

Scanning electron microscopy of vertebrate visual receptors

With its sensitivity to the details of surfaces, its high resolution over large depths of field, and its capability of providing three-dimensional perspective⁷, the scanning electron microscope has the potential of being an extremely useful tool for studying nervous-system topography. This potential can be realized, however, only if the appropriate surfaces can be exposed; and for nervous-system mapping, the appropriate surfaces are the boundaries of neurons and neuronal processes. With these surfaces properly exposed, a scanning electron micrograph could show quasi-cylindrical processes emanating from spherical cell bodies, then coursing into neuropil, branching and terminating in knobs on cylindrical processes from other cell bodies, a three-dimensional map that does not require reconstruction from serial sections. Results to date from *Aplysia* indicate that such micrographs are achievable¹⁰.

The boundaries of neurons and their processes are internal to nervous tissue. If these surfaces are to be exposed, then the tissue somehow must be made to cleave along them. In histology, the scanning electron microscope has been applied almost exclusively to naturally exposed surfaces such as those of bone³, teeth¹, insect cuticle¹², protozoa^{2,8}, bacteria⁶, red blood cells⁷, peritoneal cells¹³ and even the exposed surfaces of neurons on tissue cultures⁴. Furthermore, even in the sectioned-tissue studies published to date, cleavage along whole-cell surfaces has not been realized^{9,11}. Normal sectioning of embedded tissue leads to cleavage *through* cells rather than over their natural surfaces, and cleavage along such surfaces most likely will require drastic modifications of current histological preparation techniques. When drastic techniques are employed, one must question their effects on the integrity and proportions of both gross and fine structures and the resulting effects on topographical interpretations.

By dissecting or sectioning fresh, unfixed tissue, then fixing with buffered glutaraldehyde, extracting with moderately weak glycerin solutions, dehydrating with series of ethanol solutions, and air drying, we have been able to expose relatively clean neuronal surfaces. These have been coated with thin films of aluminum or gold to enhance secondary electron emission and to prevent charge accumulation and then placed in a scanning electron microscope (Cambridge Instrument Co. 'Stereoscan') for observation. Dissection of unfixed tissue and air drying both are extraordinary steps. To determine the extent of their effects, we applied the techniques described above to the retina of the mudpuppy (*Necturus*), and examined the readily accessible, external structures of the receptor outer segments. These structures have been examined thoroughly by Brown *et al.*⁵, both with the light microscope and with the transmission electron microscope. In addition to their characteristic shapes and sizes, the rods and cones exhibit fine structures that can be examined for alterations due to dissection and drying. According to the results of Brown *et al.*, the outer segments of the rods exhibit 22–31 deep vertical fissures separating an array of vertical columns or lobules; and a dendrite whose cytoplasm is continuous with the inner segment runs along the mouth of each fissure. The outer segments of the cones exhibit no fissures or lobules, but vertical dendrites do extend along them from the inner segments. The dimensions from the osmium-fixed receptors of Brown *et al.* are given in Table I.

TABLE I

COMPARISON OF DIMENSIONS OF RECEPTOR STRUCTURES

<i>Structure</i>	<i>Dimension (μm) from transmission electron microscopy of osmium-fixed tissue⁵</i>	<i>Dimension (μm) from scanning electron microscopy of glutaraldehyde-fixed, air-dried tissue</i>
Base of rod, diameter	12 (average)	7.5 to 9
Rod, length	30 (average)	20 (average)
Rod lobules, width	0.8 to 1.5*	0.5 to 1.0
Rod dendrites, diameter	0.25 to 0.6*	0.12 to 0.3**
Base of cone, diameter	9 (average)	5.5 to 7
Tip of cone, diameter	5 (average)	3.2 to 3.6
Cone, length	24 (average)	16 (average)
Cone dendrites, diameter	0.16 to 0.36*	0.1 to 0.25

* Dimensions taken from Figs. 3a, 3b and 4 in Brown *et al.*⁵

** The rod dendrites are considerably thicker as they emerge from the inner segment (see lower left hand corner of Fig. 4).

With air-dried retina under the scanning electron microscope, the outer segments of the rods exhibit vertical columns, with an intact dendrite emanating from the inner segment running between each column (Figs. 3 and 4). The outer segments of the cones exhibit no columns, but intact vertical dendrites extend along them from the inner segments (Figs. 1 and 2). The dimensions of these structures as seen in the scanning electron microscope are given in Table I. The scanning electron microscope was calibrated with a replica of a diffraction grating with 528 lines/mm. According to the results of calibration, the dimensions of Table I are accurate to within $\pm 3\%$. Brown *et al.*⁵ estimate that their osmium-fixed preparations have undergone shrinkage to 80% of their original size. According to Table I, the air-dried preparations have undergone further shrinkage. This shrinkage appears to have been extremely uniform, however, since 7 of the dimensions are almost exactly two-thirds of those obtained from Brown *et al.* The variants are the rod dendrites, which exhibited slightly more shrinkage. On the average, the structures of the outer segments apparently shrank to approximately 53% of their original sizes as a combined result of fixation, extraction, dehydration and air drying. In spite of this shrinkage, however, the proportions, topography and integrity seem to have been maintained extremely well, both in the large structures and in the small. In fact, the configurations in Figs. 1-4, with their three-dimensional perspective, are essentially identical to those inferred by Brown *et al.*⁵ from observations of a large number of transmission electron microscope sections.

While scanning electron micrographs can provide excellent views of cell surfaces and the external topography of multicellular systems, they cannot provide reliable images of subsurface structures. The cytoplasmic inclusions often used as landmarks or identification criteria cannot be seen. Furthermore, cytoplasmic continuity or discontinuity cannot be inferred with any certainty. Until new criteria and new rules

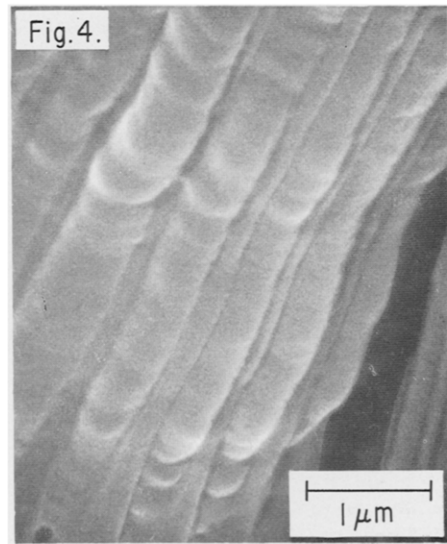
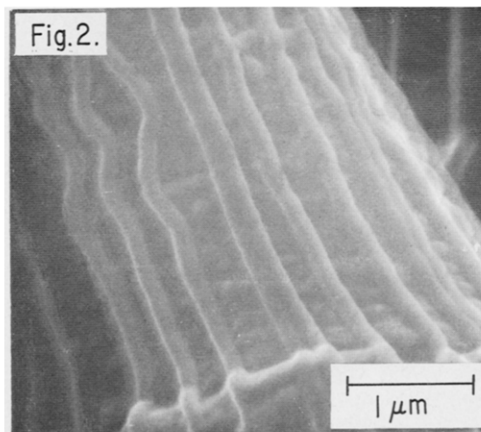
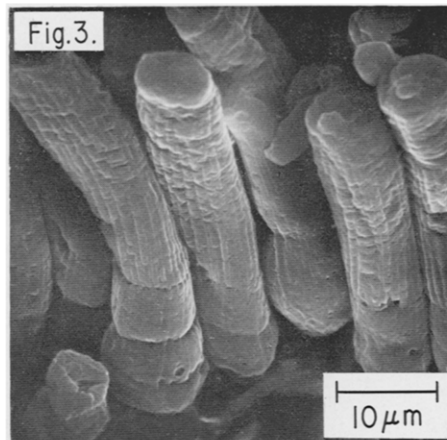
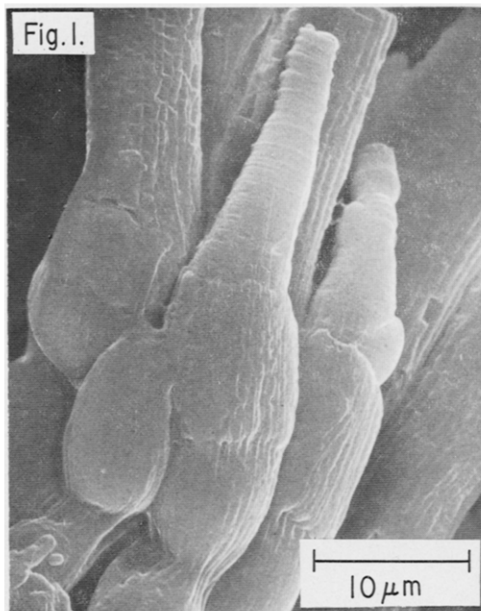


Fig. 1. Scanning electron micrograph of a cone from the retina of *Necturus*, showing the conical *outer segment*, the cylindrical *ellipsoid* and the rounded *paraboloid*.

Fig. 2. Scanning electron micrograph of cone dendrites at the junction between the ellipsoid and the outer segment.

Fig. 3. Scanning electron micrograph showing a view from above of rods in the retina of *Necturus*. The cylindrical *outer segments* with their vertical lobules are shown connected to the smoother ellipsoids and paraboloids.

Fig. 4. Scanning electron micrograph of rod lobules and dendrites just above the junction between the ellipsoid and the outer segment.

of inference have been developed, therefore, mapping with scanning electron microscopy will remain somewhat speculative unless it is accompanied by transmission electron microscopy. By the same token, inferences from transmission microscopy will be much stronger when corroborated by scanning electron micrographs such as those shown in Figs. 1-4.

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