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Scanning Electron Microscopic Observation of the Rat Cerebellar Cortex

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Summary. **The cytoarchitecture of the rat cerebellar cortex was studied by scanning electron microscopy (SEM) after exposure of cell surfaces by NaOH maceration and subsequent mild mechanical dissection. SEM observation clearly differentiated the three layers of the cerebellar cortex: the molecular layer, which consisted of a dense accumulation of thin nerve fibers; the Purkinje cell layer, in which large flask-shaped somata of neurons were arranged in a single row; and the granular layer, which contained small round neurons and nerve fibers of varying thickness. The present method provided an *en face* view of synaptic boutons of axons resting on the Purkinje cell soma, with glial septa separating each bouton. The boutons were small, round, or elliptical in shape and disposed at large intervals.**

INTRODUCTION

The central nervous tissue comprises a compact aggregate of neurons and glial cells, both interdigitating with one another via numerous processes. Scanning electron microscopy (SEM) serves as a useful method for the analysis of such complex intercellular relationships after exposure of the surfaces of the tightly interlocked elements of cells. One pioneering approach has been made by Ohtsuki,[1] who demonstrated axo-somatic synapses in the spinal cord by a resin-cracking method.[2] However, only limited portions of the neuronal elements were exposed in his study. Sheibel et al.[3] observed the cerebellar cortex after manual dissection of specimens, while other investigators examined nervous tissues by different methods of chemical digestion.[4] Nevertheless, all these methods produced considerable destruction of the cellular fine morphology.

A successful result has been obtained by Reese et al.,[5] who exhibited nerve cells and their fine processes in the cerebellar cortex after dissociation of the cells by enzymatic digestion and subsequent maceration with OsO_4. However, their method has not been repeated by themselves or by other investigators probably because of its complicated procedure for tissue treatment. On the other hand, we applied an NaOH maceration method[6] to the SEM observation of the pericellular nerve plexus in the cerebellar nuclei,[7] and demonstrated, for the first time, reticular terminals of the plexus.

The present study extends the application of this NaOH maceration to the cerebellar cortex in order to re-examine its fine cytoarchitecture and to evaluate the utility of this method in investigating the structure of complex neuropile in the central nervous tissue.

MATERIALS AND METHODS

Adult male rats of the Wistar strain weighing 180-200 g were examined in this study. The animals were anesthetized with sodium pentobarbital and perfused through the ascending aorta with Locke's solution, followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3. The cerebellum was excised and cut with razor blades into saggital sections about 1.5 mm thick. The tissue slices were immersed in the same fixative overnight at room temperature. The cerebellar cortex was cut out of the fixed specimen, and minced into small cubes containing the whole cortical layers. After being rinsed in 0.01 M phosphate buffer (pH 7.3), the tissue blocks were macerated in 6 N NaOH for 10 min at 60°C. They were then thoroughly rinsed in the 0.01 M phosphate buffer and conductive-stained by the tannin-osmium method according to Murakami.[8] The osmicated tissue pieces were dehydrated through a graded series of ethanol, transferred to isoamyl acetate, and critical point-dried using liquid CO_2. After drying, the tissue pieces were gently pricked with a thin needle and fractured into several

Fig. 1. a. Cerebellar cortex. *M* molecular layer, *P* Purkinje cell layer, *G* granular layer. **b.** Molecular layer fractured along the long axis of a cerebellar folium. Fine parallel fibers extend horizontally. Arrows indicate bundles of granule cell axons. **c.** Purkinje cell soma extending a thick dendrite (arrow) and a thin axon (arrowhead). **d.** High magnification of the Purkinje cell axon in c. The axon displays a mild thickening during its course through a pinceau of fine fibers (arrow). a ×820, b ×5,000, c ×2,000, d ×4,300

fragments. The fractured surfaces of the specimen were evaporation-coated with gold-palladium and examined in a Hitachi HFS-2 SEM at an acceleration voltage of 10 kV.

RESULTS

The three layers of the cerebellar cortex: the molecular layer, the Purkinje cell layer and the granular layer, were able to be identified on the fractured surfaces of the tissue blocks (Fig. 1a).

The molecular layer was represented by a compact accumulation of parallel fibers extending along the long axis of the cerebellar folia (Fig. 1b). The fibers measured 0.1-0.2 μm in diameter. The stems of granule cell axons, which bifurcated into the parallel fibers, vertically ascended the molecular layer in small bundles. The axonal stems were as thick as the parallel fibers.

Immediately beneath the molecular layer, the Purkinje cell layer displayed a single row of large flask-like somata of Purkinje cells, about 15 μm in diameter. Each Purkinje cell exposed a thick primary dendrite at its apical pole and a thin axon at its base, although only the proximal 5-15 μm of the processes were observed under the SEM (Fig. 1c, d). Proximal portions of the Purkinje cell axons had a mild swelling on their course in the pinceaux of basket cell axons.

The Purkinje cell somata left round impressions on the fractured surface of the complementary tissue block (Fig. 2a). The hollows revealed presynaptic terminals of nerve fibers, with glial processes intervening (Fig. 2a, b). Some terminals on the Purkinje cell soma were small and round in shape with diameters of 0.7-1.2 μm, while others were elliptical, measuring 1.0-1.4 μm along the short axis and 1.6-4.8 μm along the long axis. The former were disseminated in whole around the Purkinje cell soma, whereas the latter were restricted to the apical half of the soma. The nerve terminals were disposed at large intervals ranging from 0.5 to 2.5 μm, and isolated from one another by wide, attenuate processes of glia. The glial processes crossed and overlapped to form a pericellular network, each mesh of which contained only one nerve terminal.

The granular layer was packed with spherical perikarya of granule cells about 5 μm in diameter (Fig. 2c). They issued an attenuate axon about 0.1 μm in diameter and thick dendrites about 0.5 μm in diameter, both of which were columnar in shape and devoid of a glial sheath. The dendrites tangled about the nodular swellings of other nerve fibers, forming glomeruli measuring 8-12 μm in size (Fig. 2d). Thicker nerve fibers enclosed in glial sheaths also meandered among the granule cells. These fibers ranged in diameter from 0.5 to 1.0 μm.

DISCUSSION

The present SEM observations confirmed previous findings obtained by light microscopy (LM)[9,10] and by transmission electron microscopy (TEM)[11] concerning the cytoarchitecture of the three layers in the cerebellar cortex. The NaOH maceration enabled us to identify the parallel fibers and the bundles of their stems in the molecular layer, large perikarya of Purkinje cells and their primary processes in the Purkinje cell layer, and spherical bodies of granule cells and numerous myelinated and non-myelinated fibers in the granular layer. The SEM study by Reese et al.[5] has demonstrated all these structures excepting the vertically directed bundles of granule cell axons in the molecular layer.

Furthermore, the present study adds some information with regard to the glial sheaths and axon terminals on the Purkinje cell somata. Previous LM studies demonstrated numerous terminal branches of axons converging on the Purkinje cell somata: the pericellular plexus of basket cell axons, the climbing fibers and the recurrent collaterals of Purkinje cell axons.[9,10] However, TEM studies have described synaptic contacts between the axon branches and the somata as being restricted to small patchy areas corresponding to "fenestrations" in the attenuate glial sheath enveloping the target.[11] The present SEM observation revealed that the pericellular sheath reported in the previous studies consists of numbers of attenuate glial processes crossing and overlapping with each other, and that the "fenestrations" in the sheath are represented by gaps among the processes.

The presynaptic faces of the axons converging on a Purkinje cell soma are isolated from each other by wide glial septa, forming independent synaptic boutons, as demonstrated in the present study. In contrast to this structure, we have previously described a pericellular plexus enclosing a large ganglion cell in the cerebellar nuclei as terminating on the target with numbers of long varicose endings which crossed and overlapped with each other to form a reticulum.[7] Thus our present and previous findings indicate the occurrence of two different types of pericellular plexus, one terminating as small isolated boutons of synapses, and the other extending a huge reticular terminal woven with long varicose

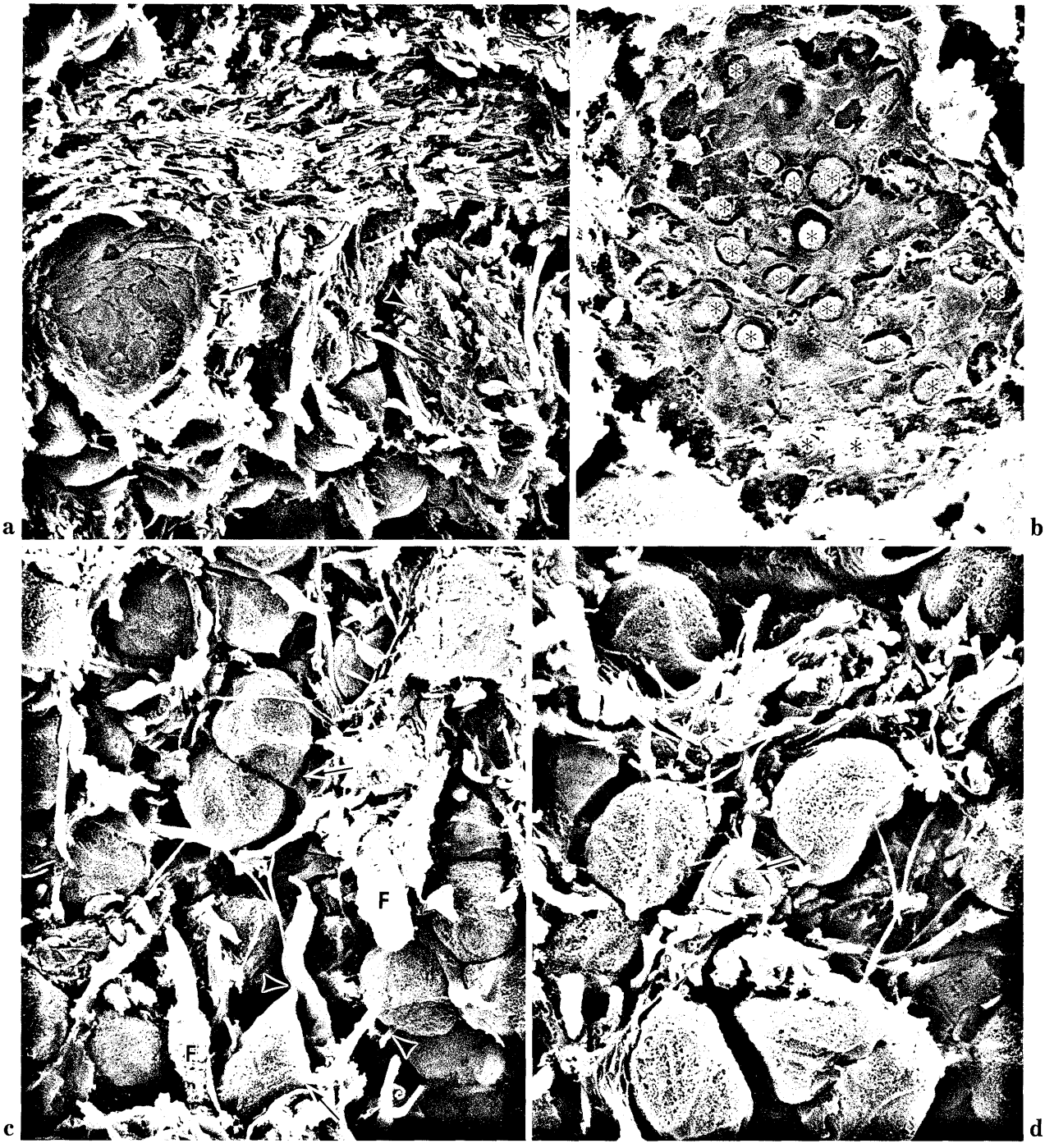

Fig. 2. a. Purkinje cell layer. Arrow indicates a hollow which had contained a Purkinje cell soma. Arrowhead Purkinje cell soma surrounded by palisades of basket cell axons. **b.** High magnification of a hollow which had enveloped a Purkinje cell soma. Small round terminals of axons are disposed sparsely (asterisks). **c.** Granular layer. Granule cells extend dendrites (arrows) and delicate axons (arrowheads). Thick nerve fibers (*F*) possess glial sheaths. **d.** High magnification of a granular layer. Nodular swelling of a presumed mossy fiber projects from a glomerulus of granule cell dendrites (arrow). a ×2,000, b ×4,900, c ×3,800, d ×5,400

synapses.

SEM observation combined with NaOH maceration thus serves as a useful method for the study of complex nerve endings on somata of different neurons and other fine structures in the central nervous tissue.

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