directly upon problems of vertebrate cartilage evolution, it is significant for study of such problems. By analogy, although cephalopod eye and brain have no direct evolutionary relationships with vertebrate eye and brain, the cephalopod organs have evolved remarkable similarities to the analogous vertebrate organs (*9*). In both classes of animals, these organs have also responded similarly to ecologic change and challenge. For this reason, study of cephalopod eyes and brains has contributed to our understanding of their vertebrate counterparts (*10*). I therefore believe that the existence of dermal cartilaginous scales in cephalopods should motivate us to open our minds to the possible existence of similar structures in the vertebrates. A critical reexamination of fossil and living vertebrates in the above perspective would surely be worth the effort (*11*).

Note added in proof: M. Moss has brought to my attention that some workers now believe that the first recognizable mineralized tissue in the vertebrates was a calcified cartilage in the dermal armor of ostracoderms (*12*). Thus, the critical reexamination suggested in my closing sentence is already under way, apparently with positive results.

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Synaptic Vesicles in Electron Micrographs of Freeze-Etched Nerve Terminals

Abstract. *Freeze-etched neuropil of the cat subfornical organ was examined with the electron microscope for synaptic vesicles. Round vesicles were found exclusively in both unfixed and aldehyde-fixed specimens. Range of diameters and histograms failed to differ significantly between freeze-etched and conventionally prepared material. The mode of distribution of diameters was approximately 500 angstroms. Round stomata (approximately 350 angstroms in diameter) were found at the outer surface of the plasmalemma of nerve terminals; they are interpreted as pinocytotic vesicles.*

The freeze-etching method, combined with electron microscopy (*1*), provides highly accurate views of profiles and surfaces of organelles and cells in the unfixed frozen state. Its application to nerve tissue has been particularly successful in the study of myelin (*2*), but no electron micrographs of freeze-etched synapses have thus far been published. We have obtained suitable pictures from the cat subfornical organ (SFO) which contains a large number of synapses in the neuropil (*3*). Twenty-eight subfornical organs were carefully dissected and half were fixed with 3 percent buffered

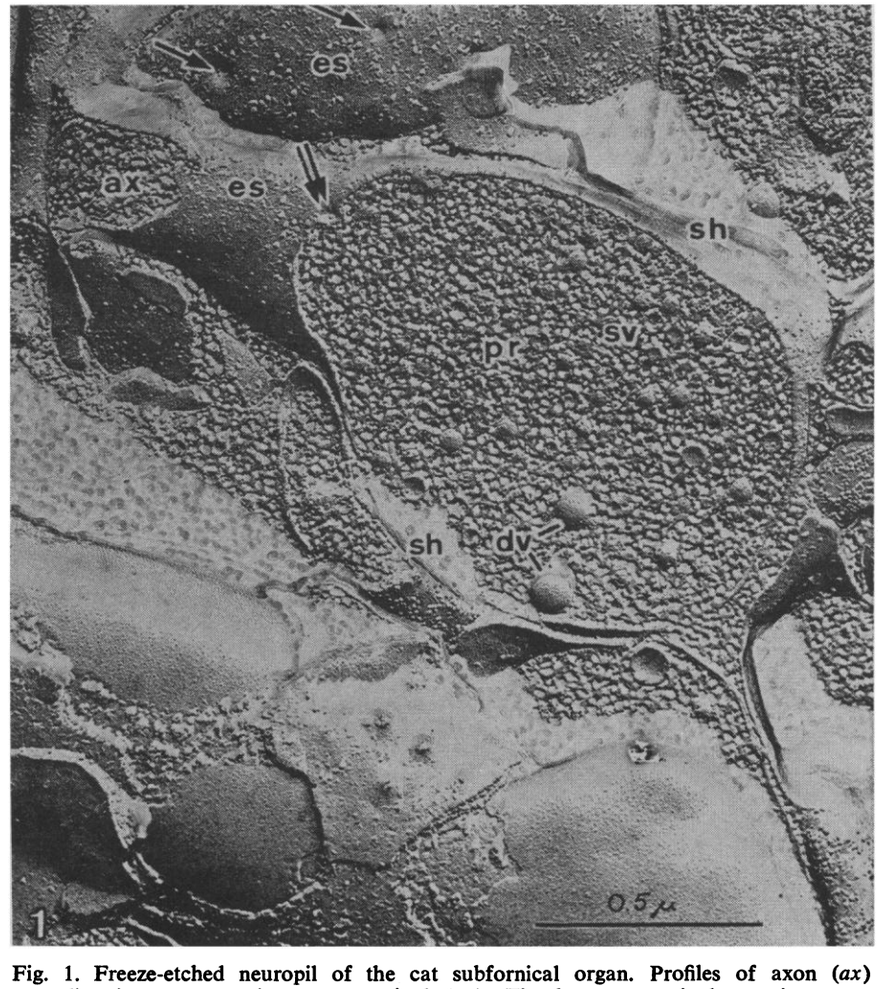

Fig. 1. Freeze-etched neuropil of the cat subfornical organ. Profiles of axon (*ax*) extending into presynaptic nerve terminal (*pr*). The bouton terminal contains many synaptic vesicles (*sv*) of various sizes. Two large concave spheric reliefs represent dark-cored vesicles (*dv*). Possible synaptic sites are blurred with shadows (*sh*). External surfaces (*es*) of axonal plasmalemma are characterized by granules and by pinocytotic stomata (arrows). One pinocytotic stoma is visible in the profile (double arrow). Glutaraldehyde fixation; primary magnification, × 20,000.

glutaraldehyde (*p*H 7.4) at room temperature for 2 hours. Before the freeze-etching procedure began, it was necessary to treat the specimens for 30 minutes in a Ringer solution containing 25 to 30 percent glycerol. Tiny tissue blocks were frozen in liquid freon (−150°C) at a rate of 100°C per second and placed in a high-vacuum freeze-etching device (Balzers BA 360 M), in which the cutting was performed. Etching was achieved by evaporating platinum from the tips of pointed carbon electrodes; it resulted in a layer, several hundred angstroms in depth, which was carefully removed by means of concentrated sulfuric acid and examined in the electron microscope (Elmiscope I). Approximately one-third of the preparations were satisfactory; casts of the fixed specimens could be lifted more successfully than those of unfixed tissue. Correspondingly, a majority of some 200 electron micrographs were obtained from fixed (and therefore slightly denaturated) material (Figs. 1 and 2A), although a sufficient number of unfixed control pictures was available for analysis.

Although findings on the surface of pre- and postsynaptic membranes, synaptic cleft, and appositional densities have been reported (*4*), we now present the observations on synaptic vesicles in more detail. The following points were examined: (i) shape of vesicles; (ii) vesicular diameters and their histogram; (iii) comparison of unfixed, freeze-etched vesicles with those of fixed, freeze-etched vesicles, and vesicles fixed in aldehyde and OsO_4; and (iv) relation between synaptic vesicles and micropinocytosis.

All the vesicles observed in unfixed material, as well as in the aldehyde-fixed, freeze-etched condition, have round profiles (Fig. 2, B and C). It seems quite impossible that disklike or cylindrical structures could have been overlooked. Thus, the synaptic vesicles in their nearest-to-native state are of spherical shape. This statement is of primary significance since the "true" shape of synaptic vesicles has been debated (*5*). The round shape of synaptic vesicles in the fixed and freeze-etched SFO neuropil is not surprising in view of our failure to observe flat (F-type) vesicles in extensive studies of conventionally prepared SFO sections. It seems, therefore, that the SFO neuropil is not suitable for examining the problem of F-type vesicles and their relation to inhibitory synaptic mechanisms (*6*).

The size of synaptic vesicles is of particular interest from the point of view of the storage capacity of transmitter synthesis and release (*7*). Previous data on size and size histograms of vesicles are based on measurements that were carried out in sections of nerve endings (*8*) and of fractionated vesicles (*9*) fixed in aldehyde and treated with OsO_4. Ranges of diameter between 250 and 750 Å, mean values of 430 to 500 Å and standard deviations of the mean of ± 110 Å, have been found. In this study, measurements with the aid of a micrometer were taken from the electron micrograph film by means of a Zeiss stereomicroscope at × 16. The data derived from three types of vesicular material are tabulated in Fig. 3. The range and peak of the histograms coincide very closely, suggesting that previous data obtained with conventional electron microscopy are not invalidated by this examination of nearly undenaturated synaptic vesicles. The range of diameters, extending from 250 to 1000 Å, of slightly more than 200 freeze-etched profiles includes not only clear vesicles but the dark-cored ones as well. The peak of the histogram (approximately 500 Å) is not necessarily in agreement with the mean diameter of the vesicular spheres; rather it represents the mode of the distribution of all diameters that were measured from randomly cut vesicular profiles. Calculation of the "true" mean diameter of vesicular spheres would require more extensive data. The diameters of dark-cored vesicular profiles varies between 600 and 1200 Å in the histogram of conventionally prepared whole-tissue sec-

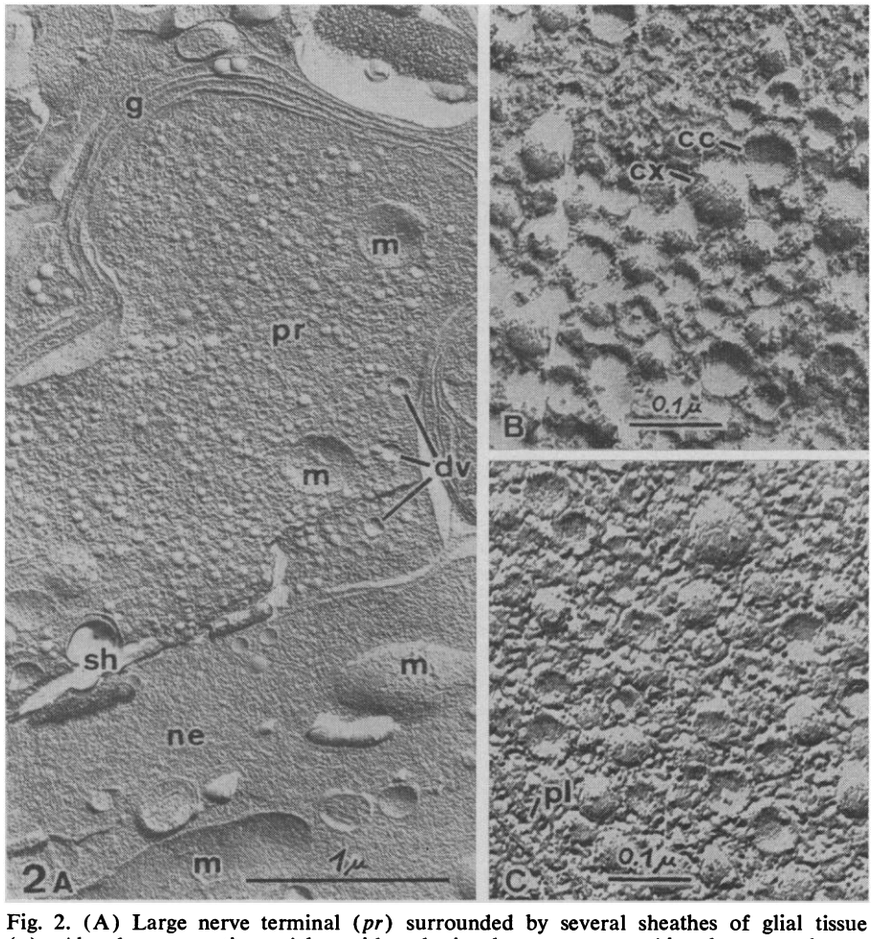

Fig. 2. (A) Large nerve terminal (*pr*) surrounded by several sheathes of glial tissue (*g*). Abundant synaptic vesicles with spheric shape are seen (*dv*, dense-cored vesicles; *m*, mitochondria; and *ne*, perikaryon of nerve cell). The possible synaptic site between nerve terminal and soma is partly blurred by shadows (*sh*). Glutaraldehyde fixation; primary magnification, × 20,000. (B) Synaptic vesicles at × 120,000. Note the concave (*cc*) and the convex (*cx*) profiles. Unfixed specimen to be compared with fixed specimen in (C). Primary magnification, × 40,000. (C) Synaptic vesicles at × 120,000. They have sizes similar to those in (B) in spite of aldehyde fixation. A slight difference in the cytoplasmic texture is noticeable; *pl*, plasmalemma of terminal. Primary magnification, × 20,000.

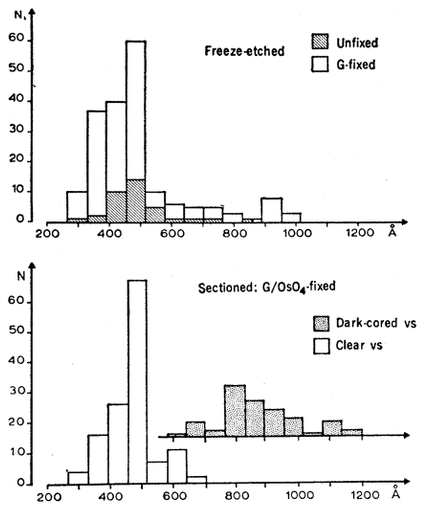

Fig. 3. Histograms of diameters of freeze-etched and conventionally prepared synaptic vesicles. (Top) Histograms of freeze-etched fixed and unfixed tissue. Note that peaks and ranges correspond closely. However, clear and dark-cored vesicles (*vs*) could not be separated. Histogram of conventionally treated vesicles (see bottom) suggests that vesicles exceeding 800 Å are dark-cored. (Bottom) Histogram of synaptic vesicles in whole tissue sections fixed in glutaraldehyde and OsO_4 (conventional electron micrographs). Note that the peak coincides with that of freeze-etched vesicles (top). The ranges of clear and dark-cored vesicles overlap slightly.

tions (Fig. 3); even larger ones may be seen occasionally. On the other hand, the diameters of clear vesicle profiles extend up to 700 Å. Thus, there is an overlap in size between the two categories of vesicles. For this reason, it may be concluded that in freeze-etched preparations only vesicles exceeding diameters of 800 Å may safely be classified as being dark-cored.

Plasmalemmal stomata that were described in nonsynaptic regions of nerve terminals in a previous communication (*4*) are confirmed in the present study (Fig. 1). These stomata have a diameter of approximately 350 Å and are interpreted as pinocytotic vesicles. Their relevance to the problem of formation of synaptic vesicles cannot be clarified with morphological studies alone.

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20 March 1969

Aphid Transmission of Tobacco Mosaic Virus

Abstract. *Aphids* (Myzus persicae *Sulz.) can acquire tobacco mosaic virus from tobacco leaves coated with a virus suspension and inoculate it into healthy leaves. Transmission depends on virus concentration, period of acquisition, previous feeding history of the aphids, and time between acquisition and transmission feedings. Aphids whose stylets are cut do not transmit the virus.*

Aphids transmit plant viruses by carrying them either internally (circulative viruses) or at their stylets (stylet-borne viruses). However, several viruses are not transmitted by aphids. Despite various attempts, no conclusive evidence has been presented why tobacco mosaic virus (TMV) cannot be transmitted by aphids, although as the result of this research some aspects of the relation between aphids and TMV could be clarified (*1–3*). For example, ingestion of TMV by aphids (*1*) and its subsequent discharge into a feeding substrate was demonstrated (*3, 4*). *Myzus persicae* Sulz. and *M. circumflexus* (Buckton) were shown to inoculate TMV when probing on leaves previously sprayed with TMV (*5*). We have found that *M. persicae* can acquire TMV from virus-covered leaves and inoculate it into healthy leaves.

Green peach aphids *M. persicae* were reared on Chinese cabbage (*Brassica pekinensis* Rupr.) or tobacco (*Nicotiana tabacum* L.). *Nicotiana glutinosa* L. was used as a test plant since it produces local lesions upon inoculation with TMV and is a more sensitive indicator when inoculated by aphids than is *N. tabacum* var. Xanthi-nc (*6*).

Leaves of *N. glutinosa* were detached, immersed in or sprayed with a suspension of purified or unpurified virus, and placed on plastic support rings in petri dishes partially filled with water. As soon as the virus suspension had dried, about 30 aphids were placed on the leaves for 1 to 3 hours. Test leaves were then cut in half; one-half was used for transmission, the other as control. Aphids were collected from virus-covered leaves into a plastic container and carefully

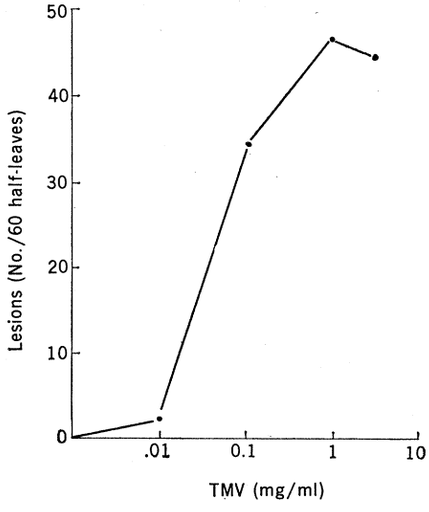

Fig. 1. Effect of virus concentration on transmission expressed as number of lesions produced by aphids that had previously probed on tobacco leaves covered with various concentrations of tobacco mosaic virus. Thirty aphids were placed on each half-leaf.