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Ultrastructure of Growth Cones in the Cerebellar Cortex of the Neonatal Rat and Cat* **

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Summary. The ultrastructure of axonal and dendritic growth cones has been examined in the cerebellar cortex of 7 days old rats and 12 days old cats. The unique feature is a bulge of the perikaryon surface or a varicosity of the growing tip of nerve processes. These cone-like areas contain large amounts of tubular smooth surfaced endoplasmic reticulum (SR) and large vacuoles. They are further characterized by filopodia (Tennyson, 1970) with a fibrillary matrix. Early cell contacts with synaptic membrane specializations are described between filopodia of mossy fiber endings and dendritic growth cones of granular cells. Synaptic vesicles appear early in synaptogenesis. While both vesicles and SR tubules are confined to separate areas of the axonal growth cone it was found that a common affinity to the ZIO staining agent exists. In contrast, the neurofilaments and microtubular components as well as the growth cone vacuoles remain consistently ZIO negative.

Key-Words: Cerebellar cortex—Axonal and Dendritic Growth Cones—Ultrastructure.

Cajal (1909) gave an early description of the growing tip of dorsal root neuroblasts and coined the term "cône de croissance". Several electron microscopic studies on growth cones (Bodian, 1966; Bodian *et al.*, 1968; Del Cerro and Snider, 1968; Tennyson, 1970) have appeared more recently. Bodian (1966) described the growing tip of axons and dendrites in the foetal monkey spinal cord as a swollen bulb about 0.5 μ in diameter containing clusters of large empty vesicles and no mitochondria or other organelles. Del Cerro and Snider (1968) made similar observations in the growing cerebellum of the albino rat. However, Tennyson (1970) reported different findings in the dorsal root neuroblast of the rabbit embryo. Filopodia were found to extend from varicosities containing tubules and sacs of smooth surfaced endoplasmic reticulum (SR), microtubules, neurofilaments and mitochondria. The filopodia appeared as long thin finger-like processes or as broad flanges of cytoplasm containing a finely filamentous matrix.

The present study is concerned with the ultrastructure of growth cones which appear in the first and second postnatal week in the cerebellar cortex of

rat and cat. The findings tend to unify and extend those of previous authors. In addition, the affinity between various subcellular components of growing nerve processes to the zinc iodide osmium tetroxide stain will be described.

Material and Methods

Eight 7 days old rats and two 12 days old kittens were used in this study. Under pentobarbital anesthesia the skull was quickly opened and the cerebellum exposed. Then 6.5% glutaraldehyde with 0.1 M phosphate buffer (pH 7.4) was poured on the surface of the cerebellar cortex of the rat to make the fixation bath, in which slices of the cerebellar vermis were obtained. Fixation of tiny pieces was continued in the same solution for 2 h at room temperature. Each specimen was then cut into 2 parts: One was treated with the zinc iodide-osmium tetroxide (ZIO) method after the washing with tris buffer at pH 7.4 (Kawana *et al.*, 1969), and the other was washed in 0.2 M phosphate buffer (pH 7.4) with 6.8% sucrose at 4° C for 14 h. The postfixation was performed with 2% osmium tetroxide in Palade buffer (pH 7.4) with 6.8% sucrose at 4° C for 2 h.

In the kitten series, the fixation fluid recommended by Tennyson (1970) was used. It consisted of a mixture of 310 mOsM of 0.1% paraformaldehyde and 1% glutaraldehyde in 175 mOsM phosphate buffer (pH 7.35) and was applied for 1 h at room temperature. The specimens were then divided into two parts: One was impregnated with the ZIO-method and the other was washed in 290 mOsM phosphate buffer (pH 7.35) for one h at 4° C. Postfixation was carried out with 2% osmium tetroxide in 290 mOsM phosphate buffer (pH 7.3) for 3 h at room temperature. The preservation of the tissue with this fixation method is nearly equivalent to that obtained with the former method. The tissue blocks were then dehydrated and embedded in Epon 812. Ultrathin sections were poststained with uranyl acetate (Watson, 1958) and lead hydroxide (Karnovsky, Method B, 1961). Electron micrographs were taken with a Siemens Elmiscope I.

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Results

Fig. 1 shows a neural perikaryon of the outer granular layer of the rat cerebellar cortex. A cone-shaped bulge in the contour of the cytoplasm of about 1.5×0.5 μ is seen. It contains a conspicuous accumulation of SR sacs and tubular elements whose profiles may give the impression of vesicles. The electrondensity of the cytoplasmic matrix is clearly diminished within this region. Further proximally to the SR complex one finds agglomerations of free ribosomes (Fig. 4). However, no intermingling of tubules and ribosomes occurs. Other organelles such as Golgi complexes, mitochondria and lysosomes are not present within the two compartments, but instead they may be found within the more proximal segment at the base of the cone (Figs. 5, 7).

Figs. 2–4 demonstrate that thin processes may protrude from the cones. These finger-like extensions have a circular or flat profile and a diameter of about 0.1 μ. Their length varies a great deal and may be as much as 1.0 μ. They contain densely packed fine fibrils (80 Å). These extensions are designated filopodia according to Pomerat *et al.* (1967) and Tennyson (1970) who described them in analogous situations. It seems noteworthy that filopodia consistently protrude from the tubular (SR) segment of the cone.

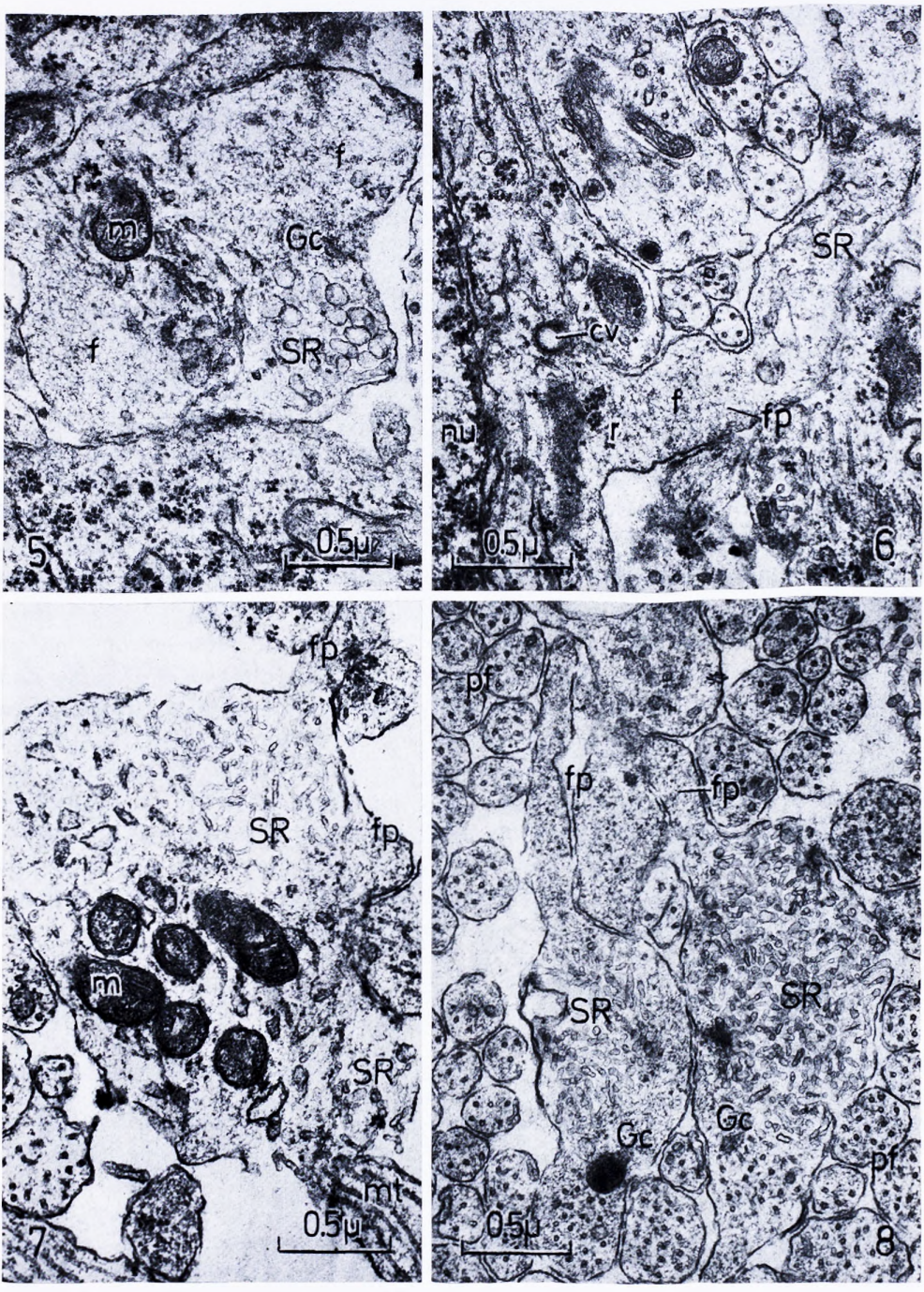


Fig. 5. Growth cone (*Gc*) containing *SR* and fine fibrillary material (*f*). *m* mitochondria, *r* ribosomes. 7 days old rat. Primary magnification, × 20000. Glutaraldehyde immersion, postfixation with OsO_4

Fig. 6. Filopodium (*fp*) extending from the soma of the external granular layer of 7 days old rat (35–20) contains fine fibrillary material (*f*). Note *SR* in the distal portion of the filopodium. *cv* coated invagination of plasma membrane, *nu* nucleus, *r* ribosomes. Primary magnification, × 20000. Glutaraldehyde immersion, postfixation with OsO_4

Incipient synapse formation (Figs. 10, 13) is of special interest because axonic growth cones providing presynaptic differentiation and dendritic growth cones with postsynaptic counterparts can be more readily identified. Both elements contain massive amounts of fibrillary material. Fig. 14 represents a more advanced step in synaptogenesis with SR complexes on both sides and large amounts of fibrillary material in the postsynaptic region. Axo-somatic synapses in a similar developmental stage are seen in Fig. 15.

Figs. 16 and 17 demonstrate the affinity of growth cone constituents to the ZIO staining compound. It turns out that synaptic vesicles and SR complexes are ZIO-positive while microtubules and fibrils remain unaffected (Fig. 18). Vacuoles in the growth cones seem to be ZIO-negative.

Discussion

Identification of Growth Cones. Cajal (1909) noted that growth cones contain neurofibrils stainable with silver nitrate and a neuroplasmic substance which was exclusively demonstrable in silver chromate preparations. Early electron-microscopic studies by Bodian (1966); Bodian *et al.* (1968) and by Del Cerro and Snider (1968) gave main emphasis to the cone-shaped tips of growing axons and dendrites. They noted the presence of SR complexes but paid little attention to filopodia, which were only recently discovered by Tennyson (1970). Her varicosities with finger-like extensions seem to correspond to what Cajal described as "gigantic growth cones" and to what Pomerat *et al.* (1967) had seen in tissue cultures ("velamentous growth cones"), although the finest tips of filopodia are below the resolution power of light microscopy. The present study in rats and cats is in full agreement with Tennyson's findings. Growth cones were observed in perikarya as well as in the tips of growing nerve processes. Glial growth cones could not be differentiated with certainty and the specific characteristics of axonal vs. dendritic growth cones could not be ascertained.

The common feature of all growth cones seem to be as follows (Fig. 19): They consist of a bulging cytoplasm from which extremely fine filopodia protrude. The main structural characteristics of the cone-shaped area is the massive convolute of SR complexes, while the filopodia are crowded with fibrils. Growth cones of perikarya reveal a threefold segmentation into (from distal to proximal) (i) SR complexes, (ii) ribosomal zone, (iii) other organelles. Axonal and dendritic growth cones resemble varicosities of adult animals. However, the richness in fibrillary material and SR complexes is a unique feature of growing fibers. The presence

Fig. 7. Nerve fiber forming a varicosity which contains mitochondria (*m*) and *SR* profiles. Cerebellar cortex of 7 days old rat. Filopodia (*fp*) extend from areas with an accumulation of *SR*. *mt* microtubules. Primary magnification, × 20000. Glutaraldehyde immersion, postfixation with OsO_4

Fig. 8. Two growth cones (*Gc*) in the molecular layer of 7 days old rat cerebellum (35–20). Filopodia (*fp*) are sectioned at a right angle to bundles of parallel fibers (*pf*). *SR* smooth surfaced endoplasmic reticulum. Primary magnification, × 20000. Glutaraldehyde immersion, postfixation with OsO_4

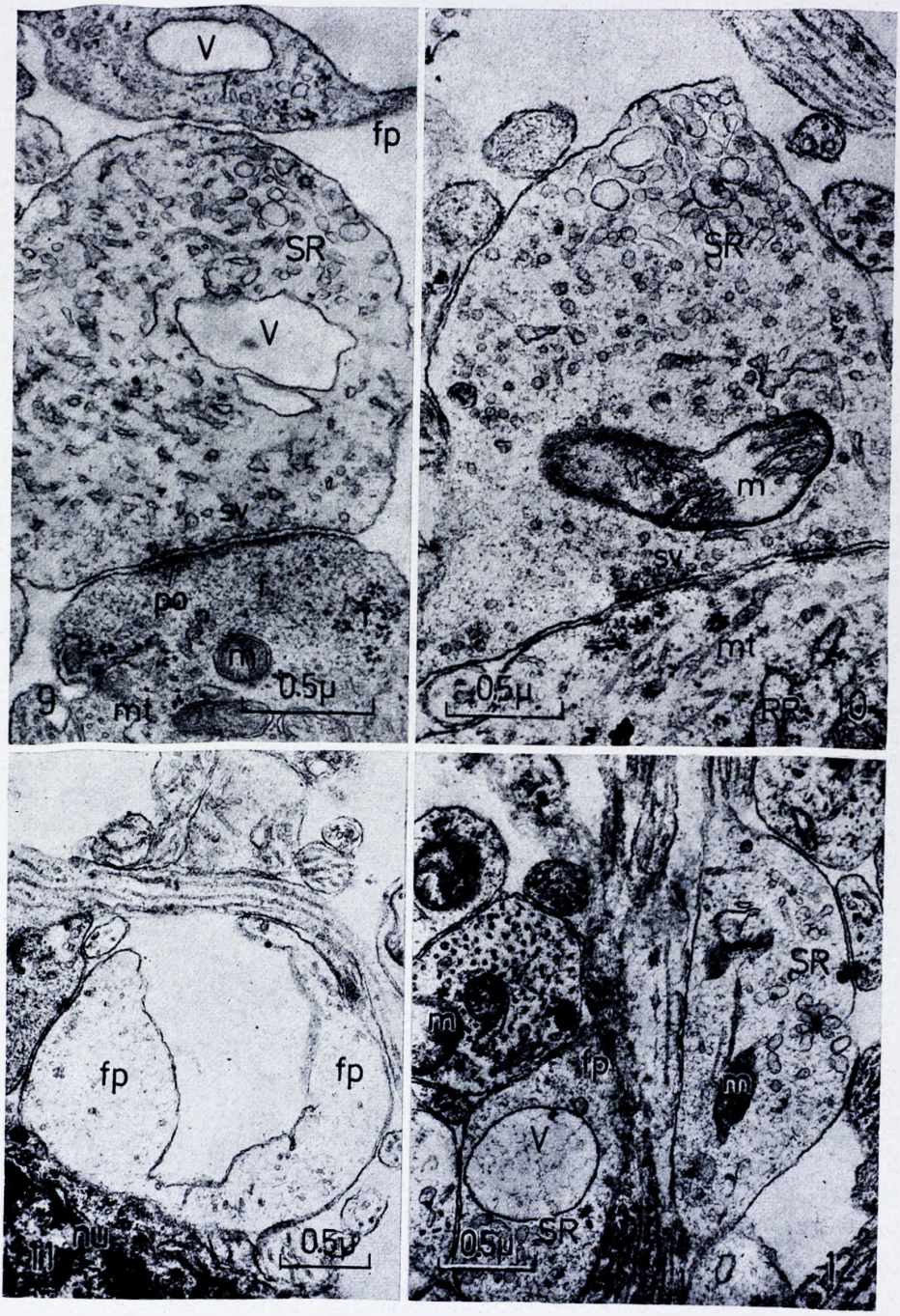


Fig. 9. Growing mossy fiber in the inner granular layer (35–50). 7 days old rat. Note early synapse formation. heaps of synaptic vesicles (*sv*) are observed near synaptic site. Postsynaptic dendrite contains fine fibrillary material (*f*). *SR* complex remains clearly separated from the presynaptic area. *fp* filopodium, *m* mitochondria, *mt* microtubules, *po* postsynaptic density, *r* ribosomes, *v* vacuoles. Primary magnification, × 20000. Glutaraldehyde immersion, postfixation with OsO_4

of free ribosomes in the immediate vicinity of SR complexes could *not* be found in the growth cones at the tip of axons and dendrites.

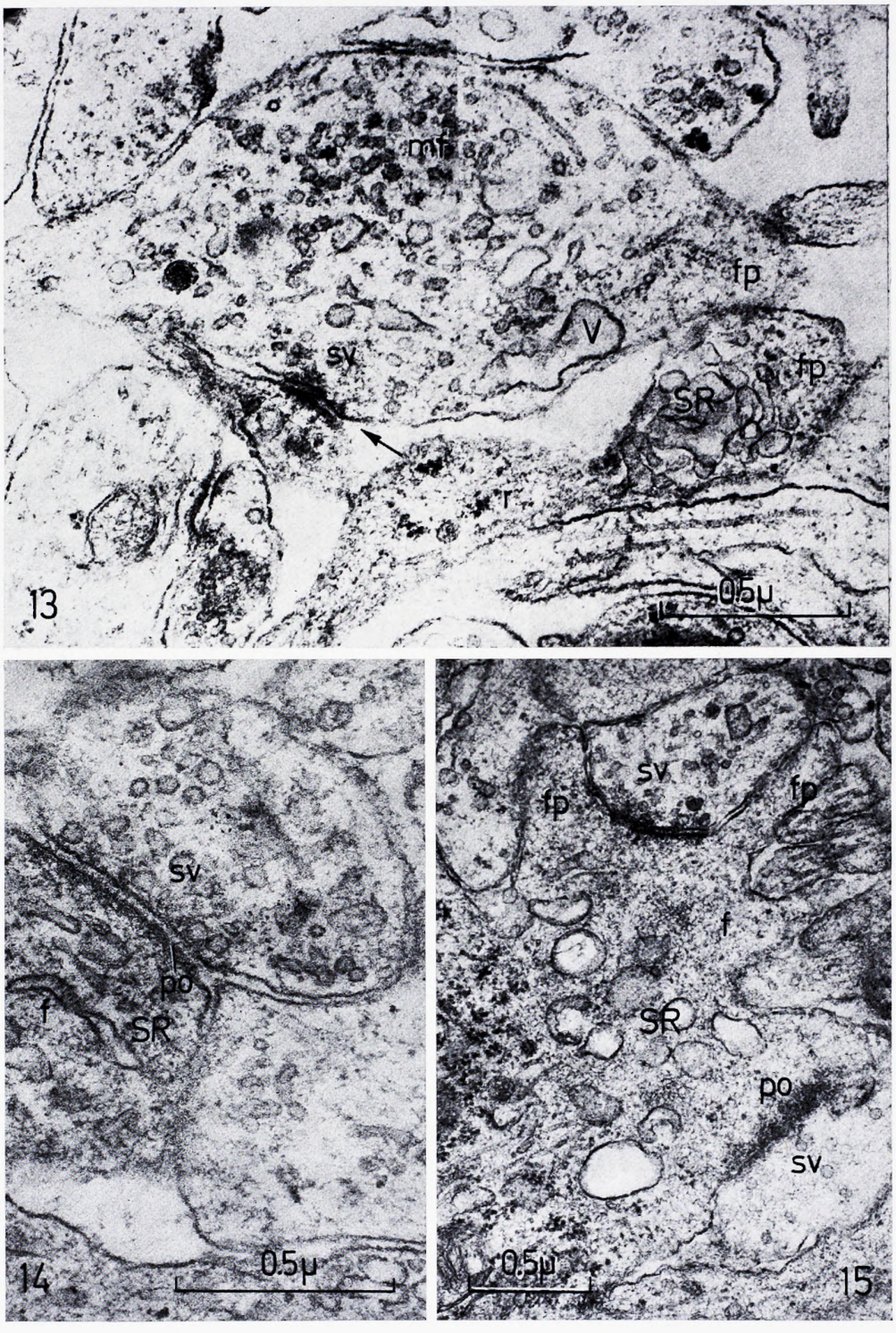
Filopodia. Tennyson's findings (1970) of the massive fibrillary content of filopodia was largely confirmed by the present study. From direct tissue culture observations by Nakai and Kawasaki (1959) and Pomerat *et al.* (1967) and more recently by Burnstock (1970) one may conclude that growing nerve fibers have mobility. This property could be reasonably correlated with the presence of fibrillary structures (Buckley and Porter, 1967). This idea receives support from studies in amoebae (Danneel, 1964: Pollard and Ito, 1970) in plasmodia (Wohlfarth-Bottermann, 1962) and in other sites where mobility was observed and correlated with fibrillary proteins (Lit. see Grimstone, 1966; Jahn and Bowee, 1969). These structures are mixtures of actinosinic and myosinic proteins and were shown to be ATP and Ca^{++} sensitive. Their origin is still obscure. While some authors have succeeded in converting tubular elements into fibrils invarious tissues by using high pressure (Tilney *et al.*, 1966) or by applying colchicine and vinblastine (Porter *et al.*, 1970), it is noteworthy that the growth cone fibrils arise consistently from the polar region where SR complexes have accumulated.

SR Complexes. Large convolutes of SR tubules and enlarged sacs have been found in earlier studies of growth cones (s. above). Tennyson (1970) mentions the possibility that the enlarged SR sacs may correspond to the endoplasmic vacuoles in growing fiber tips of tissue cultures (Pomerat *et al.*, 1967). She observed that some of the dilated sacs were apposed to the surface membrane and suspected that they play a role in exchange functions with extracellular fluid compartments. However, other aspects should be considered. Conceivably, the aforementioned vacuoles are not identical with SR complexes whose authentic shape is considered to be tubular (Ito, 1961; Fawcett, 1966). The present study seems to confirm the reservations of several authors with respect to the denaturating effect of fixatives in neonatal tissue. It turned out that tubular profiles prevail under favorable condition of tissue preservations. It should be added that the present material is not by any means considered optimal in this respect and that no decisive advantages could be obtained by the application of Tennyson's (1970) method of fixation.

The electron density of the cytoplasmic matrix within SR complexes is clearly reduced. This phenomenon may reflect a decrease in viscosity of protoplasmic components which may facilitate the mobility of the SR system. Although, still pictures cannot provide evidence concerning the origin and destination of organelles, it seems reasonable to assume that SR complexes encountered in perikaryal

Fig. 10. Early synapse at Purkinje cell soma of 7 days old rat cerebellum (35–10). *mt* microtubules, *r* ribosomes, *RR* rough surfaced endoplasmic reticulum. *SR* smooth surfaced endoplasmic reticulum, *sv* synaptic vesicles. Primary magnification, × 20000. Glutaraldehyde immersion, postfixation with OsO_4

Figs. 11 and 12. Filopodia (*fp*) containing fine fibrillary material. Note their relationship with large vacuoles (*v*). In Fig. 11 two branches of filopodium are about to fuse. 7 days old rat. *m* mitochondria, *nu* nucleus, *SR* smooth surfaced endoplasmic reticulum. Primary magnification, × 20000. Glutaraldehyde immersion, postfixation with OsO_4



Figs. 13–15

cone segments may form a continuous and dynamic system along the axis of growing nerve fibers.

SR has been associated with widely varying metabolic functions, for example detoxication as well as lipid and cholesterol metabolism in liver cells; lipid transport in intestinal epithelium; the biosynthesis of steroid hormones in endocrine tissue; secretion of chloride ions in oxyntic cells of the stomach and excitation-secretion coupling in skeletal muscle (Fawcett, 1966). Although correlated biochemical and cytological studies on SR of growth cones are still lacking we might postulate that this structural component may be related to the neogenesis of membranes or the formation of the fibrillary matrix of filopodia.

Vacuoles. As particularly well demonstrated in nerve tissue cultures by Pomerat *et al.* (1967) the growth cones contain conspicuous amounts of vacuoles whose formation may be observed in time lapse cinematography. Such vacuoles may correspond to the ones observed in the distal and central cone segments in Tennyson's (1970) study as well as in the present material. However, it is not excluded that some of the "vacuoles" appearing in the light microscope may in fact represent other organelles, e.g. accumulation of enlarged vesicles or Golgi complexes. Nevertheless, the presence of relatively large vacuoles can be considered a typical feature of growth cones. Their relationship to plasmalemma in terms of macropinocytosis has been discussed by Tennyson (1970) and Pomerat *et al.* (1967) and seems to receive further support from our present study (Fig. 11). The differential reactivity of vacuoles and SR tubules to the ZIO stain seem to speak against their causal interrelationship.

Synaptogenesis. The development of synaptic contacts has been studied in various regions of the nervous system *in vivo* and *in vitro*. Most investigators (Glees and Sheppard, 1964; Meller, 1964; Hámori and Dyachkova, 1964; Bodian, 1966; Wechsler, 1966; Meller and Haupt, 1967; Bunge *et al.*, 1967; Mugnaini, 1970) have suggested that the membrane modifications preceded the appearance of synaptic vesicles, although, it is difficult at this stage to differentiate synaptic membranes from the early components of desmosome-like contacts (Aghajanian and Bloom, 1967). Ochi (1967) claims that the formation of vesicles

Fig. 13. Mossy fiber (*mf*) with early synapse formation (arrow) at growth cone. 7 days old rat. Filopodia of early dendritic growth cones surround the mossy fiber terminal in various stages of apposition. *fp* filopodia, *r* ribosomes, *SR* smooth surfaced endoplasmic reticulum, *sv* synaptic vesicles, *v* vacuole. Primary magnification, × 40000. Glutaraldehyde immersion, postfixation with OsO_4

Fig. 14. Early synapse in the molecular layer of neonatal rat cerebellum (35–50). Postsynaptic dendrite contains *SR* convolutes and fibrillary material (*f*). *po* postsynaptic density, *sv* synaptic vesicles. Primary magnification, × 40000. Glutaraldehyde immersion, postfixation with OsO_4

Fig. 15. Early axo-somatic synapse of Purkinje cell soma (35–20). 7 days old rat. Postsynaptic density (*po*) and synaptic vesicles (*sv*) are observed. Note the persisting fibrils (*f*) and enlarged *SR* in the postsynaptic dendrite. *fp* filopodia, *r* ribosomes. Primary magnification, × 20000. Glutaraldehyde immersion, postfixation with OsO_4

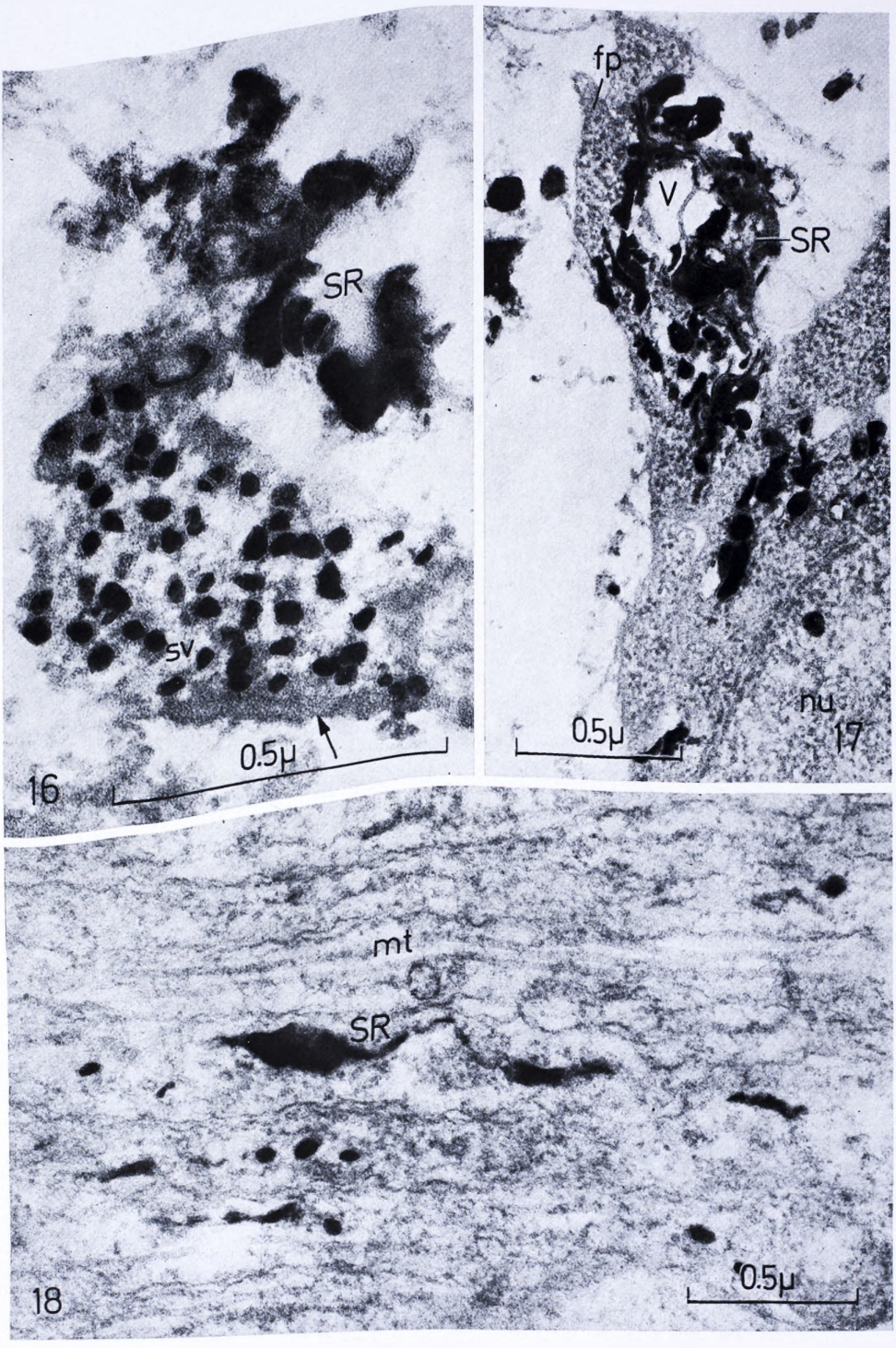


Fig. 16. Growth cone with synaptic area (arrow) in the neonatal rat cerebellum (35–2J). Synaptic vesicles (*sv*) and *SR* are topographically separated. Both structures are zinc iodide-osmium tetroxide (*ZIO*) positive. Primary magnification, ×40000. Glutaraldehyde immersion, ZIO impregnation

Fig. 17. Growth cone with filopodia (*fp*) in the neonatal rat cerebellum (35–5J). Note ZIO positive *SR* and negative vacuole (*v*). *nu* nucleus. Primary magnification, ×20000. Glutaraldehyde immersion, ZIO impregnation

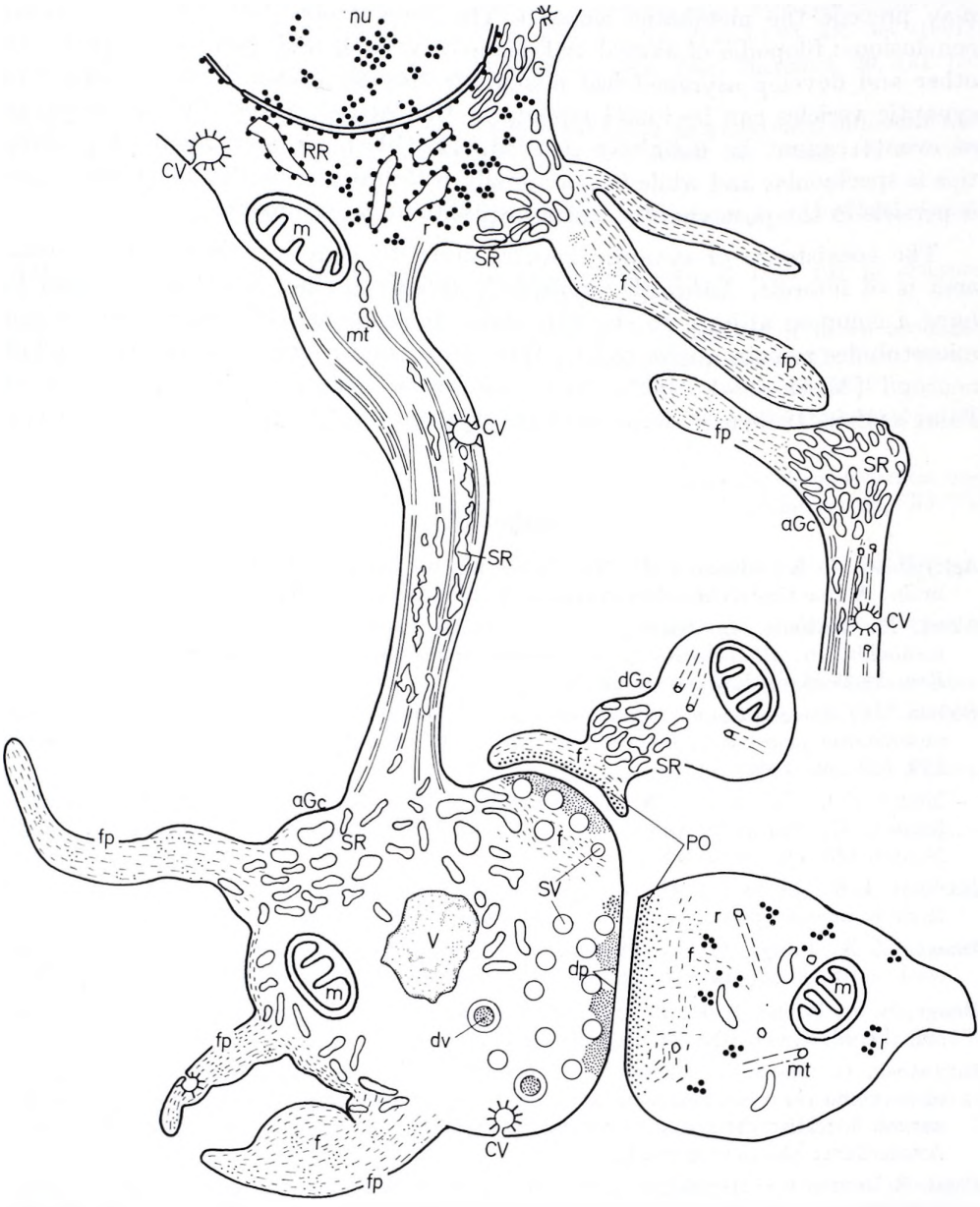


Fig. 19. Diagrammatic summary of findings. The interactions of filopodia extending from axonal (*aGc*) and dendritic growth cones (*dGc*) are represented in terms of apposition and synapse formation. Vacuoles (*v*) are possibly the result of fusion and endocytosis in filopodia (*fp*). *cv* coated invagination of plasma membranes, *dp* dense projection, *dv* dark cored vesicles, *f* fine fibrillary material, *G* Golgi complex, *m* mitochondria, *mt* microtubules, *nu* nucleus, *PO* postsynaptic density, *r* ribosomes, *RR* rough surfaced endoplasmic reticulum, *SR* smooth surfaced endoplasmic reticulum, *sv* synaptic vesicles

Fig. 18. ZIO positive smooth surfaced endoplasmic reticulum (*SR*) and ZIO negative microtubules (*mt*) in fibers of the neonatal cat cerebellum (64-J). Primary magnification, × 20000. Paraformaldehyde-Glutaraldehyde immersion, ZIO impregnation

may precede the membrane changes. The present study allows the following conclusions: filopodia of axonal and dendritic growth cones are seen to face each other and develop asymmetrical membrane specializations. A small number of synaptic vesicles can be found already at the earliest stages. Yet the sequence of events cannot be definitely decided. The fibrillary component of growing tips is spectacular and while it is gradually withdrawn from the presynaptic area it persists in the postsynaptic region until late in development.

The coexistence of synaptic vesicles and SR complexes in the presynaptic area is of interest. Although consistently separated, the two elements seem to have a common affinity to the ZIO stain. In contrast, the neurofilaments and microtubules remain unaffected by this cytochemical reagent as shown in adult neuropil (Akert *et al.*, 1970). This observation seems significant in view of Palay's (1956, 1959) early hypothesis that synaptic vesicles may be SR derivatives.

References

Aghajanian, G. K., Bloom, F. E.: The formation of synaptic junctions in developing rat brain: A quantitative electron microscopic study. Brain Res. **6**, 716–727 (1967).

Akert, K., Kawana, E., Sandri, C.: ZIO positive and ZIO negative vesicles in nerve terminals. In: Histochemistry of nervous transmission (O. Eränkö, ed.). Progr. Brain Res. Amsterdam: Elsevier (in press).

Bodian, D.: Development of fine structure of spinal cord in monkey fetuses. I. The motoneuron neuropil at the time of onset of reflex activity. Bull. Johns Hopk. Hosp. **119**, 129–149 (1966).

— Melby, E. C., Jr., Taylor, N.: Development of fine structure of spinal cord in monkey fetuses. II. Pre-reflex period to period of long intersegmental reflexes. J. comp. Neurol. **133**, 113–166 (1968).

Buckley, I. K., Porter, K. R.: Cytoplasmic fibrils in living cultured cells. A light and electron microscopical study. Protoplasma **64**, 349–380 (1967).

Bunge, M. B., Bunge, R. P., Peterson, E. R.: The onset of synapse formation in spinal cord cultures as studied by electron microscopy. Brain Res. **6**, 728–749 (1967).

Bunge, R. P., Bunge, M. B., Peterson, E. R.: An electron microscope study of cultured rat spinal cord. J. Cell Biol. **24**, 163–191 (1965).

Burnstock, G., Mark, G., Chamley, J., McConnell, J.: Time-lapse film on the formation of nervemuscle relationships in joint tissue cultures of smooth muscle and sympathetic nerves. In: Histochemistry of nervous transmission (O. Eränkö, ed.). Prog. Brain Res. Amsterdam: Elsevier (in press).

Cajal, S. Ramon y.: Histologie du système nerveux de l'homme et des vertébrés. Translated by L. Azoilay, Paris, Reissued 1952 by Consejo Superior de Investigaciones Cientificas, Madrid, chap. XXI, vol. 1, p. 589, 1909.

Danneel, S.: Identifizierung der kontraktilen Elemente im Cytoplasma von *Amoeba proteus*. Naturwissenschaften **51**, 368–369 (1964).

Del Cerro, M. P., Snider, R. S.: Studies on the developing cerebellum. Ultrastructure of the growth cones. J. comp. Neurol. **133**, 341–362 (1968).

Fawcett, D. W.: The atlas of fine structure: The cell. Its organelles and inclusion. Philadelphia and London: W. B. Saunders Comp. 1966.

Glees, P., Sheppard, B. L.: Electron microscopical studies of the synapse in the developing chick spinal cord. Z. Zellforsch. **62**, 356–362 (1964).

Gray, E. G.: The granule cells, mossy synapses and Purkinje spine synapses of the cerebellum: light and electron microscope observations. J. Anat. (Lond.) **95**, 345–356 (1961)

Grimstone, A. V.: Structure and function in protozoa. Ann. Rev. Microbiol. **20**, 131–150 (1966).

Hámori, J., Dyachkova, L. N.: Electron microscope studies on developmental differentiation of ciliary ganglion synapses in the chick. Acta biol. Acad. Sci. hung. **15**, 213–230 (1964).

Ito, S.: The endoplasmic reticulum of gastric parietal cells. J. biophys. biochem. Cytol. **11**, 333–347 (1961).

Karnovsky, M. J.: Simple methods for "staining" with lead at high pH in electron microscopy. J. biophys. biochem. Cytol. **11**, 729–732 (1961).

Kawana, E., Akert, K.: Zinc-iodide osmium tetroxide impregnation of nerve terminals during growth and secondary degeneration. In: Microscopie électronique (P. Favard, ed.). 7th Congr. Int. de Microscopie Electronique, Grenoble, vol. I, p. 407–408, 1970.

— — Sandri, C.: Zinc iodide-osmium tetroxide impregnation of nerve terminals in the spinal cord. Brain Res. **16**, 325–331 (1969).

Meller, K.: Elektronenmikroskopische Befunde zur Differenzierung der Rezeptorzellen und Bipolarzellen der Retina und ihrer synaptischen Verbindungen. Z. Zellforsch. **64**, 733–750 (1964).

— Haupt, R.: Die Feinstruktur der Neuro-, Glio- und Ependymoblasten von Hühnerembryonen in der Gewebekultur. Z. Zellforsch. **76**, 260–277 (1967).

Mugnaini, E.: The relation between cytogenesis and the formation of different types of synaptic contact. Brain Res. **17**, 169–179 (1970).

Nakai, J., Kawasaki, Y.: Studies on the mechanism determining the course of nerve fibers in tissue culture. I. The reaction of the growth cone to various obstructions. Z. Zellforsch. **51**, 108–122 (1959).

Ochi, J.: Elektronenmikroskopische Untersuchungen des Bulbus olfactorius der Ratte während der Entwicklung. Z. Zellforsch. **76**, 339–348 (1967).

Palay, S. L.: Synapses in the central nervous system. J. biophys. biochem. Cytol. Suppl. **2**, 193–202 (1956).

Palay, S. L.: The morphology of synapses in the central nervous system. Exp. Cell Res. Suppl. **5**, 275–293 (1958).

Pollard, T. D., Ito, S.: Cytoplasmic filaments of *Amoeba proteus*. I. The role of filaments in consistency changes and movements. J. Cell Biol. **46**, 267–289 (1970).

Pomerat, C. M., Hendelman, W. J., Raiborn, Ch. W., Jr., Massey, J. F.: Dynamic activities of nervous tissue in vitro. In: The neuron (H. Hydén, ed.), chapt. 3, p. 119–178. Amsterdam: Elsevier 1967.

Porter, K. R., Bennett, G. S., Junqueira, L. C.: Microtubules in intracellular pigment migration. In: Microscopie électronique (P. Favard, ed.). 7th Congr. Internat. de Microscopie Electronique, Grenoble, vol. II, p. 945–946, 1970.

Szentágothai, J.: Anatomical aspects of junctional transformation. In: Information processing in the nervous system (R. W. Gerard, J. W. Duyff, eds.). Proceedings of the internat. union of Physiological Sciences. XXII. Internat. Congr., Leiden, vol. 3, p. 119–136. Amsterdam: Excerpta Medica Foundation 1962.

Tennyson, V. M.: The fine structure of the axon and growth cone of the dorsal root neuroblast of the rabbit embryo. J. Cell Biol. **44**, 62–79 (1970).

Tilney, L. G., Hiramoto, Y., Marsland, D.: Studies on the microtubules in heliozoa. III. A pressure analysis of the role of these structures in the formation and maintenance of the axopodia of *Actinosphaerium nucleofilum* (Barrett). J. Cell Biol. **29**, 77–95 (1966).

Watson, M. L.: Staining of tissue sections for electron microscopy with heavy metals. J. biophys. biochem. Cytol. **4**, 475–478 (1958).

Wechsler, W.: Elektronenmikroskopischer Beitrag zur Nervenzelldifferenzierung und Histogenese der grauen Substanz des Rückenmarks von Hühnerembryonen. Z. Zellforsch. **74**, 401–422 (1966).

Wohlfarth-Bottermann, K. E.: Weitreichende fibrilläre Protoplasmadifferenzierungen und ihre Bedeutung für die Protoplasmaströmung. I. Elektronenmikroskopischer Nachweis und Feinstruktur. Protoplasma **54**, 514–539 (1962).

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