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A Peculiar Substructure in the Postsynaptic Membrane of *Torpedo* Electroplax

(electric organ/membranes/freeze-etching/electron microscopy)

LELIO ORCI, ALAIN PERRELET, AND YVES DUNANT

Institute of Histology and Institute of Pharmacology, School of Medicine, University of Geneva, Geneva, Switzerland

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ABSTRACT **Freeze-etching of *Torpedo marmorata* electroplax reveals an unusual substructure in one of the fracture faces of the postsynaptic membrane. This substructure consists of a lattice of closely spaced particles that are smaller than the usual 80- to 90-Å particles present in other membrane types. The lattice occurs exclusively on the B-face of the postsynaptic membrane.**

Freeze-etching is now widely recognized as a powerful morphological approach to the study of biological membranes (1, 2). This advantage comes from the fact that the freeze-etching process exposes large areas of membranes that are split in the frozen state so as to reveal their inner structure (3, 4). In freeze-etched replicas, this inner structure appears as two complementary fracture faces named the A- and the B-face (5), both of which have a common basic appearance, i.e., a smooth surface containing particles about 85 Å in diameter (1). What differs, however, from one membrane type to another (and also from one given A-face to its complementary B-face) is the total number of particles on the fracture face, as well as the disposition of the particles. Indeed, recent work has shown that particles might gather in a specific distribution on fracture faces and that such a pattern could be the marker of a specific function in this particular area of the membrane. The most distinctive patterns described so far are the hexagonal array of particles seen at the gap junctions (5–8) and the "necklace" figures accompanying certain exo- and endocytotic membrane processes (9, 10). Both gap junctions and exo-endocytotic processes are, however, not permanent differentiations of the membrane, and they probably occur in all membranes.

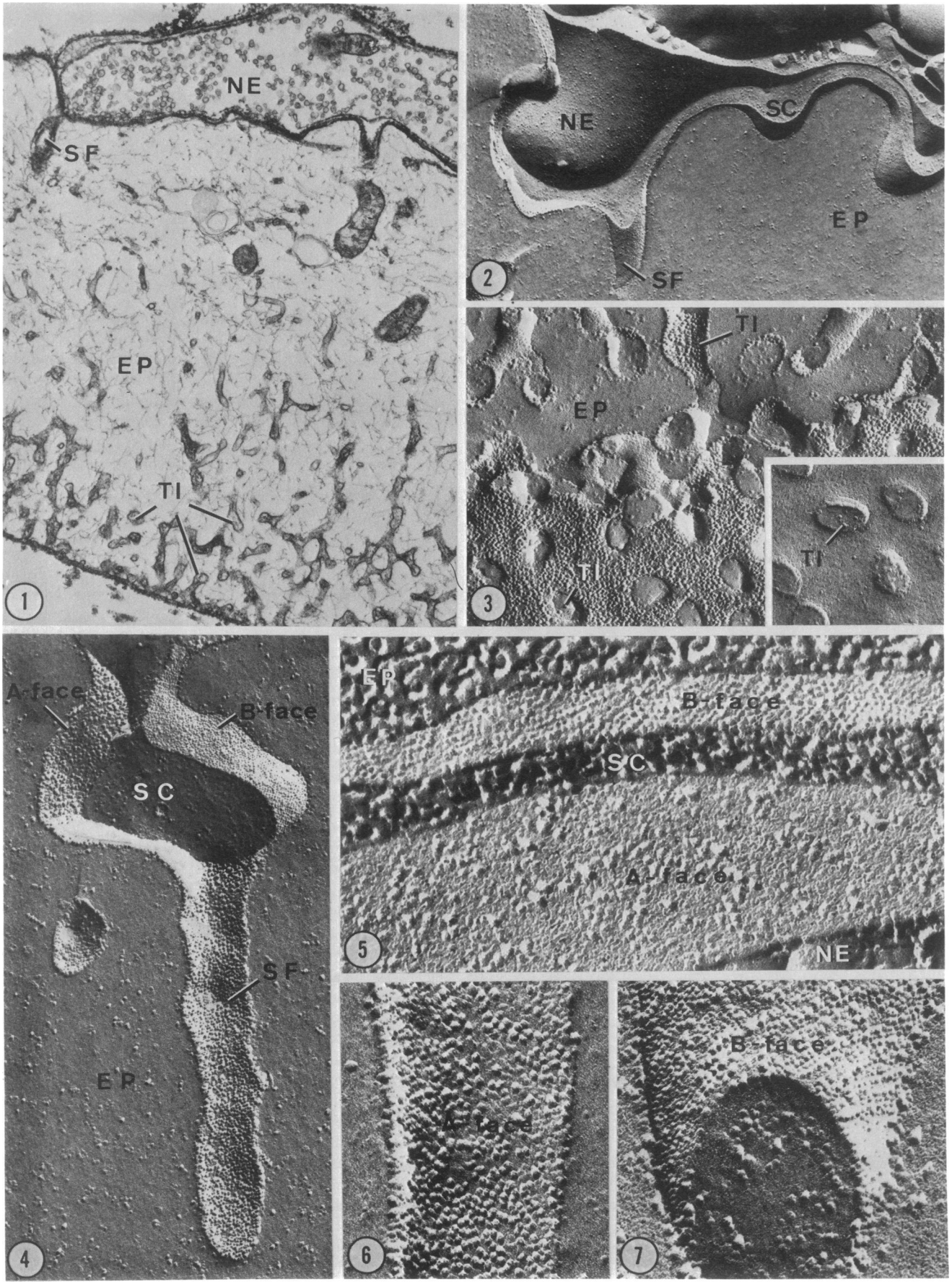
We now have evidence for a new type of morphological differentiation in cell membranes. This differentiation consists of a closely spaced lattice of particles smaller than the usual 85-Å particles; it occurs selectively in the postsynaptic membrane of the electroplax of *Torpedo*.

MATERIALS AND METHODS

Pieces of fresh electric organ of *Torpedo marmorata* were fixed in 2% glutaraldehyde buffered with phosphate, pH 7.4 (11). For freeze-etching experiments, small cubes cut out from the fixed pieces were infiltrated for a few hours in 30% glycerol buffered with phosphate, frozen in Freon 22 cooled with liquid nitrogen, and freeze-etched according to Moor *et al.* (12) in a Balzers BAF 301 device. Fracturing and etching temperature was maintained at −100°. Etching time was 1 min, after which the frozen surface was replicated with platinum and carbon evaporated from an electron gun. This device allows a reproducible amount of platinum to be evaporated. After thawing of the tissue, the replicas were cleaned with sodium hypochlorite, rinsed twice in distilled water, recovered on 150-mesh copper grids, and then examined in a Philips EM 300 electron microscope. For conventional electron microscopy, cubes of glutaraldehyde-fixed tissue were postfixed in OsO_4, dehydrated in graded alcohol concentrations, and embedded in Epon 812 (13). Thin sections of the electrical organ were observed in the electron microscope after they had been contrasted with lead citrate (14).

RESULTS

The electrical organ of *Torpedo* has been thoroughly described in conventional thin-section electron microscopy by Sheridan (15). This organ is unique in the fact that it is formed by numerous repetitive units, the electroplax, each of which is a long and thin cell having two distinctive surfaces. One of these (dorsal) has numerous invaginations of its plasma membrane, whereas the other (ventral) shows multiple differentiations similar to muscle endplates. This latter surface receives a cholinergic innervation and has been investigated as a possible source for the isolation of a cholinergic receptor (for review, see ref. 16). Due to this highly repetitive organization, the identification of the different components of the electroplax is easy to carry out in thin section as well as in freeze-etch replicas. A general view of one electroplax as seen with these two techniques is presented in Figs. 1–3. The innervated (ventral) side of the electroplax has many deep infoldings of the plasma membrane that face the axon terminals, whereas the noninnervated side of the electroplax shows a dense network of interconnected tubular invaginations. These two specializations of the plasma membrane appear clearly on freeze-etched replicas, which show, in addition, the structure of the fracture faces (the A-face of the noninnervated side of the electroplax is shown in Fig. 3). This face (representing the inner leaflet of the plasma membrane, associated with the cytoplasm of the cell) has a very large number of 85-Å particles, which are evenly distributed on the smooth background surface. It is interrupted by the fractured openings of the interconnected tubular invaginations of the membrane. The same general appearance holds also for the B-face of the non-innervated membrane (the B-face represents the outer leaflet of the plasma membrane, associated with the extracellular space), except that there are fewer particles (*inset*, Fig. 3). Unlike the noninnervated (dorsal) face of the electroplax, which is easily exposed by the freeze-fracturing, the innervated (ventral) membrane seems more resistant to fracturing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and, accordingly, is sparsely exposed. The reason for such a difference in the breaking properties of the membranes is not known; nevertheless there are enough membrane areas exposed in this region to allow a description of their fracture faces. Most of the exposed membrane consists of deep infoldings facing the axon terminals, and they appear mostly as segments of cylinders (Fig. 4). The A-face appears concave and it displays a number of 85-Å particles similar to that found on the noninnervated face of the cell (Figs. 4 and 6). However, a striking difference in the morphology of the fracture face is observed when the B-face of the synaptic infolding has been exposed (Figs. 4, 5, and 7). This face (the outer leaflet of the plasma membrane) becomes visible when the cytoplasm of the electroplax has been broken away during the freeze-fracturing process. It is seen, therefore, from the inside of the cell and usually appears convex. Unlike other B-faces that have been described so far, the B-face revealed at the postsynaptic face of the electroplax does not show the usual 85-Å particles. Instead, it appears to be entirely covered by rows of smaller particles only about 60 Å in diameter and tightly packed. The peculiarity of this surface substructure is particularly evident when the fracture plane has traversed adjacent A- and B-faces of the same infolding (see Fig. 4). As already mentioned, the postsynaptic membrane outside the infoldings is infrequently exposed. On the few areas of this membrane that were observed, a pattern similar to that described in the infoldings (A- and B-face) appears to be present (Fig. 5). On the other hand, the presynaptic membrane, i.e., that covering the nerve endings, is readily exposed by the fracturing process, as is the membrane of the synaptic vesicles (Figs. 2 and 5). The membrane faces do not differ from what has been previously described (17). The A-face of the presynaptic membrane has far less 85-Å particles than the A-face of the electroplax membrane. Accordingly, fewer particles are seen on the presynaptic B-face, and no differentiations of the type described on the postsynaptic B-face could be observed.

DISCUSSION

The freeze-etch study of the electroplax of *Torpedo* has revealed the presence of a peculiar substructure on one of the fracture faces of the postsynaptic membrane. In the description given above, we have identified the substructure as occurring on the B-face of the membrane, thus implicitly following Branton's membrane-splitting hypothesis (3). Indeed, since our freeze-etching experiments were performed in the presence of 30% glycerol and only 1 min was allowed between the end of the fracturing of the frozen tissue and the platinum coating, the possibility that the substructure would have been uncovered on the true membrane inner surface by etching is unlikely. Moreover, whenever true surfaces have been exposed by etching in the absence of glycerol (19, 20), these have been reported to be smooth, without adhering 85-Å particles. The possibility that the peculiar pattern described above might be the result of an artifactual rearrangement of membrane particles on the B-face is, of course, impossible to rule out completely since there is no other work so far reporting the occurrence of a similar specialization which would confirm or refute our results. Indeed, in their freeze-etching study of *Torpedo* electroplax, Nickel and Potter (17, 18) were primarily concerned with the characterization of the synaptic vesicles in the axon terminal and they did not pay attention to the postsynaptic membrane. Another freeze-etch study of the same tissue was performed by Cartaud *et al.* (21) on isolated membrane fragments assumed to be exclusively postsynaptic on the basis of their acetylcholinesterase content. However, their results are not comparable to ours since their images of freeze-etched postsynaptic "microsacs" showed a similar substructure on both the concave (probably A-face) and convex (probably B-face) fracture faces, a finding that is at variance with ours. Moreover, they do not report the presence of a pattern similar to the one found on the B-face of the postsynaptic membrane *in situ*. However, the two tissues examined (isolated membranes compared with membrane *in*

FIG. 1. Conventional thin-section electron micrograph illustrating the organization of the electroplax. The electroplax cell (*EP*) receives on one side a nerve-ending (*NE*) containing synaptic vesicles. The innervated side of the electroplax has also deep infoldings or synaptic folds (*SF*). The plasma membrane of the other side of the cell shows numerous tubular invaginations (*TI*). A few mitochondria and numerous filaments can be seen in the electroplax cytoplasm. Magnification: ×12,000.

FIG. 2. Freeze-etch replica of the electroplax showing the innervated side of the cell. The nerve-ending (*NE*) membrane has been exposed, as well as parts of the synaptic folds (*SF*). *EP*, electroplax cytoplasm; *SC*, synaptic cleft. Magnification: ×32,000.

FIG. 3. Freeze-etch replica of the electroplax showing the noninnervated side of the cell. The A-face of the plasma membrane has been exposed and shows a very large number of 80- to 90-Å membrane-associated particles. The openings of the tubular invaginations (*TI*) are seen, as well as their extensions within the electroplax cytoplasm (*EP*). Magnification: ×55,000. The *insert* displays the B-face of the noninnervated plasma membrane. It also shows the openings of the tubular invaginations, but in contrast with the A-face, very few membrane-associated particles. Magnification: ×126,000.

FIG. 4. Freeze-etch replica showing a synaptic fold (*SF*) at high magnification. This fold encloses a part of the synaptic cleft (*SC*) and it enters deeply within the electroplax cytoplasm (*EP*). Both faces of the synaptic-fold membrane have been exposed by the freeze-fracture and they both have a large number of particles. However, whereas the A-face of the synaptic fold has 80- to 90-Å particles, the B-face shows smaller particles, which are displayed at higher magnification in Figs. 5 and 7. Magnification: ×50,000.

FIG. 5. Freeze-etch replica of the electroplax membrane on the innervated side. The fracture has exposed the A-face of the nerve ending (*NE*) and the B-face of the electroplax (*EP*) postsynaptic membrane. These two faces are separated by the synaptic cleft (*SC*). The B-face of the postsynaptic membrane shows an array of closely spaced particles. The particles are clearly smaller than the 80- to 90-Å particles standing on the A-face of the presynaptic membrane. Magnification: ×140,000.

FIG. 6. Freeze-etch replica illustrating, at high magnification, the A-face of a synaptic fold. This face has numerous 80- to 90-Å particles. Magnification: ×138,000.

FIG. 7. Freeze-etch replica of the B-face of a synaptic fold at high magnification. The B-face is covered with small particles which are arranged in a lattice with about 60-Å spacing. The lattice also contains a few large (80- to 90-Å) particles. Magnification: ×132,000.

situ) have been treated in a very different way, and more experimental evidence is certainly needed to clarify this issue. Similarly, the question of whether the pattern of small membrane particles occurring specifically in the postsynaptic region of the electroplax might represent, at least in part, a receptor protein, remains to be answered.*

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* When this paper was being submitted for publication, a second report by Cartaud *et al.* (22) appeared which helps to resolve partially the apparent contradiction between their former results and our present ones. Again, in negatively stained or in freeze-etched fragments of isolated postsynaptic membrane of *Torpedo*, Cartaud *et al.* were able to demonstrate a lattice pattern of closely spaced particles. The spacing of the particles is about 90 Å, and each of the particles seems to be pierced by a central pit. The interpretation as to where this lattice is situated is, however, still at variance with ours. Cartaud *et al.* conclude that the lattice is situated on the true outer surface of the postsynaptic membrane.

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