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Identification of the Active Synaptic Region by Means of Histochemical and Freeze-etching Techniques

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Abstract: Synaptic fine structure was studied with recently developed histochemical methods and the freeze-etching technique. The concept of the *presynaptic vesicular grid* is supported by further evidence from various brain regions. The combined use of techniques for the specific impregnation of the two components proved especially useful. Both S- and F-type synapses have characteristic pre- and postsynaptic densities and are therefore asymmetrical. Postsynaptic densities at axosomatic synapses may be relatively thin in F- as well as in S-type junctions. Freeze-etching pictures of unfixed neuropil tend to confirm the main features of active synaptic sites in F- and S-type synapses as revealed by more conventional techniques. Conceivably, the methods used in this study may prove not only useful in describing normal synaptic morphology but in the qualitative and quantitative evaluation of experimentally induced alterations of the synaptic complex.

INTRODUCTION

Walberg (1968) has provided a critical up-to-date review of morphological correlates of postsynaptic inhibitory processes, and Szentágothai (1970) discussed in the preceding chapter of this monograph the highlights of excitatory processes in terms of synaptic organization from the point of view of functionally oriented morphology.

The aim of this presentation is to summarize structural data obtained more recently with the aid of two new procedures: (1) the use of iodine-heavy metal compounds and (2) application of freeze-etching (Steere 1959) in electron microscopic studies of synapses. Early attempts have been confined for technical reasons to the mammalian subfornical organ and several contributions have resulted from observations in this rather unknown and mysterious region of the brain (Akert, Moor, Pfenninger and Sandri 1969, Moor, Pfenninger and Akert 1969). These data will now be supplemented by those of recent studies on the neuropil of the cat's spinal cord and cerebral cortex where similar findings could be made.

METHODS

Synaptic junctions were studied in the subfornical organ (SFO), spinal cord, and cerebral cortex of rat and cat, as well as in the tectum opticum of pigeon and chicken. The majority of specimens was derived from adult animals; a small number of pre- and postnatal developmental stages was also used.

The first section deals with observations on brain material which was perfusion-fixed with paraformaldehyde, post-fixed with glutaraldehyde, and impregnated with bismuth iodide (BI), according to Pfenninger, Sandri, Akert and Eugster (1969) or with the zinc iodide-osmium tetroxide (ZIO) mixture of Akert and Sandri (1968) modified by Kawana, Akert and Sandri (1969). All sections were stained with uranyl acetate and lead hydroxide. Control sections were prepared from glutaraldehyde-osmium tetroxide (OsO_4) fixed tissue and from blocks impregnated with ethanolic phosphotungstic acid (E–PTA) according to Bloom and Aghajanian (1966). In a number of specimens the ZIO method and the BI technique were successfully combined.

The second section deals with observations on freeze etched brain tissue. The freeze etching method combined with electron microscopy (Moor and Mühlethaler 1963) provides highly accurate views of profiles and surfaces of organelles and cells in the unfixed frozen state. Its application to the neuropil of the mammalian brain (SFO, spinal cord, cerebral cortex) has been accomplished in our laboratories* (Akert, Moor, Pfenninger, Sandri 1969). Small tissue blocks were removed from glutaraldehyde perfusion-fixed or unfixed nerve tissue. Before the freeze etching began it was necessary to treat the specimens for 30 minutes in a Ringer solution containing 25 - 30 per cent glycerol. Tiny blocks were frozen in liquid freon ($-150^{\circ}C$) at a rate of $100^{\circ}C$ per second and placed in a Balzer's BA 360 M freeze-etching device, in which the cutting was performed. Etching was achieved by sublimation of ice from superficial tissue layers and by subsequent evaporating platinum from the tips of pointed carbon electrodes upon the freeze-dried surface of the specimen. This resulted in a layer of several hundred Å depth, which was carefully removed by means of concentrated sulfuric acid and examined in the electron microscope (Elmiscope I). About one half of some 500 successful electronmicrographs was obtained from unfixed material; however, the glutaraldehyde-prefixed material turned out to contain no less important information and proved useful for comparison.

* Freeze-etching was performed in the laboratory of Prof. H. Moor (Laboratory of Electron Microscopy, Department of General Botany, Swiss Federal Institute of Technology, Zürich, Switzerland).

RESULTS

A. Vesicles and densities in S- and F-type synapses

1. *The presynaptic vesicular grid:* Comparison of the results of three different staining methods (OsO_4, BI and ZIO-BI) is provided by Fig. 1. The presynaptic vesicular grid (Akert, Moor, Pfenninger and Sandri 1969) is faintly visible in synaptic cross sections (Fig. 1A). Similarly, the hexagonal arrangement on dense projections (Gray 1963) and its relationship to synaptic vesicles is blurred (Fig. 1B) because the impregnation is weak and the relatively thick sections prevent finer resolution. Nevertheless, it is important to note that the conventional glutaraldehyde-OsO_4 fixation combined with uranyl acetate and lead hydroxide post-staining allows one to identify the presynaptic densities and the crowding of synaptic vesicles within the area of the grid. Dense projections are more clearly detectable after impregnation with the BI-mixture (Fig. 1C) than in specimens treated with E-PTA (Bloom and Aghajanian 1966) or with uranyl acetate (Westrum and Lund 1966). On the other hand, the BI method fails to stain unit membranes including those of synaptic vesicles, a disadvantage not present in the combined OsO_4-PTA technique of Gray (1959). Thus, the synaptic vesicles in BI preparations are represented rather indistinctly as round holes whose contours are surrounded by an electron dense fuzz. Additional BI preparations have been presented in previous contributions (Akert and Pfenninger 1969, Pfenninger, Sandri, Akert and Eugster 1969). An important improvement is obtained when the ZIO-staining of synaptic vesicles is combined with BI impregnation (Fig. 1D). Both dense projections and synaptic vesicles can be clearly rec-

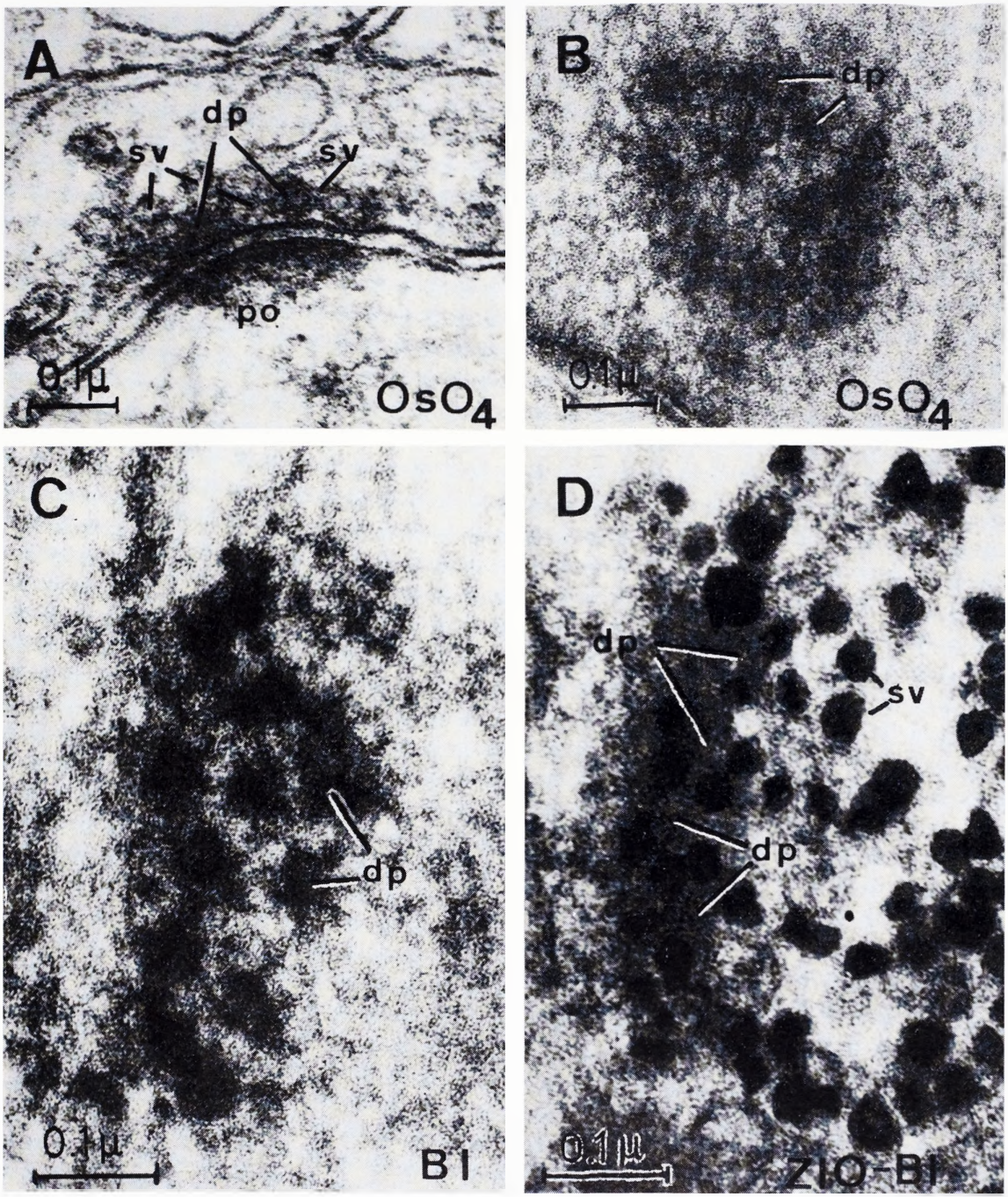

Fig. 1. The presynaptic vesicular grid as demonstrated with different techniques. *A:* Cross section of synapse with spheric vesicles in the superficial layers of the chick (4 weeks after hatching) optic tectum. Note the presynaptic dense projections (dp) surrounded by synaptic vesicles (sv). *B:* Tangential section through the presynaptic area in pigeon optic tectum. Dense projections form hexagonal array; profiles of synaptic vesicles are seen between and around dense projections. Glutaraldehyde-OsO_4; poststained with uranylacetate and lead hydroxide. *C:* Tangential section through presynaptic area of cat spinal cord. Glutaraldehyde-BI method; poststained with uranylacetate and lead hydroxide. The dense projections are clearly visible. Synaptic vesicles are not readily recognized (see text). *D:* Oblique section through presynaptic area of cat spinal cord. ZIO and BI methods combined. Spheric synaptic vesicles (sv) give positive ZIO-reaction and form rosettes around four dense projections (dp). Cf. schematic representation of arrangement in Fig. 3.

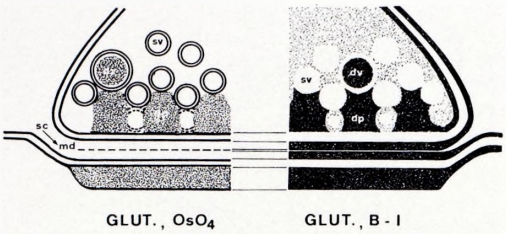

Fig. 2. Comparison of two histochemical procedures for demonstration of 'active synaptic sites' (Couteaux 1961). Both procedures have in common: perfusion-fixation with glutaraldehyde and poststaining of sections with uranyl and lead. Left: Impregnation with OsO_4 shows unit membranes at synapse and of synaptic vesicles. The so-called synaptic densities (presynaptic dense projection, intra-cleft lines and postsynaptic density) are less prominent. Right: Impregnation with BI compound fails to show unit membranes, instead emphasizes synaptic densities.

ognized and the rosette-like arrangement (Akert, Moor, Pfenninger and Sandri 1969) is amply confirmed.

The fundamental differences as well as advantages and disadvantages of the OsO_4 as against the BI method with respect to the classical components of synaptic morphology are illustrated in Fig. 2.

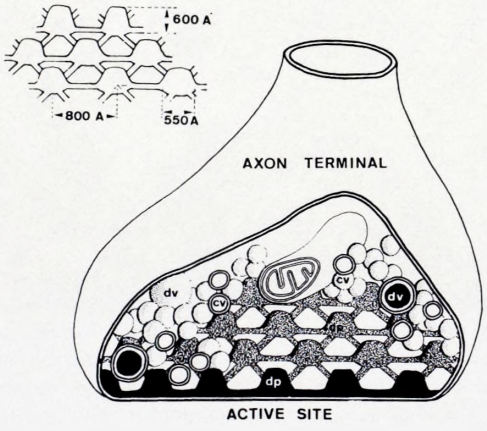

Fig. 3. Presynaptic vesicular grid as reconstructed from observations in synapses with spheric-type vesicles. Explanations see text. (From Pfenninger, Sandri, Akert and Eugster 1969).

Fig. 3 depicts the present view on organization of the presynaptic area according to Pfenninger, Sandri, Akert and Eugster (1969). A grid-like structure is provided by the fuzz subjacent to the presynaptic membrane, and the synaptic vesicles are positioned in its holes and around the peaks. The functional significance of this complex arrangement is by no means clear. It is noteworthy that similar presynaptic structures are present at the active sites of receptor junctions of vertebrates (De Robertis 1964, Wersäll 1966, Dowling and Boycott 1966, and others), and invertebrates (Trujillo-Cenoz 1965), motor endplates (Akert, Moor, Pfenninger and Sandri 1969) and insect synapses (Lamparter, Steiger, Sandri and Akert 1969). When comparing these various situations one is increasingly aware of the fact that the intimate and often very regular topographical relationship between dense projections and vesicles may reflect a highly selective mechanism which seems to have gone almost unnoticed, although it has been already demonstrated by Gray in 1963.

2. *Pre- and post-synaptic densities at S- and F-type synapses:* One problem which came up during a recent symposium on 'Synaptic Mechanisms' (see Akert, Moor, Pfenninger and Sandri 1969) is whether the *presynaptic grid formation* as demonstrated in Fig. 3 may be an exclusive feature of excitatory junctions or whether it may be similar or identical in inhibitory synapses. As the problem stands (Walberg 1968, Gray 1969), we would prefer to confine the discussion of this problem to synapses characterized by S-(spheric) and F-(flat) type vesicles (Uchizono 1965, Bodian 1966, Walberg 1966).

Fig. 4. consists of photographs taken from cat spinal cord ventral grey matter which was treated with the combined ZIO and BI techniques. Both S-type synapses (= synapses with S-type vesicles) and F-type synapses (= synapses with F-type vesicles) are seen. The positive ZIO reaction of synaptic vesicles (irrespective of shape) has been described in more detail elsewhere by Kawana, Akert and Sandri (1969). Here, it should be pointed out that F-

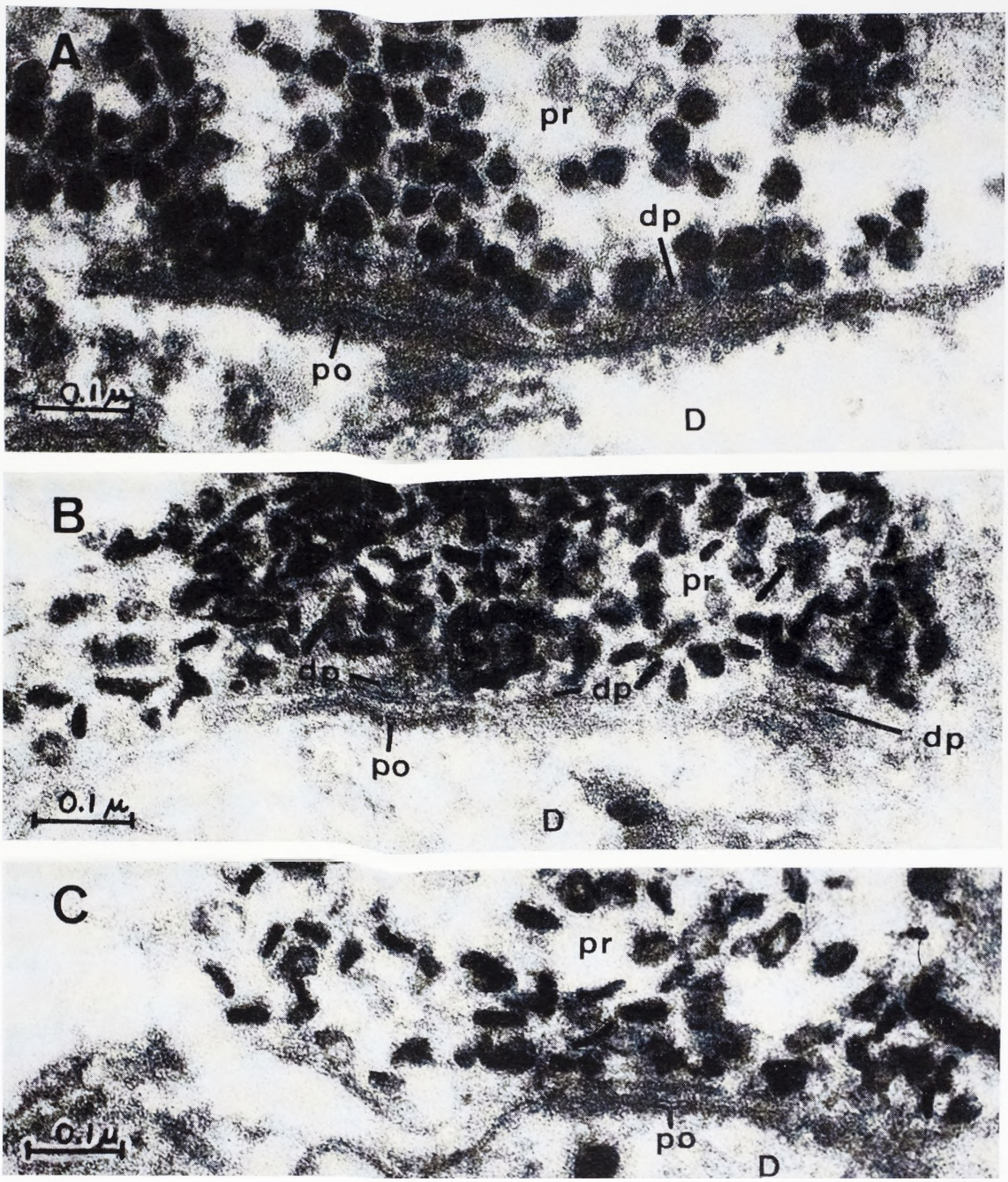

Fig. 4. S- and F-type axodendritic synapses from cat spinal cord ventral grey. Compare shape of vesicles, width of synaptic gap, width of postsynaptic density (po) and visibility of presynaptic dense projections (dp). A = S-type synapse; B and C = F-type synapse. pr = presynaptic terminal, D = dendrite. Note that both flat and spheric vesicles give positive ZIO reaction. Some vesicles remain unstained. Technique: Combined ZIO and BI methods.

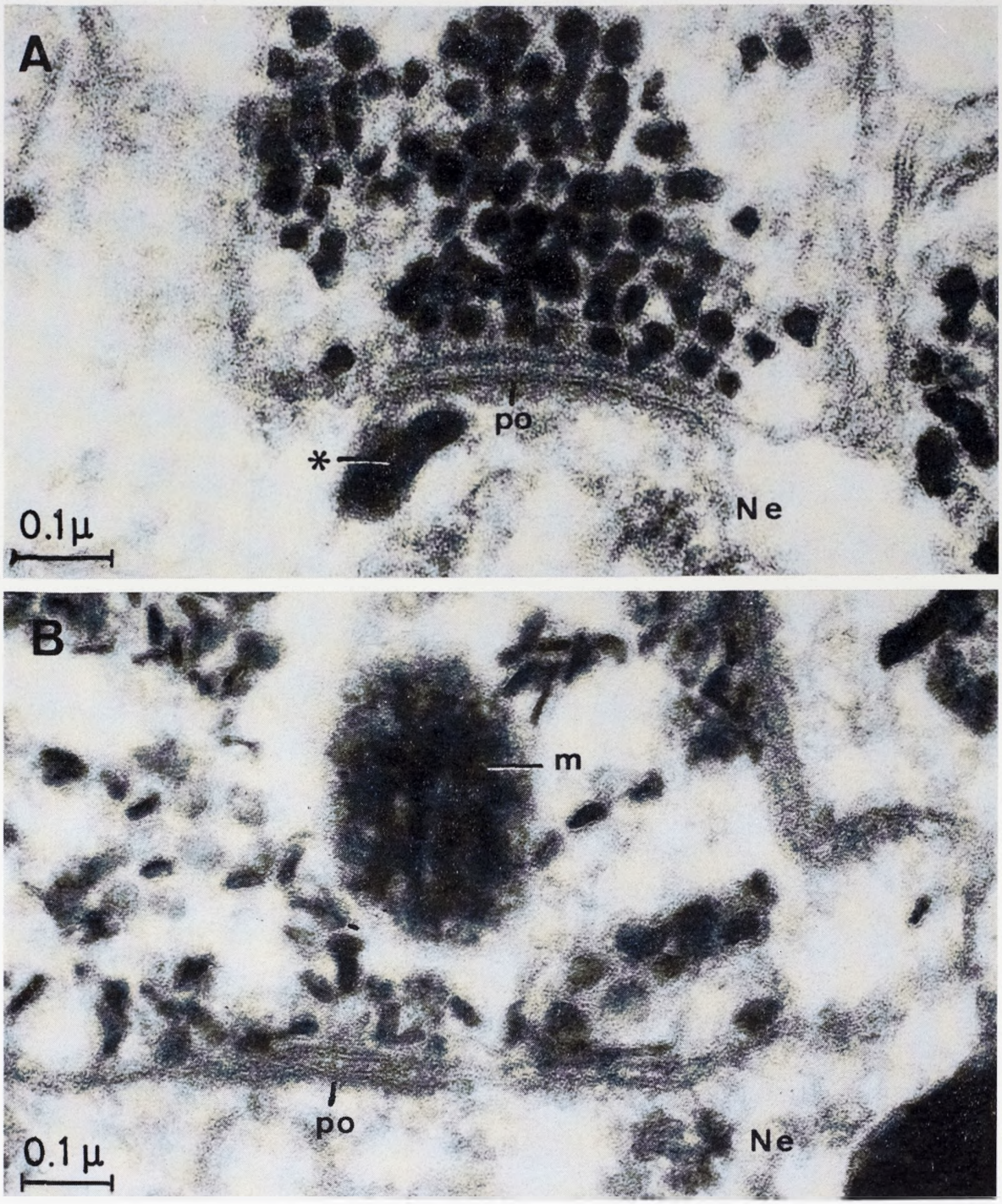

Fig. 5. S- and F-type axosomatic synapses from cat spinal cord ventral grey. Compare postsynaptic densities between *A* (S-type) and *B* (F-type) as well as with analogous photographs in Fig. 4. Asterisks indicate accumulation of ZIO-positive material. m = mitochondrium. The fact that the junctions are on soma is derived from overall review of the region in which they were taken. Technique: same as in Fig. 4.

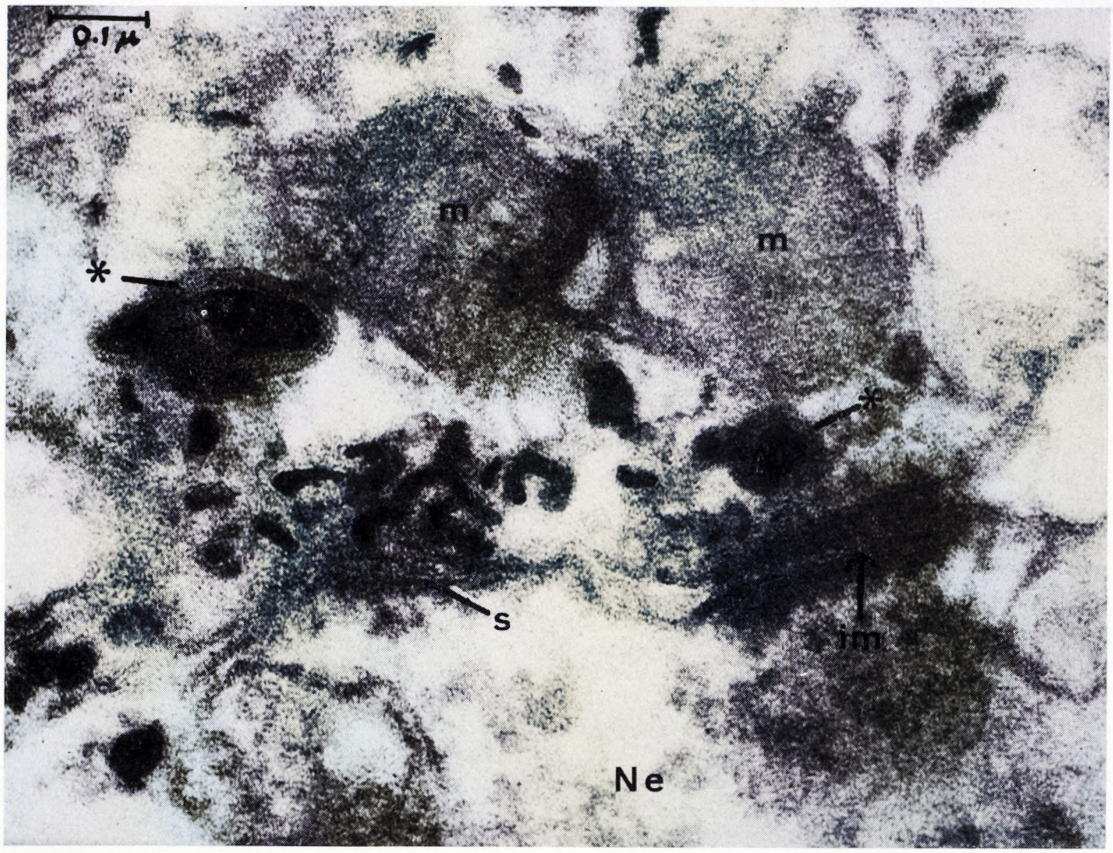

Fig. 6. F-type synapse (s) and adjacent attachment plaque (im) from neurone (Ne) cell body in cat spinal cord. The former is clearly asymmetrical in contrast to the latter. F-type synapse contains small postsynaptic thickening and dense projection. Asterisk designates accumulation of ZIO positive material in unknown structure. m = mitochondria. Technique: same as in Fig. 4.

type synapses contain presynaptic dense projections (Fig. 4B). This observation is not unexpected in view of Gray's observations (1961) that class 2 synapses (which corresponds more or less to F-type) have dense projections. Minor differences of dense projections (miniaturization of F-type grid) between the two types of synapses have been discussed in connection with recent data obtained with the aid of the BI method by Akert, Moor and Pfenninger (1969); however, these observations require confirmation by other methods.

The contrast between S- and F-synapses with respect to *postsynaptic densities* is more conspicuous and firmly established (Gray 1961, 1963, Hamlyn 1962, Colonnier 1968). Again, it seems that the BI-stained material brings out these differences more clearly than material prepared with other methods. The fact that the cytoplasmic side of postsynaptic membranes in F-type junctions is vested by a dense layer should be emphasized, since it is hardly visible in conventionally fixed and stained neuropil. Measurements in spinal cord synapses have revealed a ratio between S- and F-type postsynaptic thickenings of 2:1 with respect to mean depth. However, this ratio seems to be valid only in dendrites (Fig. 4). In contrast, examination of axosomatic junctions suggest that the difference in postsynaptic thickenings may be less prominent (Fig. 5).

Two important points seem to emerge from

the results represented in Figs. 4 and 5: 1) S- and F-type junctions differ in more than one respect (e.g. shape of vesicles, postsynaptic thickenings). 2) Both types are characterized by the presence of pre- and postsynaptic densities, a fact that is usually overlooked or underestimated in relying exclusively on osmicated material. The second point is particularly important because S-type synapses have been designated as 'asymmetrical' and F-type as 'symmetrical' (Colonnier 1968). Symmetry, however, is not to be found within the family of chemical synapses. Rather, it is typical of interneural attachment plaques (Farquhar and Palade 1963), and possibly of so-called electrotonic junctions (Robertson, Bodenheimer and Stage 1963, Hama 1961, Pappas 1966, Martin 1969). Our data show that F- and S-type synapses are both asymmetrical (vesicles, pre- and postsynaptic densities). Fig. 6 demonstrates an F-type synaptic contact side-by-side with a symmetrically arranged attachment plaque inserting at the soma of the same nerve cell. The asymmetry of the former is quite obvious and justifies our concern with terminology. The use of purely descriptive terms such as 'type 1 or 2 synapses' according to Gray or 'S- and F-type synapses' according to Uchizono (1968) seems to be adequate until more about their functional significance is known. In this respect, it is of considerable interest that S-type synapses with relatively narrow postsynaptic thickenings seem to prevail at the cell soma. Thus, the postsynaptic membrane densities at the neurone soma may differ less conspicuously than the shape of synaptic vesicles within

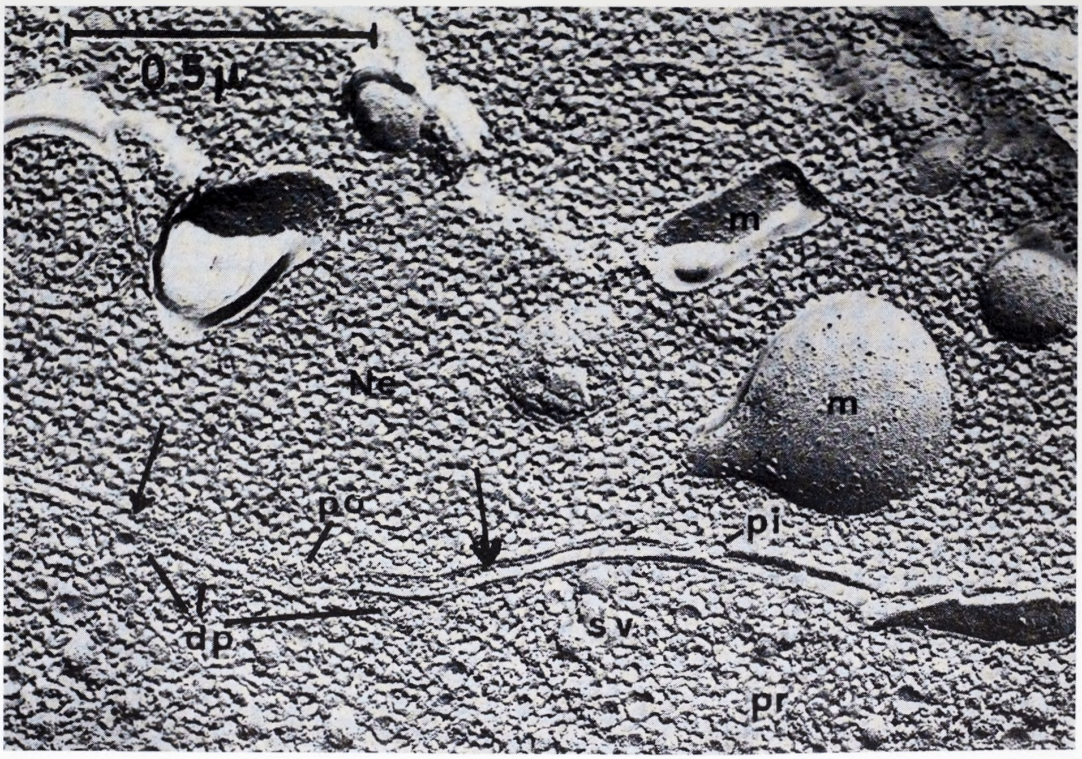

Fig. 7. Freeze-etched axosomatic synapse from cat subfornical organ. This specimen was prefixed with glutaraldehyde. Arrows point to active site. Presynaptic terminal (pr) is below. Synaptic densities may be recognized. Intracleft material seems to reveal 'in parallel' – and crosswise arrangement (see text). pi = pinocytotic vesicle at postsynaptic membrane. Note that all vesicle profiles are circular. Double leaflet of plasmalemma is recognized at arrows.

Fig. 8. Freeze-etched axosomatic synapse from cat subfornical organ. This specimen was not prefixed with aldehydes. Arrows point to active site. Presynaptic dense projections and intracleft material can be recognized in terms of finely granulated material. The postsynaptic density is not clearly seen. ax = preterminal axon. G = glial sheets. Ne = neuronal perikaryon.

the corresponding terminals. This reminds one of the fact that excitatory and inhibitory properties of synapses are – in the final analysis – determined by changes occurring at the *post*-synaptic site (postsynaptic potentials, electrolyte permeability) rather than by presynaptic events. It seems therefore unwise to classify E-type and I-type synapses too rigidly with the aid of presynaptic morphology.

B. Freeze-etching of synapses

1. *Synaptic cleft and synaptic densities:* In conventional electron micrographs the active site is mainly defined by a zone of apposition between membranes with a slightly enlarged interstitial space. On the cytoplasmic side the plasmalemma is furnished with typical densities which have been illustrated and discussed in the preceding section. The synaptic cleft itself seems to contain a finely granular or vaguely filamentous material whose orientation is crosswise arranged according to some (Van der Loos 1963, De Robertis 1964, Gray 1966), and parallel to the membranes according to other authors (Bloom and Aghajanian 1968, Akert and Pfenninger 1969). Bondareff (1967) demonstrated that the synaptic gap may be filled with densely packed granules forming an electron opaque reaction with ruthenium red. Presumably, these somewhat diverging observations reflect molecular complexities of inner and outer membrane coats at the active site.

And it seemed of interest to examine freeze-etched preparations which may reveal structural arrangements of the nearly native state. The most informative cast of a synaptic site in our collection is unfortunately one derived from an aldehyde-fixed specimen (Fig. 7) representing an axo-somatic junction in the neuropil of the cat subfornical organ.

The *synaptic gap* is about 200 Å wide and contains very fine granules whose main orientation may be along two parallel lines which are interrupted at regular intervals, thus suggesting a combined horizontal and vertical arrangement. The accumulation of granular material is limited within a certain region of about 0.5 μ, although some granulations may be encountered within adjacent segments of the interstitial space. Similar findings are represented in the region of the active site in synapses pictured in Figs. 8 and 9, which are derived from unfixed material. Unfortunately, the suggestive arrangement described in Fig. 7 is not clearly seen, and it remains for future studies to decide whether it reflects an aldehyde fixation artifact or the 'true' structural arrangement. It is noteworthy that the view of the synaptic cleft obtained in Fig. 7 seems to correspond rather closely to that in BI or uranyl acetate treated material.

Presynaptic dense projections are recognized in Figs. 7, 8, and 9 and consist of patches of granulated material whose grain size is approximately identical with that described within the gap. *The postsynaptic 'web'* is best seen in Fig. 7; its texture corresponds to that of the other densities. All three types of densities are coextensive. The fact that the postsynaptic density is relatively shallow reminds one of the similar finding made in BI treated axo-somatic synapses (see preceding section, Fig. 5). Unfortunately, an adequate picture of a freeze-etched axo-dendritic synapse is not presently available for comparison.

2. *The problem of S- and F-type vesicles:* This problem has received wide attention recently (Uchizono 1968, Gray 1969) and needs not be discussed at length here. It was hoped that examination of unfixed freeze-etched material could bring some further clarification. Preliminary studies (Moor, Pfenninger and Akert 1969) on the subfornical organ confirmed that only nerve terminals with spherical vesicles may be found in this region (the fact that vesicles are of spherical shape in the unfixed state being in itself of considerable importance). Analogous investigations of spinal cord neuropil are briefly reported here. Twelve unfixed boutons with spherical vesicles are available for comparison with three terminals which may contain elongated profiles. Thus, the present material is too small to draw definite conclusions.

Fig. 10 represents an S-type bouton which was not treated with aldehyde before freeze-etching was performed. Numerous circular profiles of synaptic vesicles are readily seen. In many instances, concave spherical reliefs can be identified. The vesicles seem to occupy a considerable portion of the total profile of the terminal. The synaptic site is covered by a shadow.

Fig. 11 may possibly represent an F-type bouton. Again, this cast is derived from unfixed material. Numerous oval and elongated profiles of vesicles are seen along with circular ones of comparable size. Aside from the shape of vesicles one may note that the terminal is less crowded with vesicles than that in Fig. 10 and that the concave reliefs of elongated vesicles are shallower than that of the spherical ones. Conceivably, the casting of F-type vesicles may meet with difficulties since the smallest dimension is no more than 150 Å (compare with Figs. 4C, 5B, and 6). This may explain the paucity of vesicular profiles in the corresponding endbulbs. It may be argued that the elongated form of vesicles is due to distortion caused by a slight curvature which is signalled by corresponding shadows at the lower border of the terminal. However, additional elongated profiles are encountered outside of this zone.

On the whole, it may be too early to conclude that boutons with flat vesicles are present in freeze-etched spinal neuropil, but further evidence may well be rapidly accumulating.

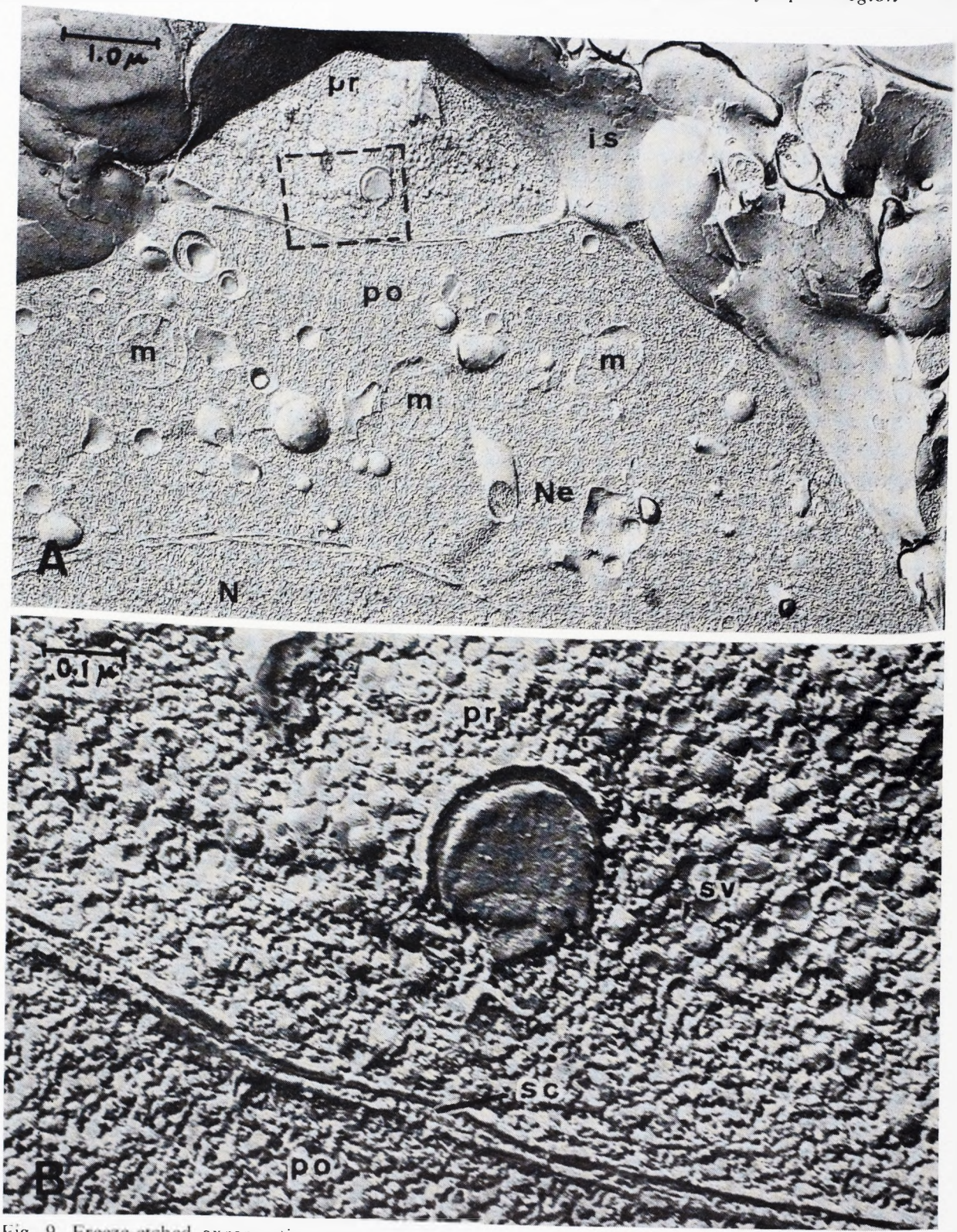

Fig. 9. Freeze-etched axosomatic synapse from cat subfornical organ. This specimen was not prefixed with aldehydes. *A* shows overall view with boutons (pr) synapsing at soma (Ne). The cytoplasm contains numerous organelles. N = nucleus. Dashed rectangle delineates area shown in *B*. The latter gives details on active site. Presynaptic area is less crowded with vesicles (sv) than the more remote areas of the end bulb. Patches of finely granulated material may indicate location of presynaptic dense projections. sc = synaptic cleft.

Fig. 10. Freeze-etched axosomatic synapse from unfixed subfornical organ of the cat. Note the crowding of spherical vesicles (sv) in the presynaptic bouton (pr). *A* shows overall view. *B* represents enlarged section delineated in *A* by dashed line. The synaptic cleft (arrow) is not visible. Further explanation see text.

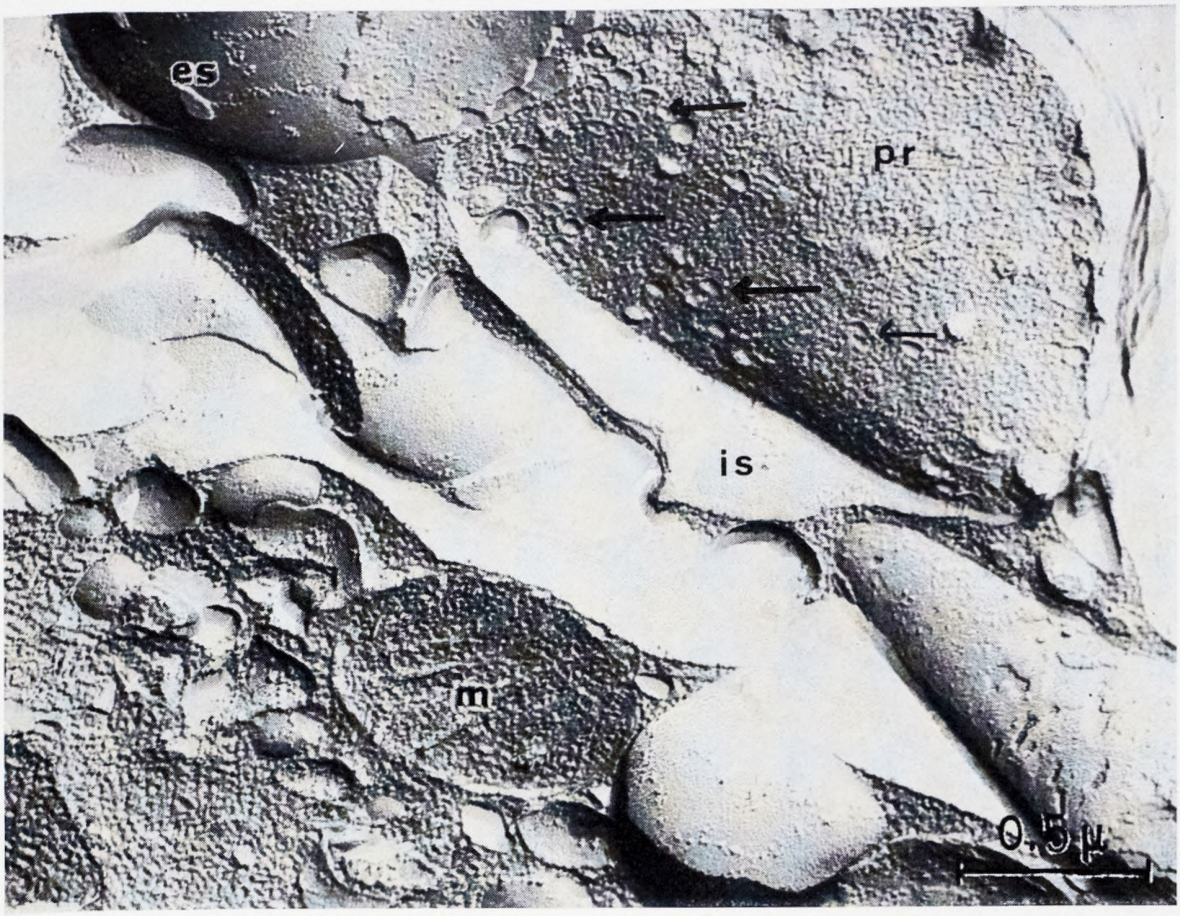

Fig. 11. Freeze-etched presynaptic terminal (pr) from unfixed spinal cord of the cat. Arrows point at groups of elongated profiles of synaptic vesicles. is = inner surface. m = mitochondrium. For further explanations see text.

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REFERENCES

Akert, K. and Pfenninger, K. Synaptic fine structure and neural dynamics. In *Cellular Dynamics of the Neuron*. I. S. C. B. Symposium, Paris, ed. Barondes, S. H., Academic Press, London, New York. In press.

Akert, K. and Sandri, C. An electronmicroscopic study of zinc iodide-osmium impregnation of neurons. I. Staining of synaptic vesicles at cholinergic junctions. *Brain Res. 7*, 286–295, 1968.

Akert, K., Moor, H., Pfenninger, K. and Sandri, C. Contribution of new impregnation methods and freeze etching to the problem of synaptic fine structure. Pp 223–240 in Akert, K. and Waser, P. (eds.). *Progress in Brain Research*, Vol. 31, Elsevier, Amsterdam, London, New York, 1969.

Akert, K., Moor, H. and Pfenninger, K. *Synaptic Fine Structure*. First Int. Symposium on Cell Biol. and Cytopharm., Venice. Raven Press, New York. In press.

Bloom, F. E. and Aghajanian, G. K. Cytochemistry of synapses: selective staining for electron microscopy. *Science 154*, 1575–1577, 1966.

Bloom, F. E. and Aghajanian, G. K. Fine structural and cytochemical analysis of the staining of synaptic junctions with phosphotungstic acid. *J. Ultrastruct. Res. 22,* 361–375, 1968.

Bodian, D. Electronmicroscopy: two major synaptic types on spinal motorneurons. *Science, 151,* 1094–1095, 1966.

Bondareff, W. An intercellular substance in rat cerebral cortex: submicroscopic distribution of ruthenium red. *Anat. Rec. 157,* 527–535, 1967.

Colonnier, M. Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscopic study. *Brain Res. 9,* 268–287, 1968.

Couteaux, R. Principaux critères morphologiques et cytochémiques utilisables aujourd'hui pour définir les differents types de synapses. *Actualités neurophysiol. 3,* 145–173, 1961.

De Robertis, E. D. P. *Histophysiology of Synapses and Neurosecretion.* Pergamon Press, Oxford, 1964, 256 pp.

Dowling, J. E. and Boycott, B. B. Organization of the primate retina: electron microscopy. *Proc. roy. Soc. B. 166,* 80–111, 1966.

Farquhar, M. G. and Palade, G. E. Functional complexes in various epithelia. *J. Cell Biol. 17,* 375–412, 1963.

Gray, E. G. Axosomatic and axodendritic synapses of the cerebral cortex: an electronmicroscopic study. *J. Anat. (Lond.), 93,* 420–433, 1959.

Gray, E. G. Electron microscopy of presynaptic organelles of the spinal cord. *J. Anat. (Lond.) 97,* 101–106, 1963.

Gray, E. G. Problems of interpreting the fine structure of vertebrate and invertebrate synapses. *Int. Rev. Gen. and exper. Zool. 2,* 139–170, 1966.

Gray, E. G. Electron microscopy of presynaptic inhibitory synapses: a brief review. pp. 141–155 in Akert, K. and Waser, P. (eds.) *Progress in Brain Research,* Vol. 31, Elsevier, Amsterdam, London, New York, 1969.

Hama, K. Some observations on the fine structure of the giant fibers of the crayfishes (Cambarus virilus and Cambarus clarkii) with special reference to the submicroscopic organization of the synapses. *Anat. Rec. 141,* 275–293, 1961.

Hamlyn, H. The fine structure of the mossy fiber endings in the hippocampus of the rabbit. *J. Anat. (Lond.) 96,* 112–120, 1962.

Kawana, E., Akert, K. and Sandri, C. Zinc iodide-osmium tetroxyde impregnation of nerve terminals in the spinal grey matter. *Brain Res. 16,* 325–331, 1969.

Lamparter, H. E., Steiger, U., Sandri, C. and Akert, K. Zum Feinbau der Synapsen im Zentralnervensystem der Insekten. *Z. Zellforsch. 99,* 435–442, 1969.

Loos, van der, H. Fine structure of synapses in the cerebral cortex. *Z. Zellforsch. 60,* 815–825, 1963.

Martin, R. The structural organization of the intracerebral giant fiber system of cephalopods. The chiasma of the first order giant axons. *Z. Zellforsch. 97,* 50–68, 1969.

Moor, H. and Mühlethaler, K. Fine structure in frozen-etched yeast cells. *J. Cell Biol. 17,* 609–628, 1963.

Moor, H. Pfenninger, K. and Akert, K. Synaptic vesicles in electronmicrographs of freeze-etched nerve terminals. *Science 164,* 1405–1407, 1969.

Pappas, G. D. Electronmicroscopy of neuronal junctions involved in the central nervous system. Pp. 49–87 in Rodahl, K. and Issekutz, B. (eds.). *Nerve as a Tissue,* Harper and Row, New York, 1966.

Pfenninger, K., Sandri, C., Akert, K. and Eugster, C. Contribution to the problem of structural organization of the presynaptic area. *Brain Res. 12,* 10–18, 1969.

Robertson, J. D., Bodenheimer, Th. and Stage, D. E. The ultrastructure of Mauthner cell synapses and nodes in goldfish brains. *J. Cell. Biol. 19,* 159–199, 1963.

Steere, R. L. Electron microscopy of structural detail in frozen biological specimens. *J. biophys. biochem. Cytol. 3,* 45–60, 1957.

Szentágothai, J. The morphological identification of the active synaptic region. pp. 9–26 in Andersen, P. and Jansen, J. K. S. (eds.). *Excitatory Synaptic Mechanisms.* Universitetsforlaget, Oslo, 1970.

Trujillo-Cenoz, O. Some aspects of the structural organization of the arthropod eye. Pp. 371–382 in Frisch, L. (ed.). *Sensory Receptors,* Vol. 30, Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, New York, 1965.

Uchizono, K. Characteristics of excitatory and inhibitory synapses in the central nervous system. *Nature (Lond.) 207,* 642–643, 1965.

Uchizono, K. Inhibitory and excitatory synapses in vertebrate and invertebrate animals. pp. 33–59 in Euler, C. von, Skoglund, S. and Söderberg, U. (eds.). *Structure and Function of Inhibitory Neuronal Mechanisms.* Pergamon, Oxford, 1968.

Walberg, F. Elongated vesicles in terminal boutons of the central nervous system, a result of aldehyde fixation. *Acta Anat. 65,* 224–235, 1966.

Walberg, F. Morphological correlates of postsynaptic inhibitory processes. pp. 7–14 in Euler, C. von, Skoglund, S. and Söderberg, U. (eds.). *Structure and Function of Inhibitory Neuronal Mechanisms.* Pergamon, Oxford, 1968.

Wersäll, J. Efferent innervation of the inner ear. pp. 123–129 in Euler, C. von, Skoglund, S. and Söderberg, U. (eds.). *Structure and Function of Inhibitory Neuronal Mechanisms.* Pergamon, Oxford, 1968.

Westrum, L. E. and Lund, R. D. Formalin perfusion for correlative light- and electronmicroscopical studies of the nervous system. *J. cell Sci. 1,* 229–238, 1966.