

FORM OF THE POSTSYNAPTIC DENSITY

A Serial Section Study

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ABSTRACT

Through the use of serial sectioning of dog cerebral cortex tissue, holes or perforations could be revealed in the larger postsynaptic densities (PSDs), in confirmation of the earlier work of Peters and Kaisermann-Abramof (1969. *Z. Zellforsch. Mikrosk. Anat.* **100**:487-506). These holes appeared in serial sections which happened to be cut both parallel and normal to the plane of the synaptic junction. Cleft material was absent in that part of the synaptic cleft opposite this hole. Sometimes the presynaptic membrane opposite the hole was indented into the presynaptic cell. In addition, most of the synaptic vesicles in the presynaptic cell close to the membrane were clustered at that part of the membrane opposite the edge of the density disk. The meaning of the hole and of the other features mentioned above for the function of the density is not known at present.

KEY WORDS postsynaptic density
structure · synapse ultrastructure · synaptic
junction ultrastructure · neurobiology

doughnut. In addition, other features of the synapse, related to the position of the hole, have been uncovered.

In a recent paper (4) we came to the conclusion that the gross structure of the postsynaptic density (PSD) resembled a disk with a large perforation or hole in the center. This belief was based on occasional *en face* views of the density in PSD fractions isolated from dog cerebral cortex (4), and on an earlier paper by Peters and Kaisermann-Abramof (10) in which serial electron microscope sections of cerebral cortex revealed that the larger densities did indeed have perforations in them. Since, to our knowledge, the results of that earlier paper have never been confirmed, we decided to repeat this type of study. And indeed, we have confirmed the earlier work of these authors, and their, and our, contention that at least the larger PSD's should be looked upon as perforated disks, somewhat similar in shape to a

MATERIALS AND METHODS

Tissue from the superficial layers of the mid-dorsal part of the cortex from dog brain was fixed by immersion first in 2.5% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.4, and then in 1% osmium tetroxide in 0.03 M barbital buffer, pH 7.4. After rinsing with the latter buffer, the tissue was stained en bloc with 0.5% uranyl acetate to enhance contrast of membranes. The tissue was then dehydrated by standard procedures and embedded in Epon, which was polymerized at 60°F for 3 days. Thin sections were cut on a Porter-Blum MT2B microtome DuPont Instruments-Sorvall, DuPont Co. (Wilmington, Del.); ribbons containing ~13 sections were placed on slotted grids and then stained sequentially with 8% uranyl acetate and 4% lead citrate. Sections were examined with a Hitachi HU-11B electron microscope. From two dog cortices, ~50 series, consisting of 13 sections in each, were examined. Of these, 25 series were photo-

graphed, and representative samples were selected for presentation.

RESULTS AND DISCUSSION

It was not too difficult to obtain serial sections of cerebral cortex that indicated that the PSD is a ringlike structure. In agreement with the earlier observation (10), we too find that most of the larger densities (400–500 nm in diameter) are perforated by a hole. The electron microscope images produced were of two types, those in which the plane of the section was normal to that of the synaptic junction and those in which the plane was parallel. The latter type is shown in Fig. 1. To indicate the serial nature of the images, we have included in the micrographs views of a mitochondrion (*m*), and another density (*p*) which is cut normal to the plane of the section. Fig. 1*a* shows a cut through the presynaptic cell at the site of the junction. Clearly seen (*k*) are large particulate aggregates which are probably the dense knobs or projections noted by others (1, 6, 7, 8, 12). The numerous synaptic vesicles (*s*) identify the cell as being presynaptic. The granular material in the center of the ring formed by the presynaptic projections is possibly membrane material. Fig. 1*b* shows the section in which we believe the postsynaptic density is seen as a doughnut-shaped structure. Still clearly seen, however, is a ring of synaptic vesicles around the density, with still a vestige of the presynaptic knobs being visible. The appearance of the vesicles in the picture can be explained by the observation that at the end of dendritic spines, the postsynaptic membrane is convex with respect to the presynaptic element; a section at this site would thus still contain synaptic vesicles at the outer edges of the PSD. Since the sections are ~50 nm in thickness, it is thus not surprising that a section shows both the postsynaptic density, 30–50 nm thick, as well as the presynaptic elements appearing at the edge of the synaptic junction.

Fig. 1*c* shows a view of the density farther into the postsynaptic cell while Fig. 1*d* indicates the filamentous material extending from the compact part of the density into the postsynaptic cell, material considered to be part of the density (3–5, 13). Also, in some cases, larger PSD's with multiple perforations were discerned, in agreement with the findings of Peters and Kaisermann-Abramof (10).

Fig. 2 shows a typical series in which the sections are normal to the synapse. Again, to

demonstrate the serial nature of the images, views of a large vacuole (*v*) in the postsynaptic cell are included in the pictures. It is clear from the images that Fig. 2*a* and *d* are sections through the two edges of the densities, while Figs. 2*b* and *c* are images of a cut through the center of the density. The "hole" in the center is thus ~100 nm in width, not surprising since the entire density is from 300 to 500 nm in width; thus the hole seen in this plane is about the same size as that seen *en face* in Fig. 1*b*. Of interest is the observation (Fig. 2*b* and *c*) that the cleft material is absent in that part of the cleft opposite the hole in the PSD, and that the synaptic vesicles in the presynaptic cell seem to be clustered in that part of the cell opposite the edges of the density (cf. Fig. 2*b*). This lack of cleft material, as well as the distribution of synaptic vesicles, is also seen in Fig. 3*a* and *b* and Fig. 4*a* and *b*, images taken from two more series of serial sections. In addition, Figs. 3*b* and 4*b* show another feature which is occasionally encountered, the indentation into the presynaptic cell of that part of the presynaptic membrane opposite the hole in the PSD. It is interesting that Pfenninger et al. (11) indicated an indented area at this point in freeze-fracture pictures. Not known at present is whether the indentation represents a functional aspect, i.e., an addition to the presynaptic membrane at this site of membrane of fused synaptic vesicles, or whether it represents an artifact, i.e., a loosening of the synapse at this site due to the absence of cleft material. Also of interest are those images that show that filamentous material which is seemingly lying unattached in the postsynaptic cell (Fig. 2*b*, *c*, and *d*, and Figs. 3*a* and 4*a*) is really seen to be attached to the central mass of the PSD in the next serial section (Figs. 2*a*, 3*b*, and 4*b*).

Finally, Fig. 5*a–h* show serial sections through three densities, cut normal to the synapse. In agreement with Peters and Kaisermann-Abramof (10), no indication could be found of holes in the PSD of these small densities, though there may be a perforation in density p_3 in Fig. 5*c*. We agree with these authors that densities below a certain size, ~200 nm in width, do not show any indication of perforations; perforations may be there but the thickness of the sections may preclude their being visible.

It is of interest that Landis et al. (9) pointed out in one of their freeze-fracture images, a ring of 8–9 nm particles that they interpreted as an annular specialization of the postsynaptic membrane at the

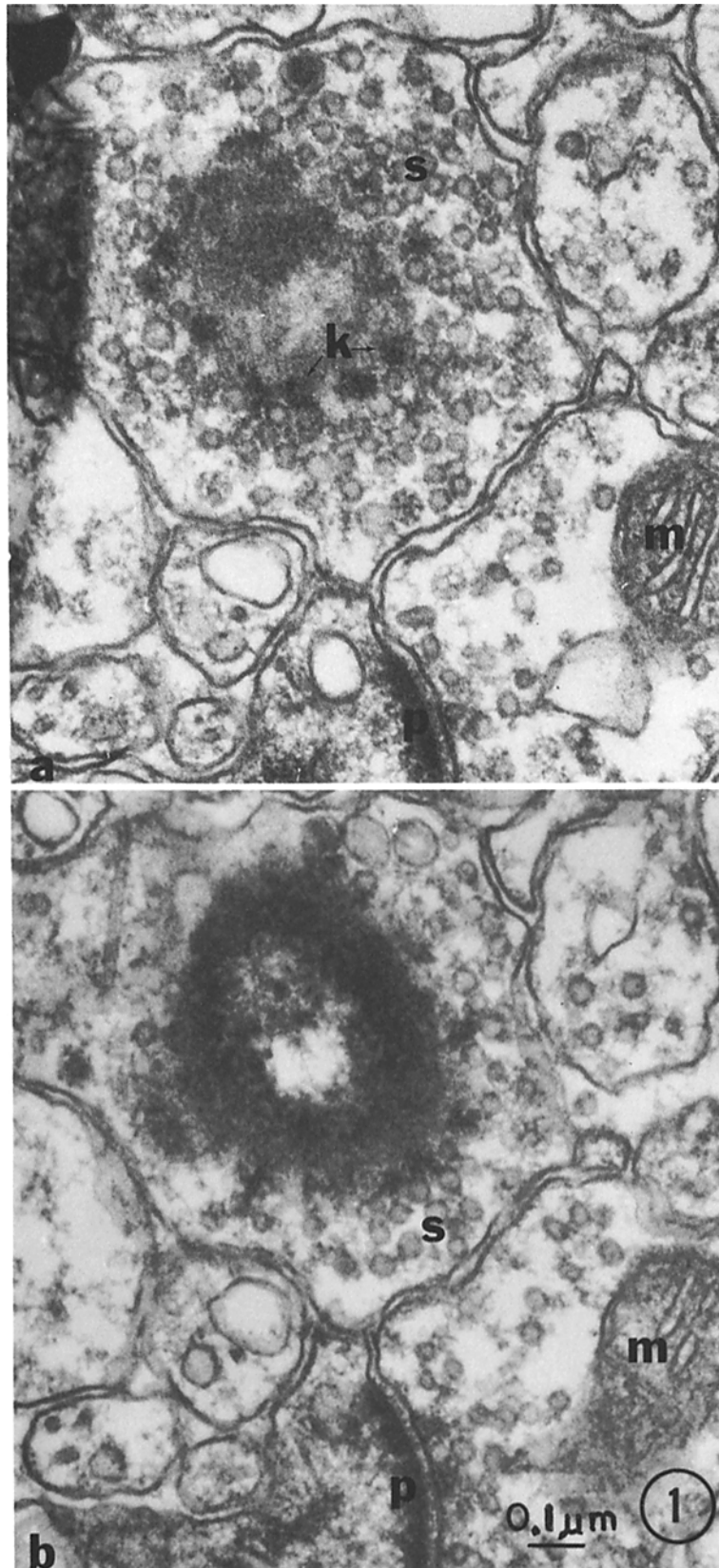
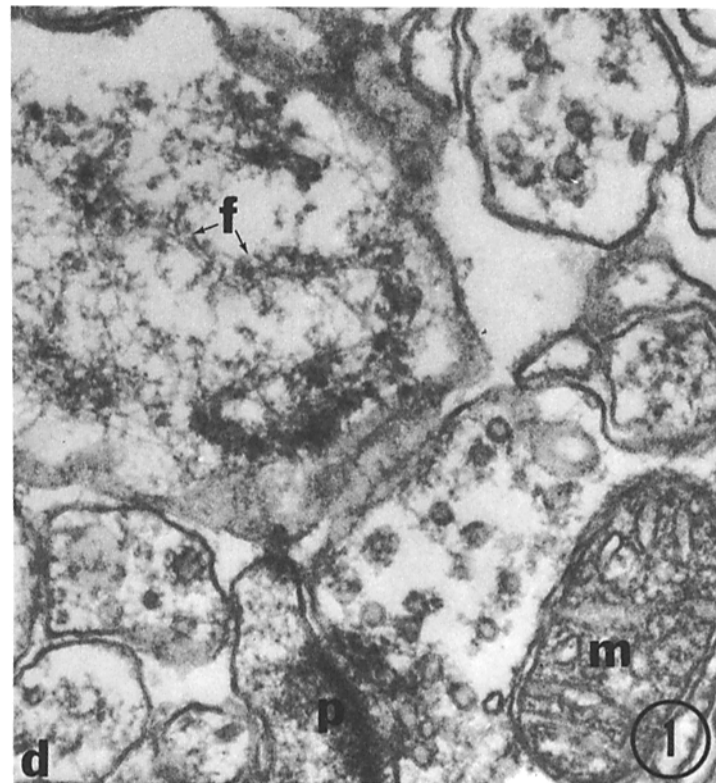
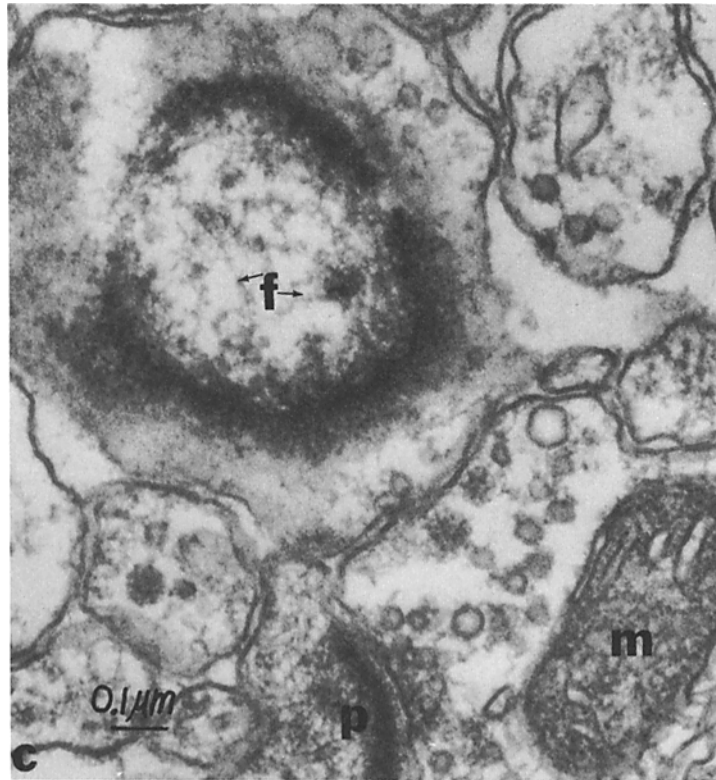


FIGURE 1 Four serial sections cut parallel to the plane of the synaptic junction. *k*, Presynaptic dense knobs or projections; *s*, synaptic vesicles; *m*, the same mitochondrion in all sections; *f*, postsynaptic filaments; *p*, PSD sectioned normal to the plane of the synaptic junction and seen in all sections. $\times 75,000$.



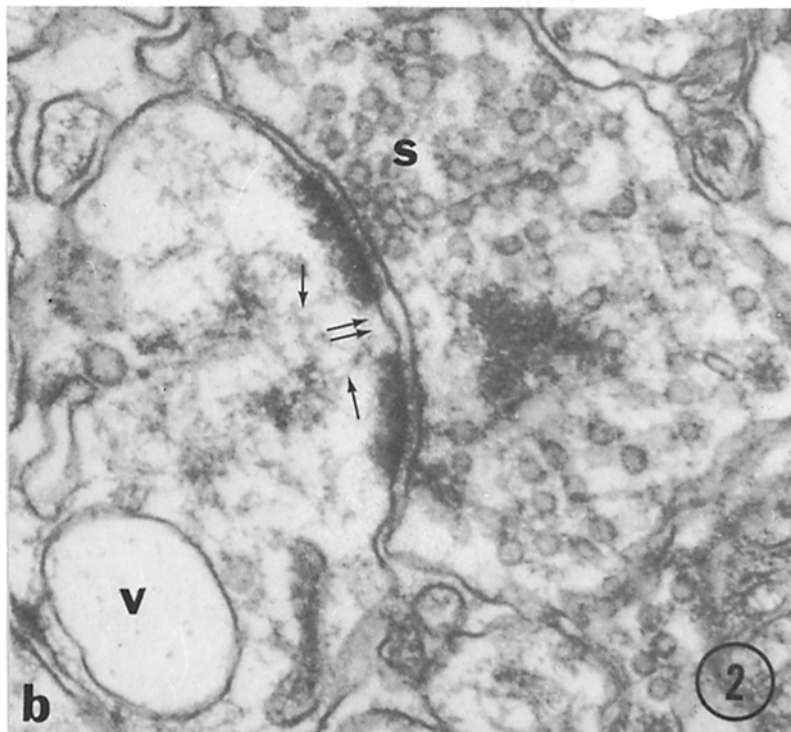
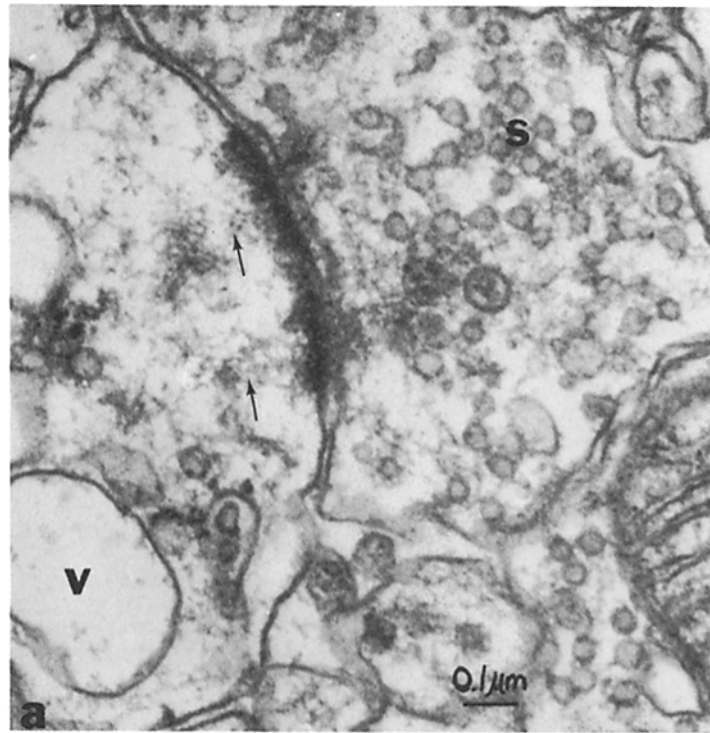
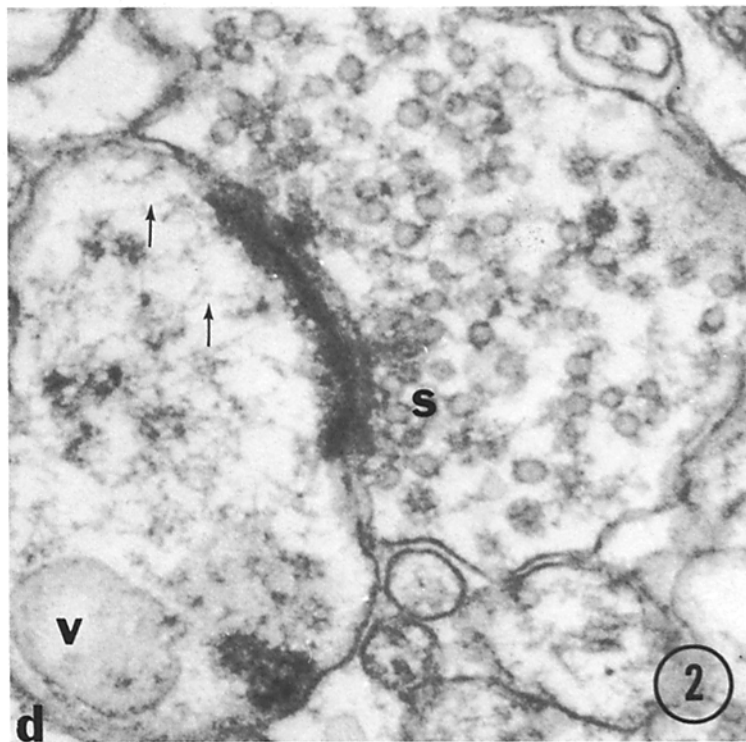
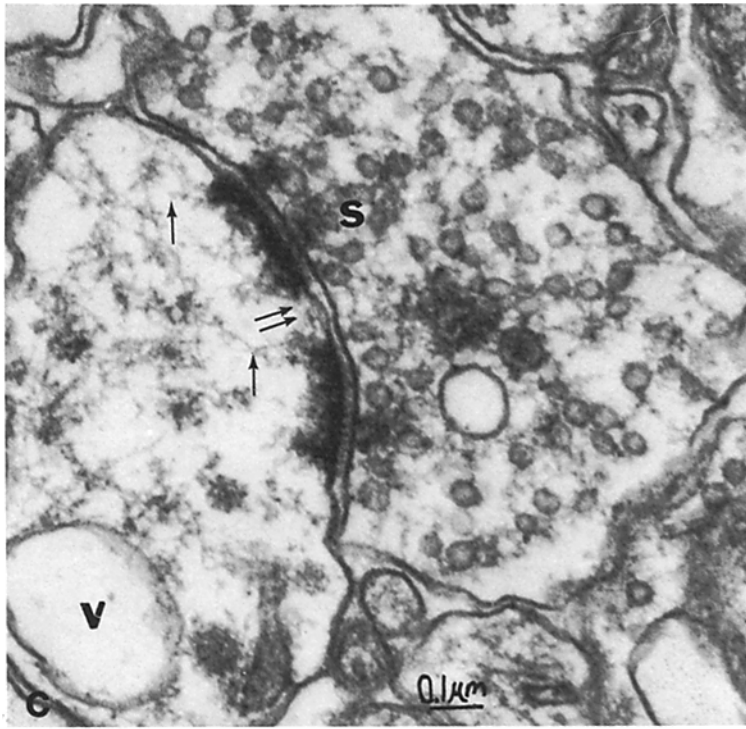


FIGURE 2 Four serial sections cut normal to the plane of the synaptic junction. *s*, Synaptic vesicles; *v*, vacuole in postsynaptic cell and seen in all sections. Double arrows indicate hole in PSD and the absence of cleft material at this site. Single arrows indicate postsynaptic cell filamentous material seen to be attached to the central mass of the PSD. $\times 75,000$.



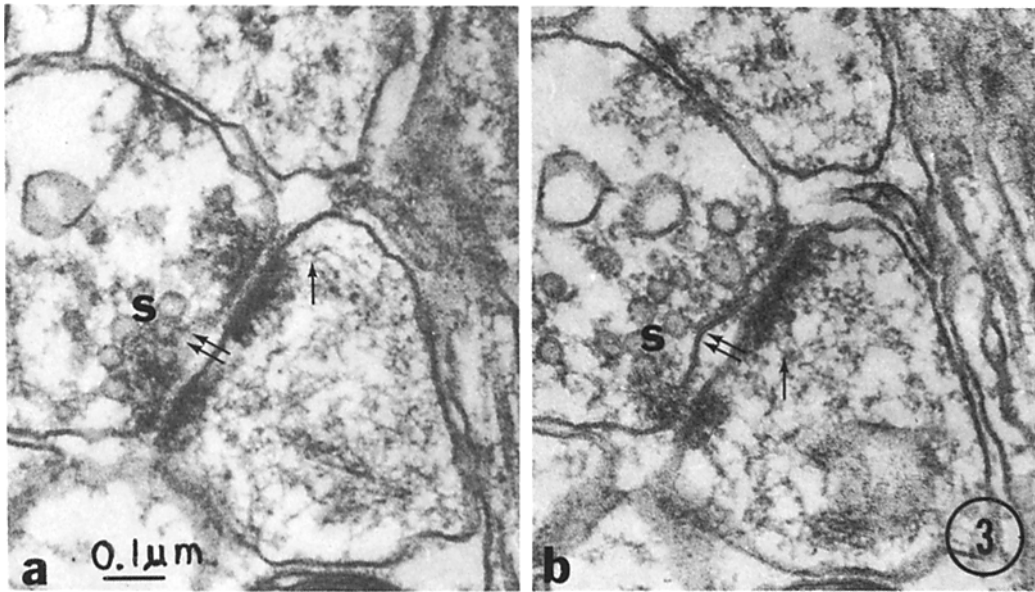


FIGURE 3 Two images from a serial section series cut normal to the plane of the synaptic junction. *s*, Synaptic vesicles. Double arrows indicate indentation of presynaptic membrane at site opposite the hole in the PSD and absence of cleft material at this site. Single arrows indicate postsynaptic cell filaments attached to the central mass of the PSD. $\times 75,000$.

site of the junction. The annular configuration of these particles in the membrane may reflect the annular shape of the PSD.

While occasional *en face* views of isolated PSD's did show perforated disk-shaped PSD's (4), we have not been successful in obtaining a good serial section series of isolated PSD's showing transverse sections of the structure. There are two possible explanations: one is that, without extraneous identifying "markers" such as mitochondria (cf. Figs. 1 and 5) to establish the serialization of the sections, it is difficult to relate a PSD in one section to an image of the same PSD in another, and since the isolated PSD fraction consists of tumbled and somewhat damaged PSD's packed into a pellet, it becomes difficult to follow the same PSD through more than two sections. Nevertheless, we believe that the PSD's isolated in the previous work (4) are very similar in their gross architecture to the structure seen *in situ*, a disk with a hole in the center, since occasionally we have seen the *en face* views of such a structure, as pictured previously (4).

At present, we have no evidence regarding the functional significance of the architecture of the PSD. We can ask questions but must admit that we have no ready answers. Does the occurrence of the clusters of synaptic vesicles opposite that

part of the postsynaptic cell membrane to which are attached the edges of the PSD reflect the occurrence of transmitter-receptors at that postsynaptic membrane site? Does the absence of cleft material opposite the hole of the PSD, and its presence at other parts of the junction, reflect a role for this cleft material in transmitter discharge? Finally, in a previous paper (2) we postulated that the PSD may have a role in amplification, modulation, or control of the transmitter-generated signal within the postsynaptic cell, by means of movements of some of its proteins relative to each other and relative to some membrane proteins. If so, does the occurrence of a hole in the center of the larger PSD's reflect a static representation of a pulsating structure, with openings and closings of the hole due to centrifugal or centripetal movements of its proteins? Thus, the smaller densities, which show no indication of a hole (Fig. 5), may be contracted forms of the larger densities, contractions either due to random fixation artifacts, or due to real differences in the metabolic states of the densities at time of fixation.

We would like to thank Lois Lynch and Roland Blichke for their assistance. This work was supported by National Institutes of Health (NIH) postdoctoral fellowship I-F22-NS-00742-MBY to R. S. Cohen and NIH grant PHS-NS 12726-01A2 to P. Siekevitz.

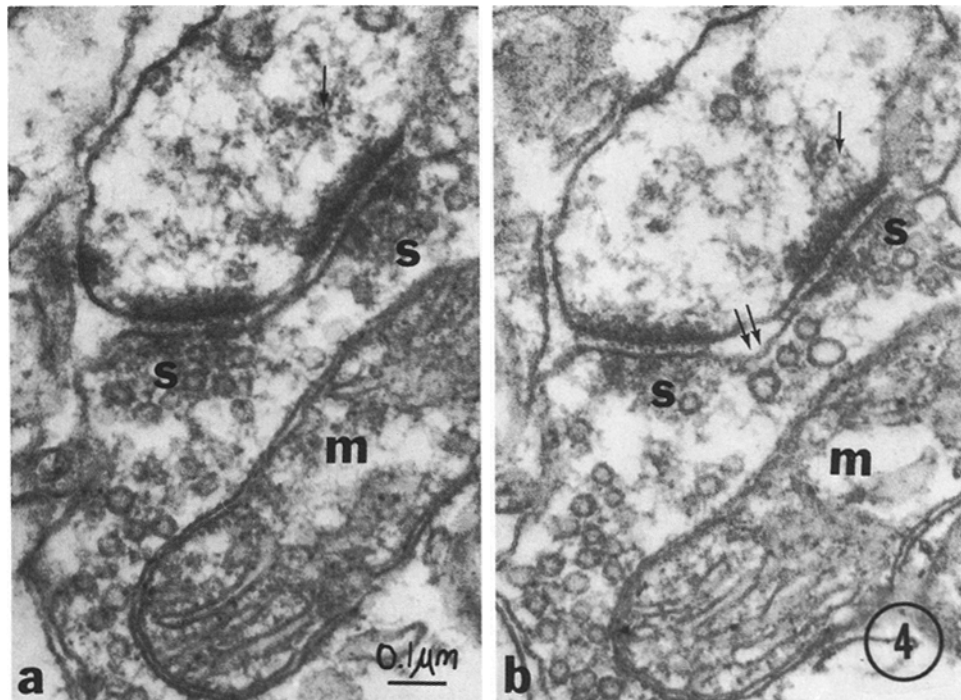


FIGURE 4 Two images from a serial section series cut normal to the plane of the synaptic junction. *s*, Synaptic vesicles; *m*, a mitochondrion seen in both sections. Double arrow indicates indentation of presynaptic membrane at site opposite the hole in the PSD and absence of cleft material at this site. Single arrows indicate postsynaptic cell filaments which appear detached from the isolated PSD structure in one section (Fig. 4*a*) and appear attached to it in the other section (Fig. 4*b*). $\times 75,000$.

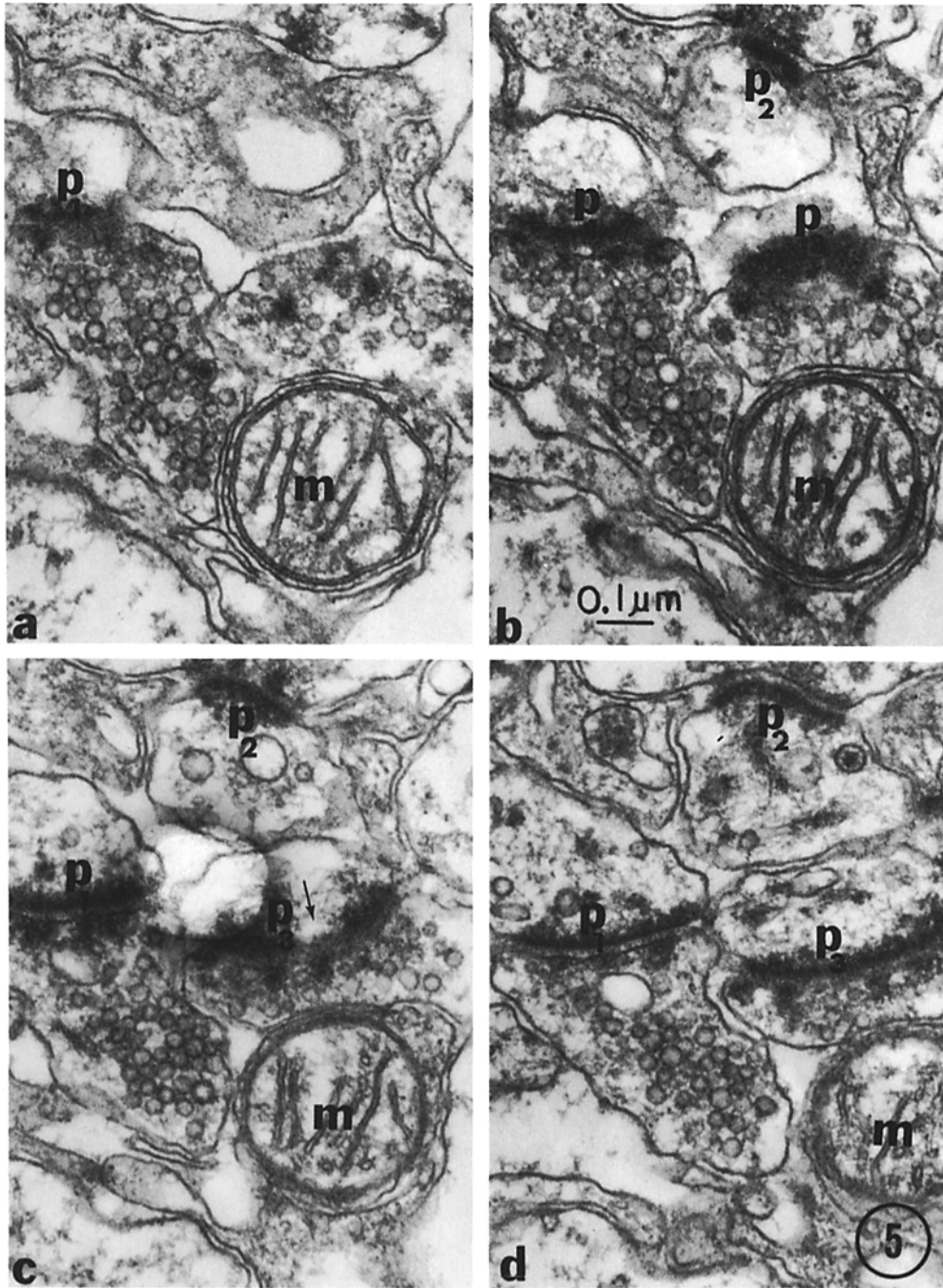
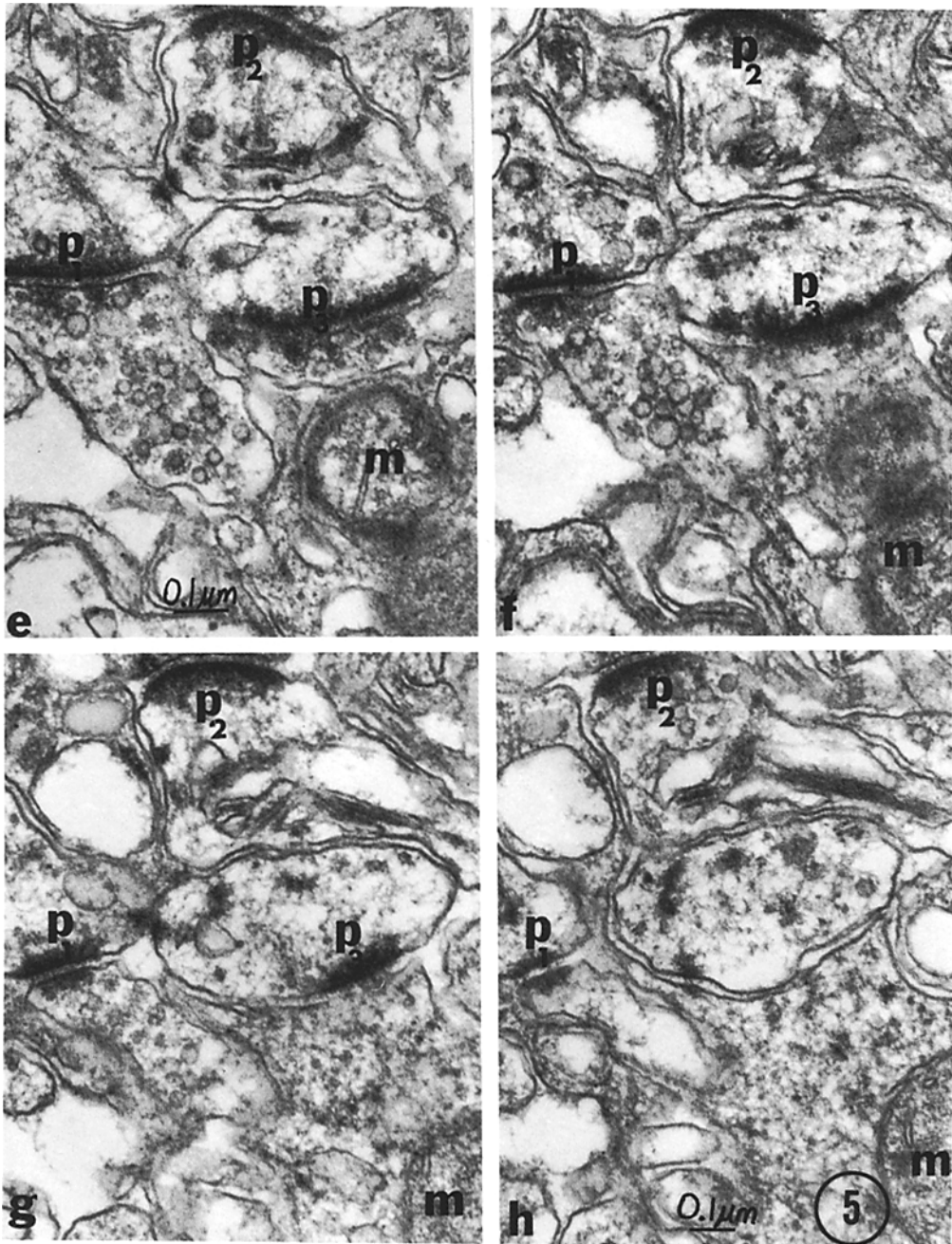


FIGURE 5 Eight serial sections cut normal to the plane of three synaptic junctions. p_1 , p_2 , and p_3 are three densities, while m is a mitochondrion seen in all the sections. Arrow in Fig. 5c indicates a possible hole in density p_3 . $\times 75,000$.



Received for publication 29 November 1977, and in revised form 24 February 1978.

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