L. C. (1953). Relationship of the Purkinje cells to the parallel fibres *Anat. Rec.* **115**, 308.

FOX, C. A., UBEDA-PURKISS, M., IHRIG, H. D. & BIAGIOLI, D. (1951). Zinc chromate modification of the Golgi technique. *Stain Tech.* **26**, 109–114.

FOX, C. A., UBEDA-PURKISS, M. & MASSOPUST, L. C. (1950). Structure of the cerebellar cortex in the adult monkey (*Maccaca mulatta*): a Golgi study. *Abstracts of Communications, International Anatomical Congress*, pp. 69–70. Oxford.

GOLGI, C. (1894). *Untersuchungen über den feineren Bau des centralen und peripherischen Nervensystems.* Trans. by R. Teuscher. Jena: Fischer.

HATAI, S. (1903). The finer structure of the neurones in the nervous system of the white rat. *Univ. Chicago Decenn. Publ.* **10**, 179–189.

HELD, H. (1897). Beiträge zur Structur der Nervenzellen und ihrer Fortsätze. *Arch. Anat. Physiol., Lpz.*, **21**, 204–294.

HENLE, J. (1879). *Handbuch der Nervenlehre des Menschen.* Bd. **3**, Abt. 2, Braunschweig: Vieweg.

HILL, A. (1896). The chrome-silver method. A study of the conditions under which the reaction occurs and a criticism of its results. *Brain*, **19**, 1–42.

HILL, A. (1897). Note on 'thorns', and a theory of the constitution of gray matter. *Brain*, **20**, 131–137.

KÖLLIKER, A. (1896). *Handbuch der Gewebelehre des Menschen.* Bd. **2**, Aufl. 6, Leipzig: Engelmann. (Cited by A. Hill, 1897.)

LORENTE DE NÓ, R. (1934). Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *J. Psychol. Neurol., Lpz.*, **46**, 113–177.

MACHNE, X. & SEGUNDO, J. P. (1956). Unitary responses to afferent volleys in amygdaloid complex. *J. Neurophysiol.* **19**, 232–240.

MEYER, S. (1895). Die subcutane Methylenblauinjection, ein Mittel zur Darstellung der Elemente des Centralnervensystems von Säugethieren. *Arch. mikr. Anat.* **46**, 282–290. (Cited by A. Hill, 1897.)

MEYER, S. (1896). Ueber eine Verbindungesweise der Neuronen nebst Mittheilungen ueber die Technik und die Erfolge der Methode der subcutanen Methylenblauinjection. *Arch. mikr. Anat.* **47**, 734–748. (Cited by A. Hill, 1897.)

OBERSTEINER, H. (1869). Beiträge zur Kenntniss vom feineren Bau der Kleinhirnrinde, mit besonderer Berücksichtigung der Entwicklung. *S.B. Akad. Wiss. Wien*, Bd. **60**, Abt. II, 101–114.

OBERSTEINER, H. (1888). *Anleitung beim Studium des Baues der nervösen Zentralorgane im gesunden und kranken Zustände.* Leipzig and Wien: Toeplitz and Deuticke.

OBERSTEINER, H. (1890). *The Anatomy of the Central Nervous Organs in Health and in Disease.* Trans. by A. Hill. Philadelphia: Blakiston.

RAMÓN Y CAJAL, S. (1891). Sur la structure de l'écorce cérébrale de quelques mammifères. *Cellule*, **7**, 123–176. (Cited by S. Ramon y Cajal, 1909.)

RAMÓN Y CAJAL, S. (1895). *Les nouvelles idées sur la structure du système nerveux chez l'homme et chez les vertébrés.* Trans. by L. Azoulay. Paris: Reinwald.

RAMÓN Y CAJAL, S. (1896). Las espinas colaterales de las celulas del cerebro tenidas por el azul de metileno. *Rev. trim. microgr.* **1**, 123–136. (Cited by A. Hill, 1897.)

RAMÓN Y CAJAL, S. (1903). Un sencillo metodo de coloracion selective del reticulo protoplasmico. *Trab. Lab. Invest. biol. Univ. Madr.* **2**, 129–221. (Cited by S. Ramón y Cajal, 1926.)

RAMÓN Y CAJAL, S. (1909). *Histologie du système nerveux de l'homme & des vertébrés*, vol. I. Trans. by L. Azoulay. Paris: Maloine.

RAMÓN Y CAJAL, S. (1911). *Histologie du système nerveux de l'homme & des vertébrés*, vol. II. Trans. by L. Azoulay. Paris: Maloine.

RAMÓN Y CAJAL, S. (1926). Sur les fibres mousseuses et quelques points douteux de la texture de l'écorce cérébelleuse. *Trab. Lab. Invest. biol. Univ. Madr.* **24**, 215–251.

RAMÓN Y CAJAL, S. (1934). Les preuves objectives de l'unité anatomique des cellules nerveuses. *Trab. Lab. Invest. biol. Univ. Madr.* **29**, 1–137.

RAMÓN Y CAJAL, S. (1954). *Neuron Theory or Reticular Theory?* Trans. by M. Ubeda-Purkiss & C. A. FOX. Madrid: Consejo Superior de Investigaciones Científicas, Instituto Ramón y Cajal.

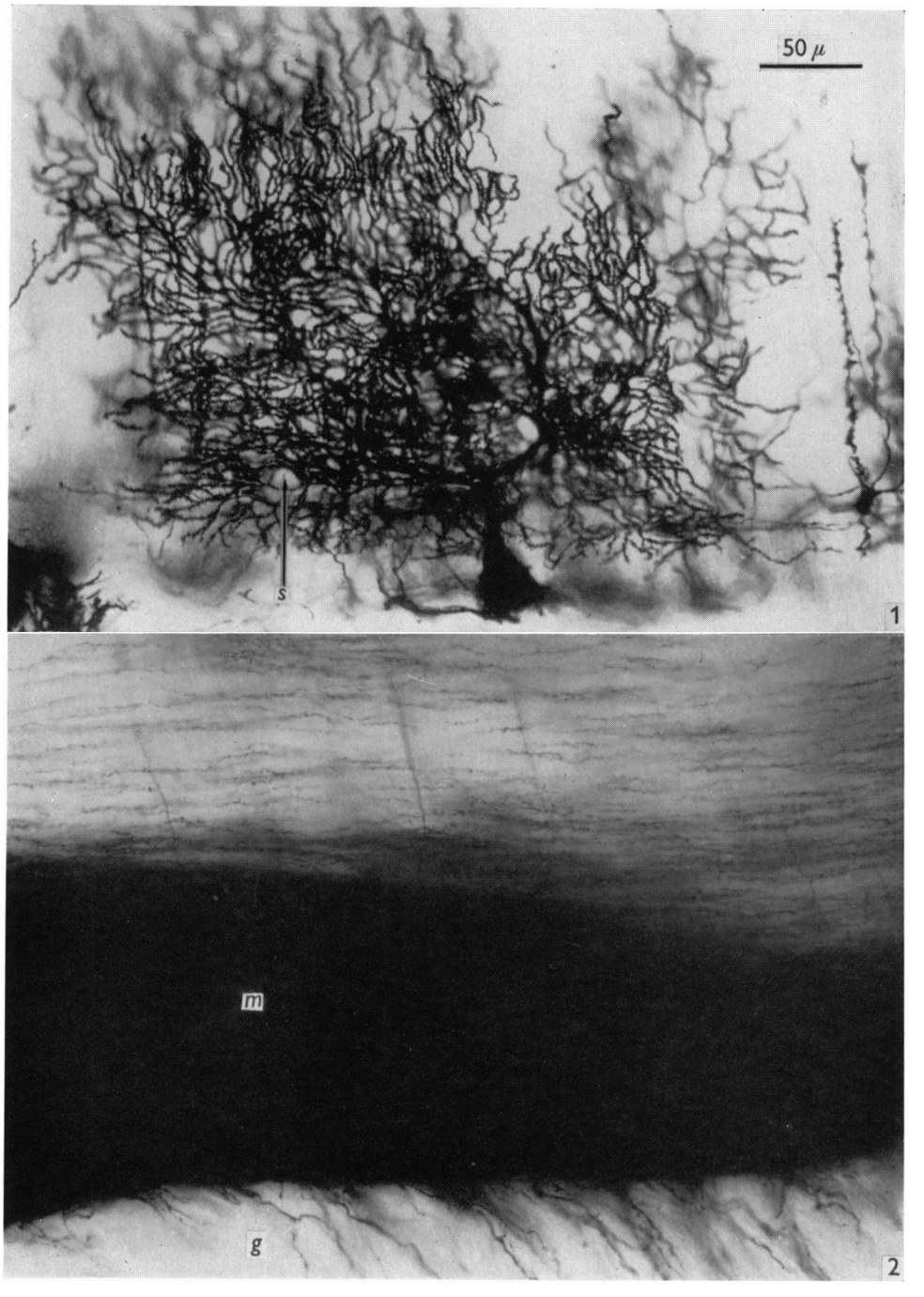
SCHEIBEL, M. E. & SCHEIBEL, A. B. (1954). Observations on the intracortical relations of the climbing fibres of the cerebellum. A Golgi study. *J. comp. Neurol.* **101**, 733–764.

SCHEIBEL, M., SCHEIBEL, A., MOLLICA, A. & MORUZZI, G. (1955). Convergence and interaction of afferent impulses on single units of reticular formation. *J. Neurophysiol.* **18**, 309–331.

SEGUNDO, J. P. & MACHNE, X. (1956). Unitary responses to afferent volleys in lenticular nucleus and claustrum. *J. Neurophysiol.* **19**, 325–339.

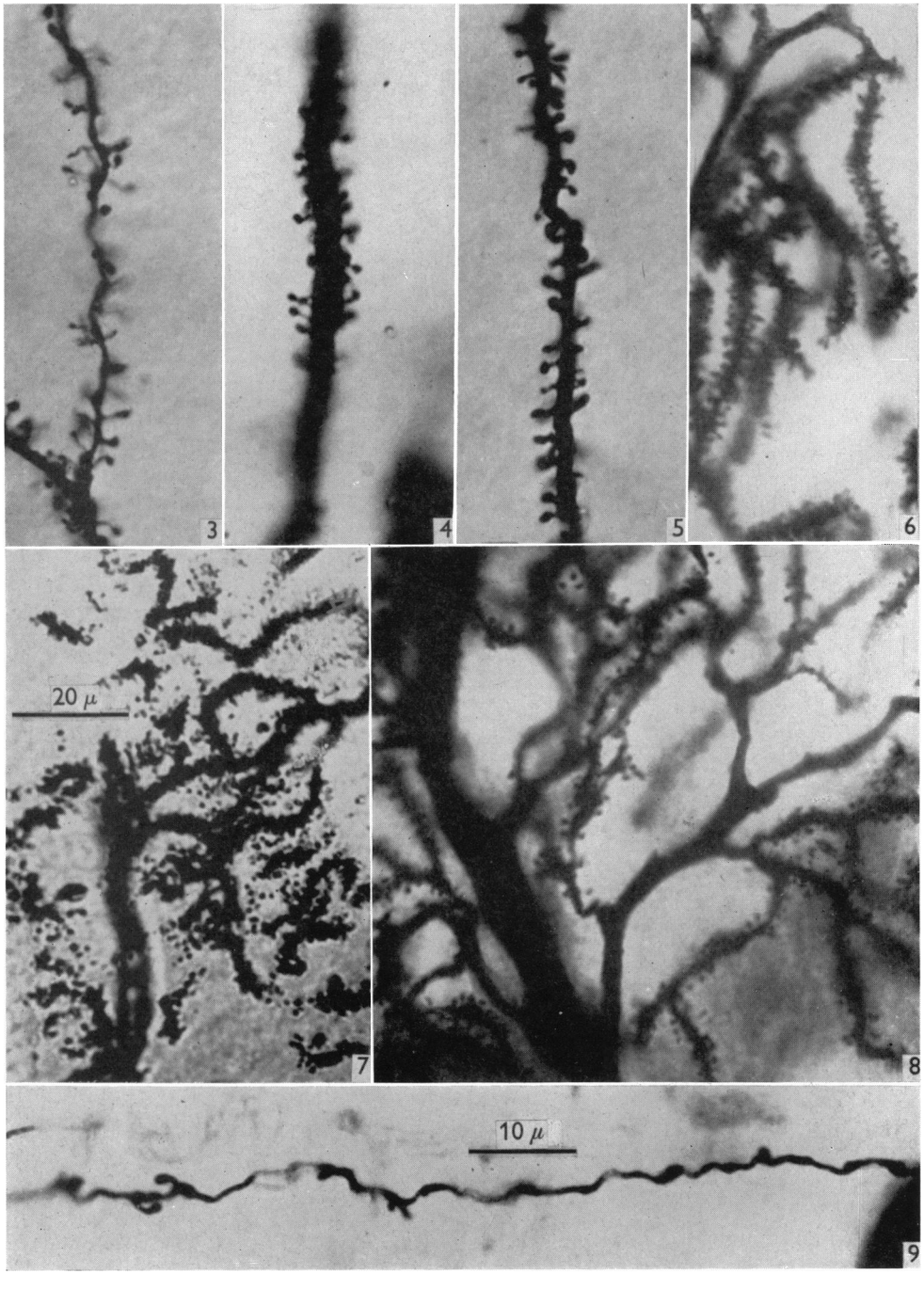
SHERRINGTON, C. S. (1929). Some functional problems attaching to convergence. Ferrier Lecture. *Proc. Roy. Soc.* B, **105**, 332–362.

SNIDER, R. S. & ELDRED, E. (1948). On the maintenance of spontaneous activity within the cerebellum. *Amer. J. Physiol.* **155**, 470.

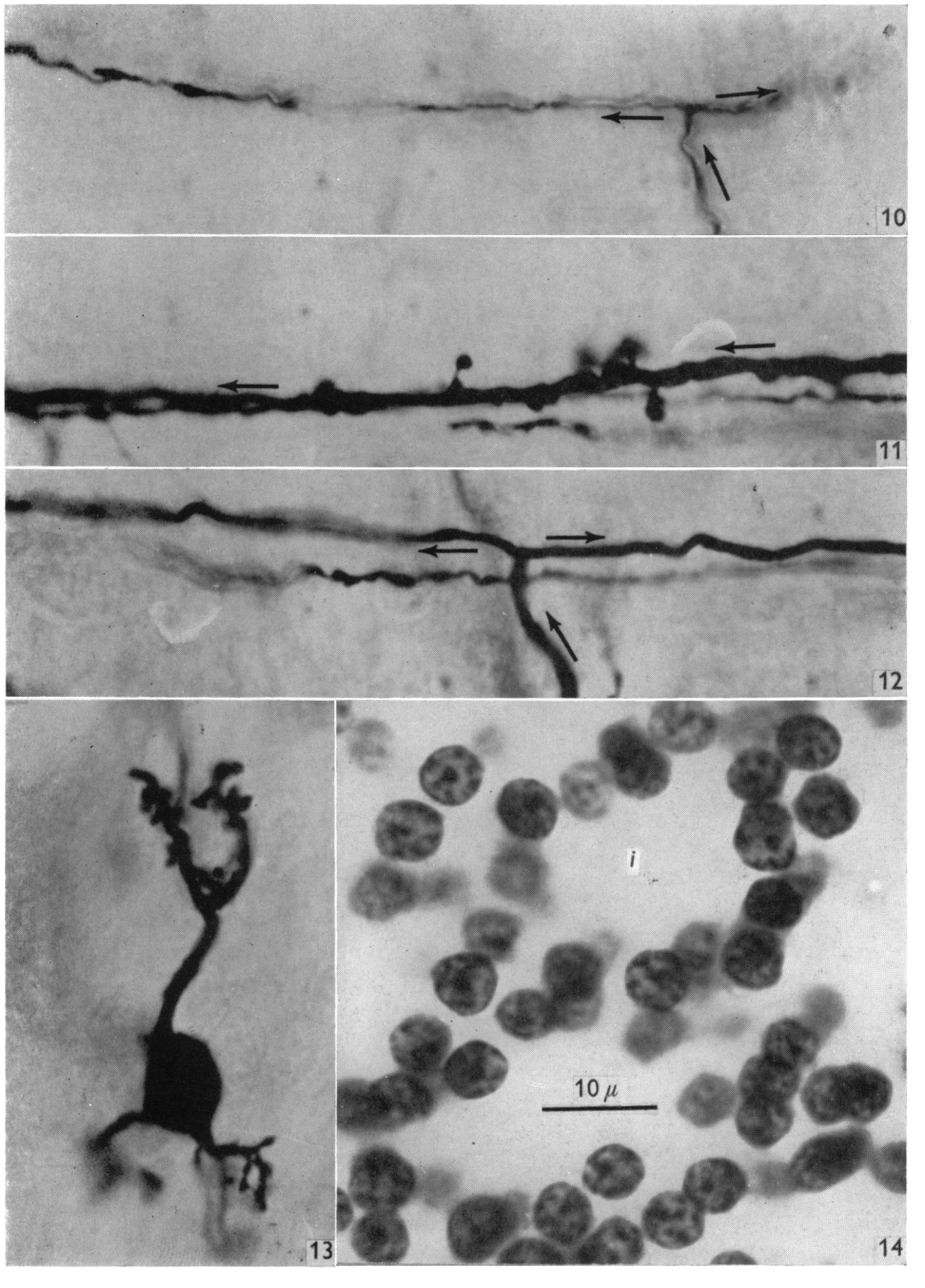


FOX AND BARNARD—QUANTITATIVE STUDY OF THE PURKINJE CELL DENDRITIC BRANCHLETS

(*Facing p.* 312)



FOX AND BARNARD—QUANTITATIVE STUDY OF THE PURKINJE CELL DENDRITIC BRANCHLETS



FOX AND BARNARD—QUANTITATIVE STUDY OF THE PURKINJE CELL DENDRITIC BRANCHLETS

STIEDA, L. (1864). Zur vergleichenden Anatomie und Histologie des Cerebellum. *Arch. Anat. Physiol., Lpz.*, 407–433.

TURNER, J. (1901). Observations on the minute structure of the cortex of the brain as revealed by the methylene blue and peroxide of hydrogen method of staining the tissue direct on its removal from the body. *Brain*, **24**, 238–256.

LIST OF ABBREVIATIONS

g	granular layer.	*m*	molecular layer.
i	cerebellar island.	*s*	space for stellate cell.

EXPLANATION OF PLATES

The photomicrographs on Pl. 1 were taken under medium power and reproduced at the same magnification. All photomicrographs on Pls. 2 and 3 were taken under oil immersion and reproduced at the same magnification, except Pl. 2, fig. 7, which was taken under high power.

PLATE 1

Fig. 1. The Purkinje cell in which the total length of the spiny branchlets was calculated. Golgi preparation.

Fig. 2. A dense impregnation of parallel fibres running longitudinally in the folia. Impregnation is incomplete in the upper portion of the molecular layer. Some concept of the convergence on a Purkinje cell's branchlets may be had by imagining this stream of fibres turned at right angles to the dendritic system of the cell in fig. 1. Golgi preparation.

PLATE 2

Fig. 3. A branch of an apical dendrite of a small cortical pyramid, showing spines. Golgi preparation.

Fig. 4. The dendritic shaft of the same cell as in fig. 3, showing spines. Golgi preparation.

Fig. 5. Dendritic spines on a cell of the caudate nucleus. Golgi preparation.

Fig. 6. The spiny branchlets of a Purkinje cell. Golgi preparation.

Fig. 7. Crushed spiny branchlets of a Purkinje cell. The knob-ends of the spines, dispersed like gunshot, are rather uniform in size. Golgi preparation. High power.

Fig. 8. A portion of the dendritic system of a Purkinje cell showing the sharp contrast between the smooth branches and the spiny branchlets. Golgi preparation.

Fig. 9. A parallel fibre in a section parallel to the surface of the molecular layer. The hook-like endings are larger than the Purkinje cell's dendritic spines. Golgi preparation.

PLATE 3

Fig. 10. A superior parallel fibre, approximately $0{\cdot}2\mu$ in diameter, showing its T-shaped bifurcation. Its warty rosette endings are larger than the spines on a Purkinje cell's branchlets. Would these endings appear hook-like, if they were cut in the same plane as the fibre in fig. 9? Golgi preparation.

Fig. 11. An inferior parallel fibre showing sessile boutons, which are much larger than the Purkinje cell spines. This fibre is the direct continuation of the fibre in Pl. 3, fig. 12. Golgi preparation.

Fig. 12. An inferior parallel fibre approximately 1μ in diameter. Notice there are no endings near the T-shaped bifurcation. The first ending on this fibre is shown in Pl. 3, fig. 11, 90μ from the bifurcation. Golgi preparation.

Fig. 13. A granule cell with one dendrite and its digitiform endings in focus. The small protoplasmic processes on the digits are comparable in size to the spines on the dendrites of cells in the caudate nucleus (fig. 5). In the background unimpregnated granule cell nuclei are visible. Golgi preparation.

Fig. 14. A thin cresyl-violet section showing granule cell nuclei clustering around cerebellar islands. There is no recognizable Nissl substance in these cells.