MEMBRANE SPECIALIZATIONS OF DENTRITIC SPINES AND GLIA IN THE WEAVER MOUSE CEREBELLUM: A FREEZE-FRACTURE STUDY

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ABSTRACT

Electron microscopy of thin-sectioned and freeze-fractured preparations of the cerebellum of the weaver mouse indicates that the dendritic spines are morphologically identical to those of their normal littermates. The weaver dendritic spines have been characterized as "unattached" since the synaptic input from the parallel fibers is absent (8–10). The entire region around the dendritic spines is taken up by astrocytic processes in the weaver. The outer fracture face of a normal dendritic spine contains aggregations of 10-nm wide particles in the immediate postsynaptic region. Similar particle aggregations occur in the unattached spines of the weaver.

Freeze-fracture preparations reveal rectilinear arrays of particles, having a 7-nm center-to-center distance in the glial membranes. Rectilinear arrays are apparently distributed throughout the astrocyte membrane.

It has been generally assumed that during synaptogenesis both elements of the synaptic complex are necessary for complete differentiation (1, 3, 7, 29). Recently, however, dendritic spines have been found in the murine mutant weaver which appear to have formed fully developed postsynaptic features in the absence of any presynaptic elements (8, 9, 14, 20, 28).

The dendritic spine is characterized by dense material which is associated with the cytoplasmic surface of the membrane (5, 13, 18, 19). The technique of freeze-fracture has been utilized to elucidate the morphology of the postsynaptic membrane (2, 17, 25). Aggregations of particles, which are approximately 10 nm in width, were found to be characteristic of the postsynaptic membrane specialization. The purpose of this investigation was to freeze-fracture the cerebellum of the mutant weaver and determine if the dendritic spines are in fact fully developed postsynaptic elements.

MATERIALS AND METHODS

Weaver mice and their normal littermates were obtained from the Jackson Laboratories, Bar Harbor, Maine. The mice were decapitated, the skull was opened, and the cerebellum was immersed in 5% glutaraldehyde in 1/15 M phosphate buffer, pH 7.4. The tissue was cut into thin slices and stored in glutaraldehyde for 30 min. After fixation, tissue from each mouse was prepared for both thin sectioning and freeze-fracture. The tissue to be sectioned was postfixed in Dalton's chrome osmium (4) for 45 min, dehydrated in an ascending series of alcohols, and embedded in Luft's Epon (15) after two changes in propylene oxide. Ultrathin sections were prepared and examined in the electron microscope.

Freeze-Fracture

Slices (~0.5 mm thick) of tissue were soaked in 25% glycerol in distilled water for 30 min at room temperature. The tissue was then rapidly frozen in liquid Freon 22 at −150°C and kept in liquid nitrogen at −196°C. The tissue was then freeze-fractured in a Balzers BAF 301 apparatus (Balzers High Vacuum Corp., Santa Ana,

Calif.) according to the method of Moor and Mühlethaler (16). Replicas were made by the evaporation of approximately 2 nm of platinum and 20 nm of carbon. The replicas were cleaned of adhering tissue by floatation on Chlorox, methanol, and two changes of distilled water. The cleaned replicas were then picked up on Formvar-coated grids and examined in a Philips 200 electron microscope.

RESULTS

Thin sections of cerebellum from both normal and weaver mice were examined by electron microscopy. Our findings do not differ from those previously published (9, 10, 14, 21, 22). Synapses in the control littermates exhibit all of the fine structural characteristics of normal fully developed synapses, including clusters of synaptic vesicles, a 20-nm wide synaptic cleft and densely stained cytoplasmic material closely adjacent to the postsynaptic membrane (Fig. 1). The weaver cerebellum shows a marked paucity in the parallel fibers and their presynaptic elements. As a result, full synaptic complexes are very rare. Although the Purkinje cells lack an orderly alignment, the dendritic spines of the Purkinje cells are numerous and quite often exhibit the dense cytoplasmic material close to their membranes which is characteristic of fully developed postsynaptic elements (Fig. 2). The apposing astrocytic processes show no junctional specializations at these sites.

In the replicas of the normal littermates, fully developed synaptic complexes are readily identifiable. This relative ease of identification is due to the presence of synaptic vesicles, the concavity of the active zone in the presynaptic element, and the presence of the synaptic cleft (Fig. 3).

The outer fracture face (B face) of the plasma membrane of the Purkinje cell dendrite and the dendritic spines is generally smooth and relatively free of particles, except in the areas of postsynaptic specialization. In the regions identified as being postsynaptic, there is an aggregation of particles which average 10 nm in width (Fig. 3). These areas of specialization were especially evident since the other areas of the membrane are relatively free of particles. These aggregations of particles can only be found in the areas of postsynaptic specialization. Such particle aggregations have been found to correspond to the postsynaptic region of transmitter sensitivity and are characteristic of specific postsynaptic sites in axodendritic synapses (25). The inner fracture face (A face) of the dendrite and the dendritic spines contain numerous particles 8 nm in width. Consequently, the determination of any specializations on this fracture face was difficult and at best inconclusive.

On very rare occasions, complete synaptic complexes were observed in replicas of the weaver cerebellum (Fig. 4). The presynaptic element contains synaptic vesicles and exhibits the concavity of the "active zone". The dendritic spine exhibits a smooth outer leaflet which is relatively free of particles except in the region immediately adjacent to the synaptic cleft. In the postsynaptic region, there is an aggregation of particles averaging 10 nm in width. These synapses are indistinguishable from those observed in the normal littermates.

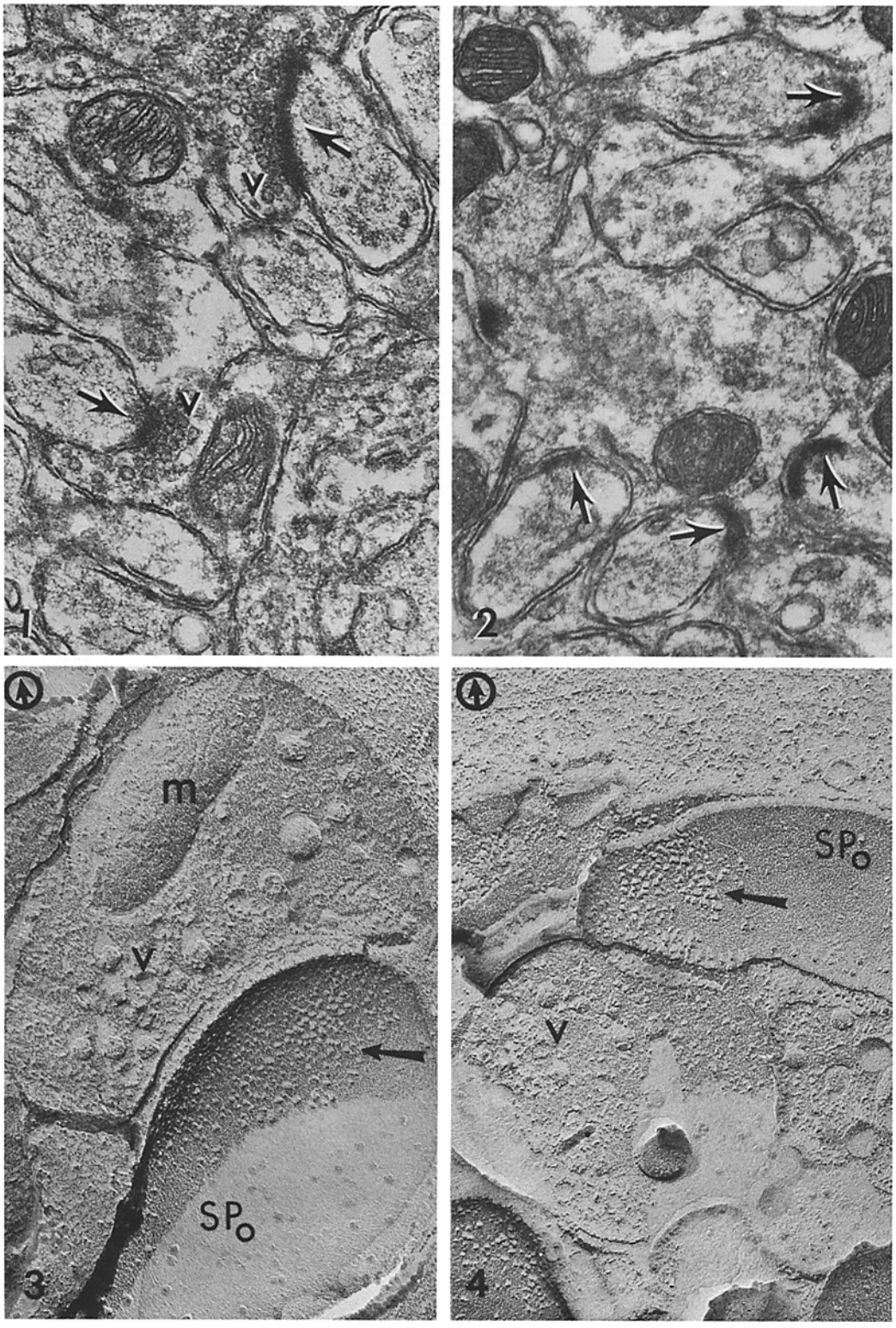
The dendritic spines in the weaver have been characterized by the absence of a presynaptic element (8, 10, 14, 20, 28). However, the unattached spines exhibit the membrane specialization that is present in the normal postsynaptic mem-

FIGURE 1 Electron micrograph of a thin section from a normal mouse cerebellum. Processes containing vesicles (V) are presynaptic to dendritic spines. Cytoplasmic dense material can be seen associated with the postsynaptic membrane (arrows). × 40,000.

FIGURE 2 Thin section from the cerebellum of a weaver mouse (normal littermate shown in Fig. 1). Unattached dendritic spines exhibit cytoplasmic dense material (arrows) which characterize processes as postsynaptic elements in the normal condition. No presynaptic elements are present. × 40,000.

FIGURE 3 Freeze-fracture preparation of a dendritic spine complex from a normal mouse cerebellum. The presynaptic terminal is characterized by the presence of synaptic vesicles (V). An aggregation of 10-nm wide particles (arrow) is seen on the outer fracture face of the postsynaptic spine (SPo). Apart from this area, the membrane is relatively free of particles. Mitochondrion, *m*. The encircled arrowhead indicates the direction of shadowing in this and subsequent micrographs. × 86,000.

FIGURE 4 Freeze-fracture preparation of a rare dendritic spine synaptic complex in the cerebellum of a weaver mouse. The presynaptic process contains vesicles (V) and the outer fracture face of the dendritic spine (SPo) contains aggregations of 10-nm wide particles at the area of the synaptic junction. × 86,000.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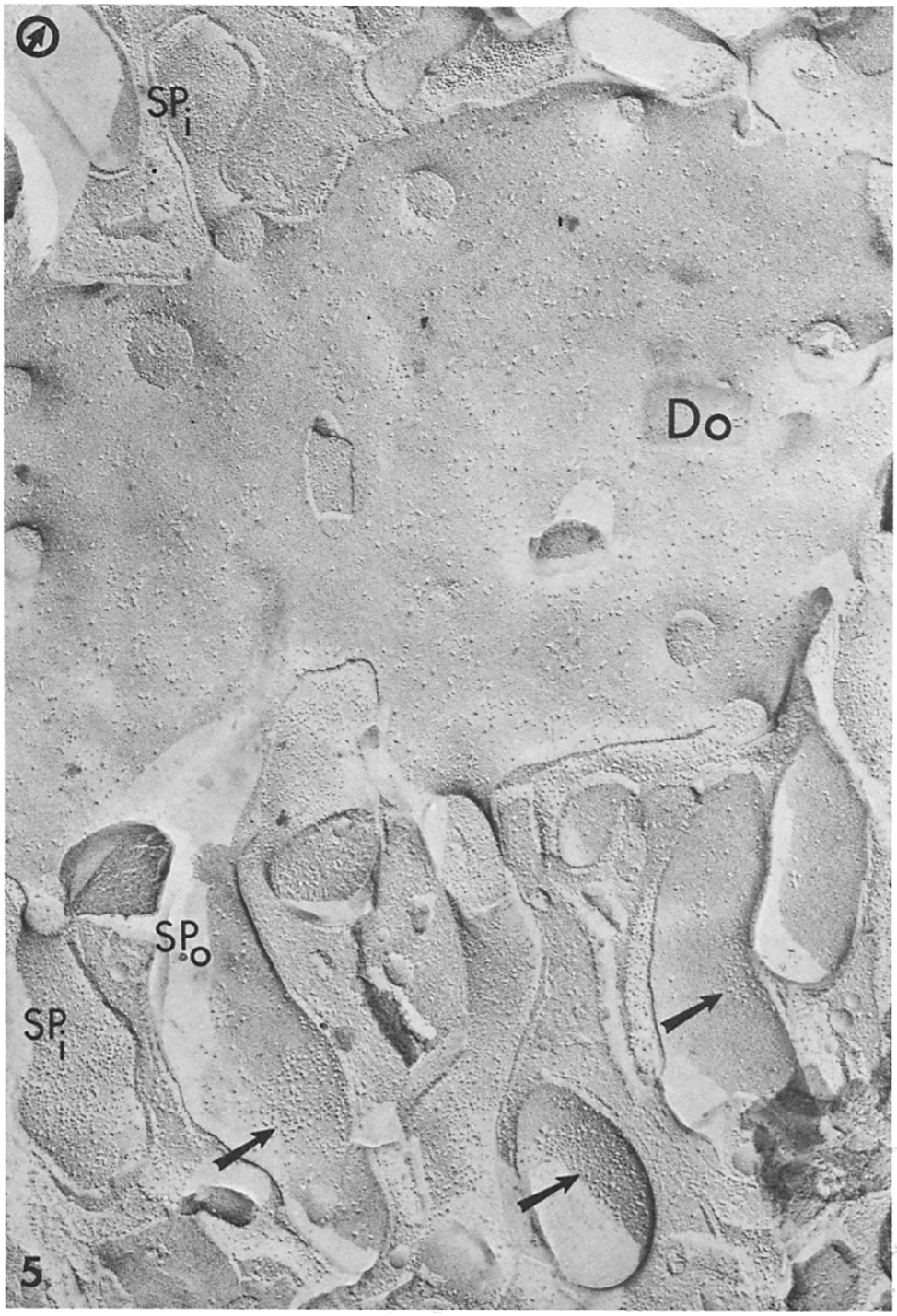

FIGURE 5 The outer fracture face of a portion of a dendrite (Do) from the cerebellum of a weaver mouse. A spine outer fracture face (SPo) as well as spine inner fracture face (SPi) can be seen in continuity with the dendrite. Aggregations of particles (arrows) can be seen in the outer fracture faces of dendritic spines (arrows). These appear identical to particle aggregations found in the outer fracture face of the postsynaptic spines of the normal littermate mouse shown in Fig. 3. × 53,000.

brane (Figs. 5–9). The outer fracture face of the dendritic spines is smooth and free of particles except in specialized areas which exhibit an aggregation of particles 10 nm in width. Morphologically, these dendritic spines could not be differentiated from those observed in the normal littermate and which had a presynaptic input. The inner fracture face of the dendritic spines is composed of numerous particles 8 nm in width (Fig. 5) and, as in the normal littermate, the determination of specialized areas was again very inconclusive.

The glial membranes in both the normal littermates and the weaver cerebellum were observed to have particles that are arranged in rectilinear arrays (Figs. 9–11). The particles are always observed on the A face with the arrays of complementary pits being found on the B face. The arrays consisted of anywhere from 4 to 70 particles which had a center-to-center spacing of approximately 7 nm. The frequency of the arrays also varies greatly from isolated arrays to large expanses of membrane which seem to be entirely made up of the rectilinear arrays (Fig. 10). Another characteristic of the glial membranes, observed in both thin sections and freeze-fracture, was the frequent presence of gap junctions (Fig. 11).

DISCUSSION

Identification of the postsynaptic element in freeze fracture is usually accomplished through its juxtaposition with the more readily identifiable presynaptic element and its constituent synaptic vesicles (2, 17, 25). In the normal littermate, identification of the postsynaptic membrane was accomplished using this criterion, but in the weaver this is not possible due to the absence of the presynaptic elements and, therefore, other criteria have to be used. In certain instances, spines could be identified because of their continuity with a cross-fractured dendrite (Fig. 7). In those cases, the appearance of the spine and the dendrite is quite similar to that seen in thin sections (compare Figs. 6 and 7). Often, spines exhibiting postsynaptic specializations were observed, but, due to the plane of fracture, could not be found to be continuous with a dendrite (Fig. 8). In sectioned material likewise the attachment of the spines to the dendrite is often not seen due to the plane of section (Fig. 2).

In all instances, the areas of membrane specialization of the unattached dendritic spines in the weaver cannot be distinguished from the postsynaptic membrane specialization in the normal littermate. Thus, the unattached dendritic spines in the weaver may be considered to be morphologically fully developed postsynaptic elements.

The rectilinear arrays appear to be identical with those previously found in the plasmalemma of certain astrocytes in the mammalian central nervous system (12). In nonneuronal tissue, similar arrays have been found in rat diaphram and intestinal epithelium (23, 24, 27). The arrays were observed more frequently in the weaver than in the normal, but this may be attributed to the fact that the weaver cerebellum contains significantly more glial processes than does the normal. The significance of the presence of rectilinear arrays in membranes is obscure at this time.

It has been suggested that a correlation exists between the development of dendritic arborization of cerebellar Purkinje cells and the synaptic response of these cells to parallel fiber stimulation (26). It may well be that in genetically normal animals experimental alterations interfering with parallel fiber formation may inhibit dendritic arborization and spine formation.

Our present findings, however, substantiate and extend the earlier reports of Hirano and Dembitzer (8–10) that, in the weaver mouse, spines are not only present but are morphologically identical to those of normal mice. Apparently, in the weaver mouse, at least, the Purkinje cell has the independent ability to form "normal" spines having the morphological characteristics of a postsynaptic process in the virtual absence of the presynaptic element (i.e., parallel fibers). Other speculations have been made that secondary input from the climbing fibers is necessary for spine formation (6). It has also been suggested that the primary input from the rare synaptic contact of the parallel fibers may be sufficient to initiate spine formation both in vivo (9) and in vitro (11). The concept of the formation and subsequent degeneration of parallel fibers is contradicted by the findings of Hirano and Dembitzer (9) on the development of the weaver cerebellum. Our study does not provide any additional information as to whether factors other than genetic may play a role in spine formation and differentiation in the weaver. It can only be concluded that the dendritic spines of Purkinje cells have the morphological appearance of fully developed postsynaptic elements in the weaver mouse.

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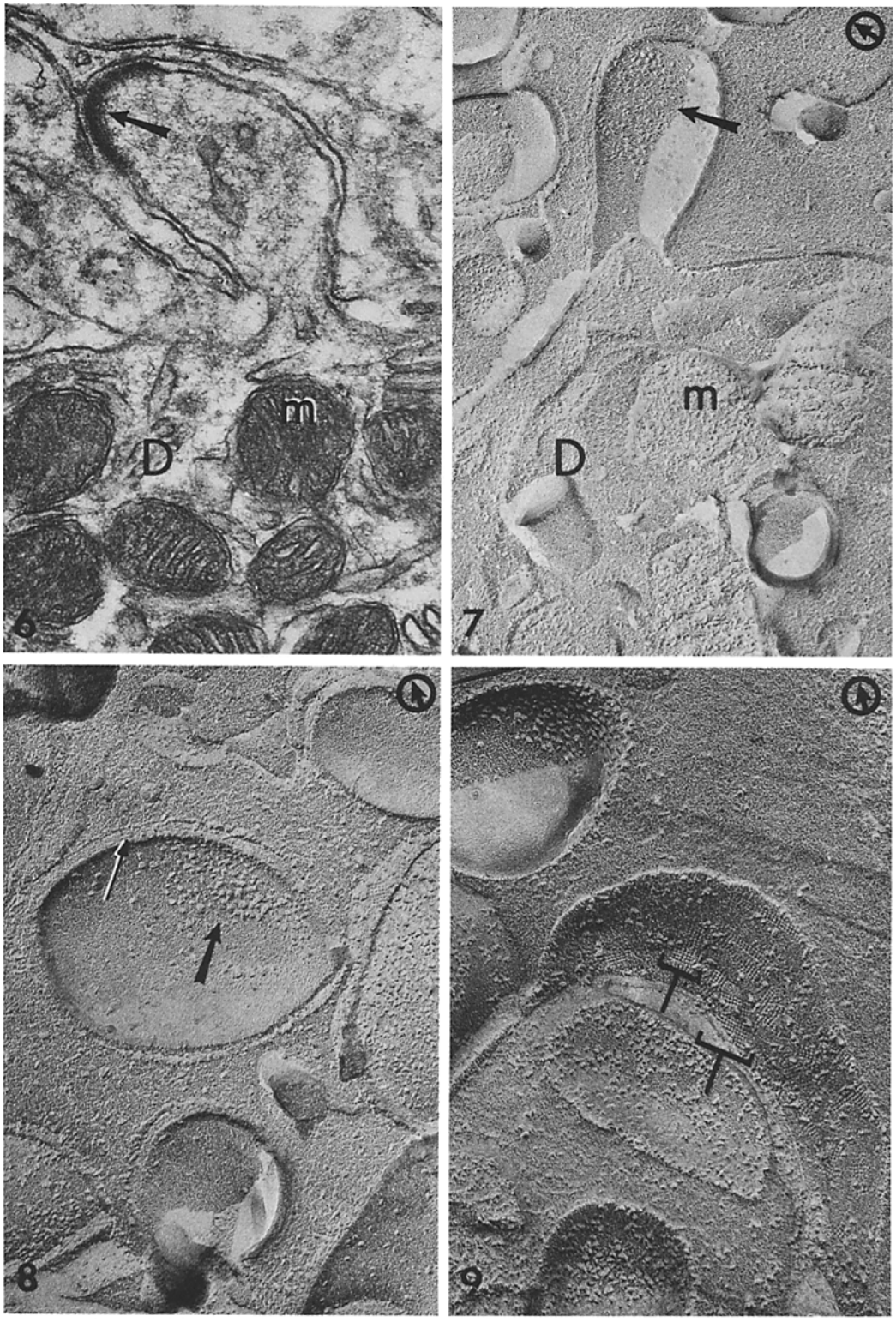
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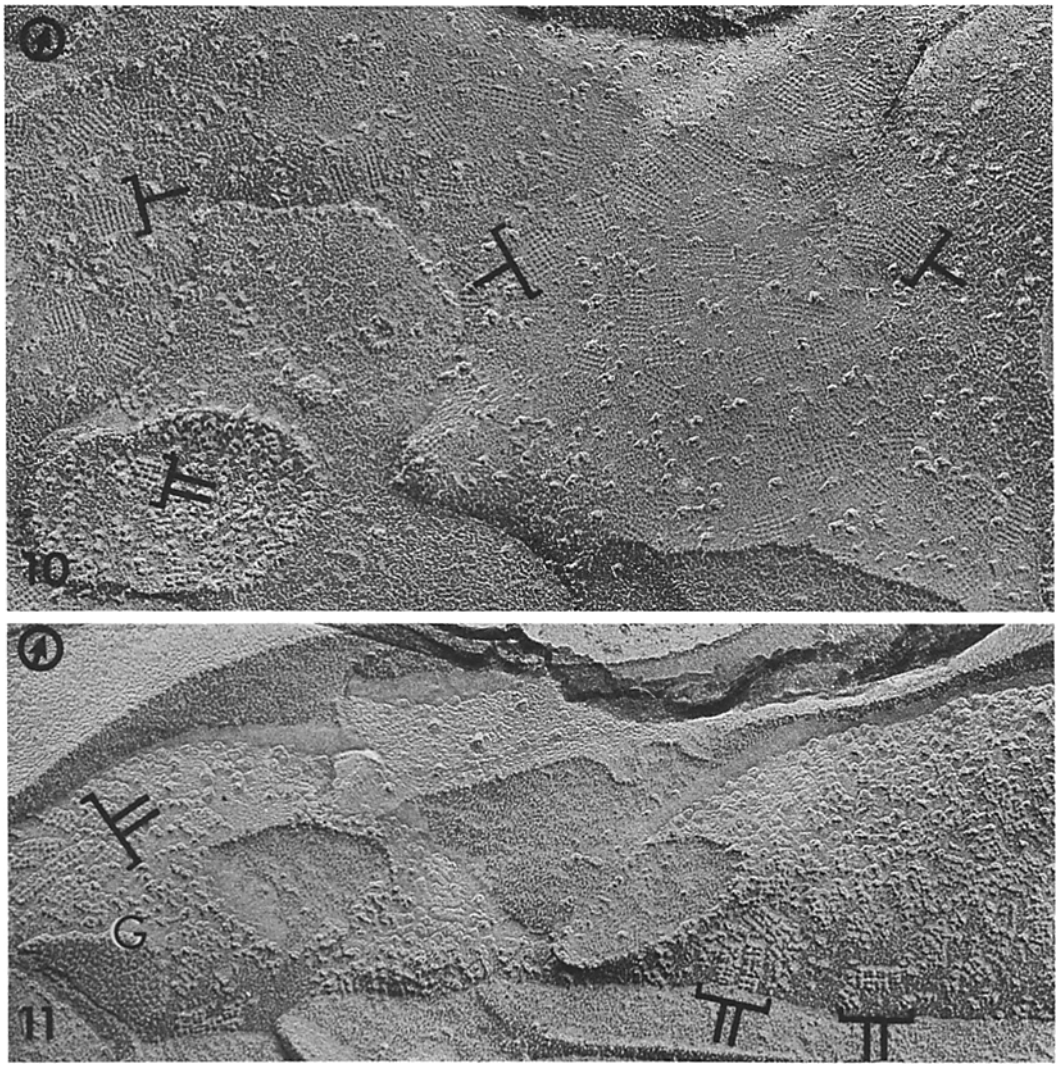

FIGURE 10 The rectilinear arrays are always observed as depressions (single brackets) on the outer fracture face of the glial membrane and as particles on the inner fracture face (double brackets in lower left of micrograph). The particles have a center-to-center spacing of 7 nm. Numerous rectilinear arrays can be found to cover large areas of glial membrane in the weaver mouse cerebellum (of which only a few are indicated by brackets). × 113,000.

FIGURE 11 Rectilinear particle arrays are often found near gap junctions (G). Particles comprising the rectilinear arrays are somewhat smaller than those found randomly scattered in the glial membrane. × 113,000.

FIGURE 6 and 7 Electron micrographs from the cerebellum of the same weaver mouse, showing dendritic spines in continuity with dendrites (D). Arrows indicate the morphological characteristics of postsynaptic membranes as seen in thin-sectioned material, cytoplasmic dense material (Fig. 6); and freeze-fractured preparations, particle aggregations (Fig. 7). The dendritic spines are "unattached", as the presynaptic processes of the parallel fibers are lacking. Mitochondria, *m*. × 61,000.

FIGURE 8 Freeze-fracture preparation of a spine outer fracture face of the weaver cerebellum showing characteristic aggregation of particles (large arrow). Small arrow indicates the cleft or space between the spine membrane and that of the surrounding astrocytes. × 88,000.

FIGURE 9 Freeze-fracture of the glial membranes surrounding the dendrites and dendritic spines exhibiting rectilinear arrays (brackets). × 66,000.

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