ARRAYS OF PARTICLES IN FREEZE-FRACTURED ASTROCYTIC MEMBRANES

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INTRODUCTION
Plasma membranes can be split in freeze-fracture preparations, revealing details of their internal structure (1, 2, 7, 9). The membrane faces so exposed typically are dotted with globular particles, apparently arranged at random over most of their surface. At cell-to-cell junctions, however, the particles are often in aggregates coextensive with the junction. At gap junctions, for instance, large particles are closely packed in an hexagonal array (5). In the course of a freeze-fracture study of synaptic junctions in the mammalian central nervous system, we have found a distinctive type of particle array which characterizes the plasma membrane of astrocytes. These arrays, which we refer to as "assemblies," are distinguished by the small size of their subunit particles and by the orthogonal packing of these particles. Furthermore, the distribution of assemblies does not coincide with any junction or other previously recognized ultrastructural feature of the astrocytic plasmalemma.

METHODS
Morphine sulfate (20–100 mg/kg) was injected into the peritoneal cavity of adult mice, rabbits, or chinchillas before cardiac perfusion with 0.08 M cacodylate or phosphate-buffered formaldehyde-glutaraldehyde for 10 min at 37°C. Selected regions of brains were immediately dissected, rinsed in buffer, equilibrated with 20% glycerol, frozen in Freon-22 cooled by liquid nitrogen, fractured in a Balzers 360 M apparatus (Balzers High Vacuum Corp., Santa Ana, Calif.) at −119°C, and etched 15–90 s. A platinum-carbon replica of the fractured surface was subsequently prepared with an electron-beam gun, cleaned in Clorox and mounted on a Formvar-coated slot grid.

RESULTS
Three regions were selected for study: olfactory bulb, cerebellar cortex, and anteroventral coch-
Figure 1 Surface of the cerebellar cortex. From above to below are: subarachnoid space (S), a thin layer of pial collagen (c), two adjacent marginal astrocytic processes (A), and the neuropil of the molecular layer (N). The plane of fracture exposes the cytoplasmic half of the plasma membrane (A face) (asterisks) and the cytoplasm of both marginal glial processes. Cytoplasmic membrane half (A face) marked by asterisk at right is enlarged in Fig. 2. × 14,000.

Figure 2 Numerous assemblies (arrows) on the cytoplasmic membrane half (A face) of the marginal glial process illustrated at far right of Fig. 1 (asterisk). Interspersed between the assemblies are single particles of various sizes and small patches devoid of particles. × 92,000.
nuclear nucleus. In each region processes of astrocytes were identified by their shape and position (Figs. 1 and 2) or because they were joined to similar processes by gap junctions (Fig. 3). We presumed that freeze-fracturing actually splits membranes, generating two complementary fracture surfaces; the outside surface of the cytoplasmic half of the membrane (the A face) and the inside surface of the external half of the membrane (the B face). This presumption was useful for interpreting all our results and is consistent with conclusions derived from freeze-fracturing other cell membranes (1, 2, 7, 9). In many instances we were able to distinguish the cytoplasmic half of the membrane from the external half of the membrane on the basis of associated cross fractures (Figs. 1–2) or previously available information on the characteristic appearance of fractured membrane specializations, such as gap junctions (5) (Fig. 3). Globular particles, 7–12 nm in diameter, were present on both cytoplasmic and external halves of neuronal and astrocytic membranes, (Figs. 1–3) but were more frequent on the cytoplasmic membrane half. Pits on external membrane halves were apparently complementary to the particles on the cytoplasmic half of the membrane (Figs. 3 and 5).

Distinctive arrays of particles, which we refer to as assemblies, were present on the cytoplasmic membrane half of certain astrocytic processes (Figs. 1–5). Each assembly was a rectangular structure composed of from 4 to 60 subunit particles packed in an orthogonal array with a periodicity of approximately 6–7 nm. If the particles were assumed to be touching, this figure could represent their actual diameter within the membrane. The subunit particle appeared to be smaller than most of the individual particles on the adjacent cytoplasmic half of the astrocytic membrane but there was no way to determine the actual diameter of individual isolated particles (Fig. 3). The external membrane half of astrocytic membranes in regions rich in assemblies were studded with arrays of pits apparently complementary to the assemblies (Fig. 4). Assemblies were identical in the three regions of the brain and in the three mammalian species.

The number of assemblies per unit area of membrane varied with the location of the astrocytic process. The highest concentration of assemblies was present in the luminal membranes of perivascular and marginal astrocytic processes (Figs. 4 and 5). The number of subunit particles per assembly tended to be larger in these areas with more assemblies. Marginal and perivascular astrocytic processes had assemblies in the abluminal membrane as well, but in smaller numbers. Astrocytic processes enveloping large neuronal somata and, in the anteroventral cochlear nucleus, large synaptic calyces had fewer assemblies than perivascular astrocytic processes. It was difficult to obtain extensive fractures through membranes of the convoluted astrocytic processes within neuropil, but where these membranes could be examined a few assemblies were found.

Not all astrocytic membranes had assemblies. The astrocytic processes investing the nerve fiber layer immediately adjacent to the glomerular layer of the olfactory bulb were devoid of assemblies. On astrocytic cell bodies seen in cerebellar cortex (including Bergmann astrocytes) and olfactory bulb no assemblies were found. It appears that processes with assemblies may arise from cell bodies on which they are very sparse or lacking. No assemblies were found.

**FIGURE 3** Assemblies and a gap junction in astrocytic processes from the cerebellar granular layer. The orthogonally arrayed subunit particles of assemblies (arrows) are distinct from the larger particles of the gap junction (G). Imprints of assemblies on the external membrane half (B face) are evident below asterisk, and polygonal arrays of the complementary pits of the gap junction particles are present above and to the left of the same asterisk. × 138,000.

**FIGURES 4 and 5** Cytoplasmic half (Fig. 4) and external half (Fig. 5) of the luminal plasmalemma of a perivascular astrocytic process from cerebellar cortex. Assemblies adherent to the cytoplasmic membrane half (A face) are numerous and randomly oriented with respect to each other (Fig. 4). Each assembly is a closely packed orthogonal array of a variable number of particles, each smaller than the individual particles occasionally found between assemblies (arrow). Only imprints of assemblies are left on the external membrane half (B face) (Fig. 5). On the external membrane half are also a few large individual particles (upper arrow) as well as imprints of the individual large particles on the cytoplasmic membrane half (lower arrow). × 140,000.
in myelin sheaths or elsewhere in oligodendroglial membranes.

DISCUSSION

Structures similar or identical to astrocytic assemblies were first noted in freeze-fractured mouse hepatocytes in the vicinity of bile canaliculi (4). They were subsequently found in the apposed cell membranes of intestinal epithelial cells, and there considered to be an unusual type of gap junction (8). Dermietzel (3) recently described small rectangular arrays of particles in astrocytic membranes around the fourth ventricle of cats, and suggested that they may represent subunits of functional nexuses in different stages of integration and disintegration. Our observations indicate, however, that astrocytic assemblies are present where junctions would not be likely to occur; for example, in the astrocytic plasmalemma adjacent to neuronal somata and on the outer plasmalemma of marginal astrocytic processes where they face the cerebrospinal fluid.

The nature of particles associated with freeze-fractured membrane surfaces is uncertain, but at least some are likely to be proteins intercalated or sequestered in the membrane lipid bilayer (2, 6). The isolation and characterization of assemblies may be facilitated by their readily recognizable configuration, and could serve as a useful approach to the analysis of membrane particle composition. Assemblies may also be used as a means to identify astrocytes in freeze-fracture preparations because they apparently are not found on neurons.

Astrocytic assemblies are probably identical to the structures observed in hepatocytes and intestinal epithelial cells and therefore could be involved in the transport or permeability of certain substances yet to be defined. The striking localization of assemblies in astrocytic processes around blood vessels and adjacent to the subarachnoid space could represent a differentiation of these processes to control the concentration of certain substances in the interstitial fluids of the brain.

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