REMODELING OF NEURONAL MEMBRANES AS AN EARLY RESPONSE TO DEAFFERENTATION

A Freeze-Fracture Study

R. L. GULLEY, R. J. WENTHOLD, and G. R. NEISES

From the Laboratory of Neuro-otolaryngology, NINCDS, National Institutes of Health, Bethesda, Maryland 20014, and the Department of Anatomy, Case Western Reserve University Medical School, Cleveland, Ohio 44106

ABSTRACT

The early effects of deafferentation on the postsynaptic membrane beneath the end bulb of Held in the anteroventral cochlear nucleus (AVCN) were studied with the freeze-fracture technique. Three distinct responses were seen on the external membrane leaflet after cochlear ablation. Within 12 h the number of nonaggregate particles increased 147% by the addition of new particles to the membrane. The increase in number of nonaggregate particles continued until 4 days after cochlear ablation. The other responses occurred later, after degenerative changes were present in the end bulb. Between 1 and 2 days after cochlear ablation, the number of perisynaptic aggregates surrounding the postsynaptic active zone decreased to 10% of normal numbers. By 4 days, all perisynaptic aggregates had disappeared from the membrane. Coated vesicles may be involved in removing these aggregates. Between 1 and 3 days, the number of junctional aggregates decreased, but the size of the aggregates increased, apparently as a result of coalescence of nearby junctional aggregates. The total number of particles in junctional aggregates in the membrane was not altered during the first 6 days after cochlear ablation. The three separate responses suggest the existence of at least three different types of intramembranous particles on the external leaflet of the principal cell membrane, with each type dependent upon different cues for its maintenance in the membrane.

KEY WORDS deafferentation · postsynaptic membrane · freeze-fracture · auditory system · synapses

At peripheral cholinergic synapses, only junctional regions of the postsynaptic membrane are sensitive to acetylcholine (19, 28, 36) and contain receptors (3). After denervation, the distribution of acetylcholine sensitivity (1, 19, 27, 28, 36) and cholinergic receptors (16) is altered. There is little change in the junctional region of the membrane (28); however, there is a marked increase in sensitivity and receptors in nonjunctional regions of the membrane. With the freeze-fracture technique, specializations of the postsynaptic membrane at the synaptic junction of these terminals are found on the cytoplasmic leaflet (11, 34, 37, 38). After denervation at the neuromuscular junction (13) and electroplaque (9), there is no change in the large, junctional particles; however,

there is little information about changes in the nonjunctional region of the membrane where the background density of membrane particles is high.

In the central nervous system, there is indirect evidence that supersensitivity occurs after disuse and denervation (5, 39, 40). At excitatory chemical synapses in the central nervous system, the postsynaptic membrane specializations are found on the external leaflet (20, 21, 35)[1] where the background density of particles is low in nonjunctional regions. Thus, any changes in the distribution of intramembranous particles in both junctional and extrajunctional regions of the membrane after deafferentation should be easy to detect. In this report, the freeze-fracture technique has been used to study the response of the postsynaptic specializations of the principal cell in the rostral anteroventral cochlear nucleus (AVCN) immediately after cochlear ablation. The central projection of the auditory nerve on the principal cell of the rostral AVCN[2] is well suited for studying the response of the postsynaptic membrane to denervation and other experimental conditions. The large calyceal terminal of this projection, the end bulb of Held (17, 33), covers half of the principal cell soma, and can easily be distinguished from other axosomatic synapses in thin-sectioned (21) and freeze-fractured material (see fn. 1). In addition, the postsynaptic membrane of this terminal has two distinct types of specializations, each with a characteristic distribution on the external leaflet beneath the end bulb (see fn. 1). Moreover, the presynaptic component of this synapse can be easily destroyed by cochlear ablation or the level of activity in the terminal can be closely regulated by controlling the intensity and duration of acoustic stimulation (see fn. 1).

The objectives of this study are: (*a*) to determine whether deafferentation can alter the organization of the postsynaptic membrane in the central nervous system; (*b*) to identify the factors that might mediate the response of the membrane, and (*c*) to correlate any change in the central nervous system postsynaptic membrane with that which has been described biochemically and physiologically in the peripheral nervous system.

MATERIALS AND METHODS

The left cochleae of 18 National Institutes of Health strain guinea pigs were ablated surgically by the procedures previously described (46). The animals were sacrificed by intracardiac perfusion at 12 h (two animals), 1 day (four animals), 2 days (four animals), 3 days (four animals), 4 days (two animals), and 6 days (two animals) after cochlear ablation. The perfusion solutions consisted of 0.1 M sodium cacodylate, 1% sodium nitrite, and 20 mM $CaCl_2$ followed by 3% glutaraldehyde, 2% paraformaldehyde in 0.1 M sodium cacodylate with 20 mM $CaCl_2$. The temperature of both solutions was 37°C. After perfusion the head was removed, the skull opened and placed in the aldehyde fixative for 2 h. The brainstem was then dissected and 225-um sections cut through the hemisected brainstem with a Smith-Farquhar tissue chopper. The AVCN, rostral to the midlevel of the vestibular nerve, were dissected and placed in 20% buffered glycerol for 2 h. The AVCN from the lesioned and control sides were frozen in Freon 22 (Phillips Manufacturing Co., Chicago, Ill.) and stored in liquid nitrogen before fracturing in a Balzers 301 (Balzers High Vacuum Corp., Santa Ana, Calif.) at −119°C. Platinum-carbon replicas were made with electron beam guns. The replica thickness was standardized with a quartz crystal monitor. The replicas were cleaned in successive changes of methanol, distilled H_2O, Clorox, and distilled H_2O before picking them up on Formvar (Monsanto Company, St. Louis, Mo.) carbon-coated grids. The replicas were examined in a Philips 201 electron microscope.

At least two additional animals with ablated cochleae from each survival period were used to determine, in thin sections, the time-course and sequence of changes that occur after cochlear ablation. This data and the preparative techniques used have been reported (46). Additional thin-section data reported here came from these experimental animals. Thin-section and freeze-fracture data from control cochlear nuclei were taken from normal animals (see fn. 1) and from the AVCN contralateral to the lesion in experimental animals. No differences were seen between the synapses and synaptic membranes of these two control groups.

Photomicrographs of over 100 μm^2 of the external membrane leaflet from all regions of the principal cell were randomly selected for quantitation from each survival period. Membrane from at least 10 different neurons from each animal were included in the sample. The number of nonaggregate particles and junctional and perisynaptic aggregates was counted. In many cases two observers made independent counts to eliminate bias. Standard errors were calculated to allow for variation due to differences in shadow angle caused by the contours of the fractured surface, in replica thickness as well as variation between different cells. Counts were also made of the particles on the cytoplasmic membrane leaflet. No differences were found between the particle distribution on the cytoplasmic membrane leaflet in

[1] Gulley, R. L., D. M. D. Landis, and T. S. Reese. 1977. Internal organization of membranes at end bulbs of Held in the anteroventral cochlear nucleus. *J. Comp. Neurol.* In press.

[2] *Abbreviations used in this paper:* AVCN, anteroventral cochlear nucleus.

normal animals and in animals after cochlear ablation (Table I).

Fifty aggregates were randomly chosen from samples of the external leaflet of animals 2 and 3 days after cochlear ablation. The packing density and the size of the particles in these aggregates were measured and compared to similar measurements of junctional and perisynaptic aggregates in normal animals. Based on these two criteria, two populations of aggregates could be distinguished in the experimental animals which corresponded to the junctional and perisynaptic aggregates in normal material (Table II). These criteria were used to identify the two types of aggregates when their relationship to the end bulb was not visible or after the end bulb had been removed from the membrane.

The fracture plane, in control groups or in experimental groups within 24 h after cochlear ablation, typically jumps from the postsynaptic external membrane leaflet near the synaptic junction to the cytoplasmic membrane leaflet of the end bulb. Thus, fractures exposing entire junctional aggregates are infrequent. Inasmuch as every example of a junctional aggregate that was seen was only at the synaptic junction, the number of junctional aggregates per μm^2 in these groups was calculated by counting the number of these junctions in the sample chosen for quantitation. This figure was similar to the density of synaptic junctions of the end bulbs on the principal cell in thin sections. Similar difficulties were encountered in determining the number of particles per junctional aggregates in these groups, because only parts of many aggregates were encountered. The number of particles per junctional aggregate for these examples was computed by determining the density of particles per μm^2 in a partial aggregate and multiplying this figure by the average size of the junction ($0.07\ \mu m^2$). In each of the three groups where this problem was encountered, at least six examples were found where the entire junctional aggregate was exposed. In these examples, the number of particles counted per junctional aggregate compared well with the calculated value for the other aggregates.

RESULTS

Control Cochlear Nuclei

In freeze-fractured specimens, three distributions of particles are found on the external leaflet

TABLE I

Changes in the Particle Distribution on the Postsynaptic Membrane After Deafferentation

	Normal	12 h	1 day	2 days	3 days	4 days	6 days
A. Cytoplasmic membrane leaflet							
Nonaggregate particles	1,470 ±132	1,389 ±117	1,403 ±111	1,346 ±149	1,486 ±109	1,396 ±99	1,256 ±121
B. External membrane leaflet							
Junctional aggregates/um²	1.99 (±0.13)	2.03 (±0.22)	2.12 (±0.11)	1.82 (±0.09)	1.49 (±0.14)	1.46 (±0.08)	1.39 (±0.13)
Particles/junctional aggregate	130.7 (±24.6)	141.3 (±23.7)	137.7 (±19.4)	159.8 (±13.3)	186.3 (±17.0)	181.2 (±9.8)	189.4 (±14.3)
Total junctional particles/um²	260.1	286.8	291.9	290.5	277.6	264.6	263.3
Perisynaptic aggregates/um²	3.21 (±0.67)	3.25 (±0.74)	3.33 (±0.79)	0.32 (±0.41)	0.16 (±0.34)	0	0
Nonaggregate particles/um²	53.2 (±18.4)	131.2 (±20.6)	138.2 (±21.4)	159.7 (±19.8)	304.0 (±36.4)	346.5 (±29.3)	357.2 (±39.1)
Particles/perisynaptic aggregate	79.60 (±9.4)	80.59 (±12.8)	80.29 (±8.4)	77.77 (±14.6)	83.97 (±13.6)	–	–
Total nonjunctional particles/um²	308.7	393.1	405.6	184.2	317.1	346.5	357.2

TABLE II

Comparison of the Density and Sizes of Particles in Aggregates on the Principal Cell Membrane Before and After Deafferentation

	Density of particles	Percentage of particles sized		
		6–10 nm	*10–14 nm*	*14 nm*
Perisynaptic aggregate				
Normal	1,205 ± 288	37.4	50.2	12.4
2 days after cochlear ablation	1,271 ± 196	35.3	54.4	10.3
3 days after cochlear ablation	1,312 ± 212	37.0	56.3	6.7
Junctional aggregate				
Normal	1,888 ± 156	7.2	27.4	65.4
2 days after cochlear ablation	2,010 ± 213	8.3	23.2	68.5
3 days after cochlear ablation	2,150 ± 170	6.4	26.5	67.1

of the principal cell membrane beneath the end bulb (see fn. 1). The first is found at the synaptic junction. It is a circular aggregate of particles, 0.25–0.35 μm in diameter. The aggregate fills a shallow circular depression of the external leaflet which corresponds to the evagination of the principal cell membrane into the end bulb at the synaptic junction. This evagination is lined with the postsynaptic density. In replicas where the fracture crosses the cytoplasm before exposing the external leaflet at the level of the synaptic junction, thin striations of the cytoplasm corresponding to the postsynaptic density are coextensive with this aggregate. The aggregate consists of many large, angular particles (Table II). These particles are tightly packed, and frequently appear to be arranged in rows (see fn. 1). A few smaller, round particles are interspersed among the large particles. Because of its association with the synaptic junction, it is called the *junctional aggregate*. The second is a particle aggregate distributed around the synaptic junction, typically over the enlarged channels of extracellular space surrounding a junction or along the edge of an end bulb. This *perisynaptic aggregate* is irregularly shaped. It varies considerably in size, typically between 0.055 and 0.065 μm^2. The aggregate consists of particles of variable sizes (Table II). The particles typically are smaller than those in the junctional aggregate; however, some large, angular particles are found in the perisynaptic aggregate (Table II). In thin sections no dense material is associated with the region of the membrane where this aggregate is distributed (see fn. 1). In addition to the two particle aggregates, single, *nonaggregate* particles are randomly distributed over the membrane. These data are schematized in Fig. 1.

Day One after Cochlear Ablation

In thin sections, few endings are structurally altered by 12 h. However, 24 h after cochlear

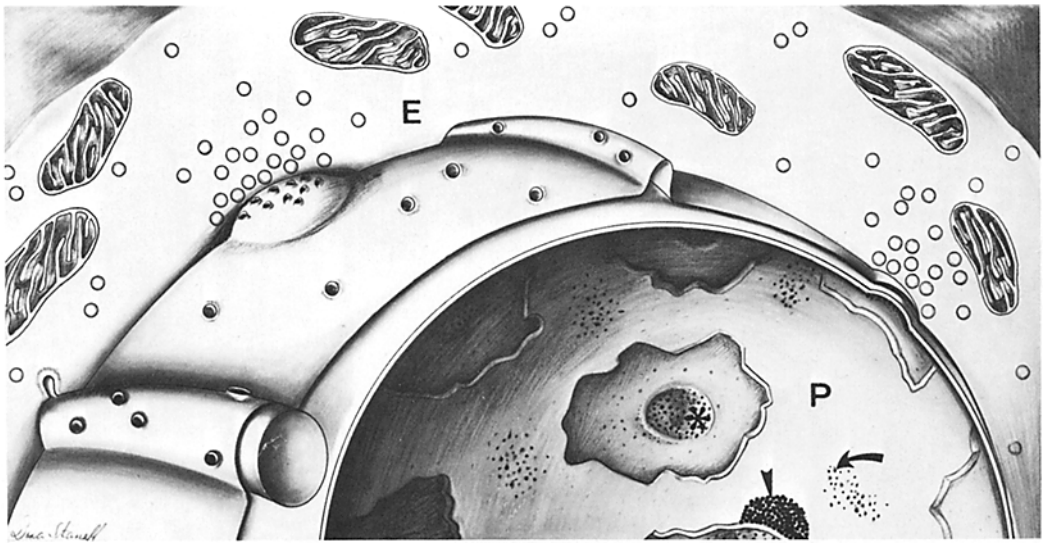


FIGURE 1 A schematic diagram summarizing the membrane specializations on the postsynaptic membrane of the principal cell opposite an end bulb (*E*). The perspective of the drawing is as if the cytoplasm and cytoplasmic leaflet of the plasmalemma of the principal cell has been removed, permitting the external leaflet (*P*) of the principal cell to be viewed. Windows in this leaflet allow the cytoplasmic leaflet of the end bulb near an active zone (*) to be seen. Two types of membrane specializations are found on the external leaflet of the postsynaptic membrane. The first is a circular aggregate found at the active zone (arrowhead). It consists of large, tightly packed particles. The second is a smaller, irregular perisynaptic aggregate (arrow) of loosely packed particles of varying sizes. Three to five of these aggregates surround each active zone. The outer surface of the principal cell, seen on the right between the soma and the end bulb, does not represent an observed fracture plane; it is included in the illustration only to help achieve the proper three-dimensional perspective.

ablation most endings show early changes characteristic of granulofilamentous degeneration (10, 14, 46). In freeze-fracture replicas, there are no changes in the membrane of the *pre*synaptic end bulb 12 h after cochlear ablation. The active zones are clearly delineated, but no vesicle sites are found within them. 24 h after cochlear ablation, the plasmalemmae of most end bulbs are altered (see Fig. 4). The active zones are flattened and indistinct, and the number of large particles on the cytoplasmic leaflet of the active zone is decreased.

After 12 h, the *post*synaptic membrane is altered. The number of nonaggregate particles on the external leaflet has increased from 53 particles per μm^2 on the membrane of principal cells in controls to 131 particles per μm^2 on the membranes of deafferented principal cells (Table I, Fig. 2). However, the number of junctional and perisynaptic aggregates is unchanged (Table I, Figs. 2, 3). 24 h after cochlear ablation, the distribution of particles on the postsynaptic membrane is similar to that found 12 h earlier (Table I, Figs. 2-5).

Two Days after Cochlear Ablation

Two days after cochlear ablation, many end bulbs have been removed from the postsynaptic membrane. Remnants of the end bulb often lie in shallow depressions of the principal cell plasmalemma (see Figs. 6-14, esp. Figs. 11, 12) attached to the principal cell at the former synaptic junctions (Figs. 6, 9, 11, 13, 14). Between the postsynaptic densities the plasmalemma is frequently deeply invaginated (Fig. 9). The plasmalemma at the depth of the invagination is often lined by a cytoplasmic coat (Fig. 9), and coated vesicles are common in the cytoplasm near the remnants. The coated invaginations are found adjacent to the postsynaptic density (Figs. 6, 9, 11), but they seldom include the former synaptic junction.

In freeze-fracture specimens, the number of perisynaptic aggregates is decreased (Table I, Figs. 2, 5). At normal end bulbs, the density of these perisynaptic aggregates is over 3.2 aggregates per μm^2, with up to five aggregates associated with each postsynaptic active zone. Two days after cochlear ablation, the ratio of junctional to perisynaptic aggregates has increased (Fig. 5), and the density of perisynaptic aggregates in the membrane has fallen to 0.32 aggregates per μm^2 (Table I, Fig. 2). The number of particles in each aggregate is similar to controls (Table I). Many remaining perisynaptic aggregates are found on

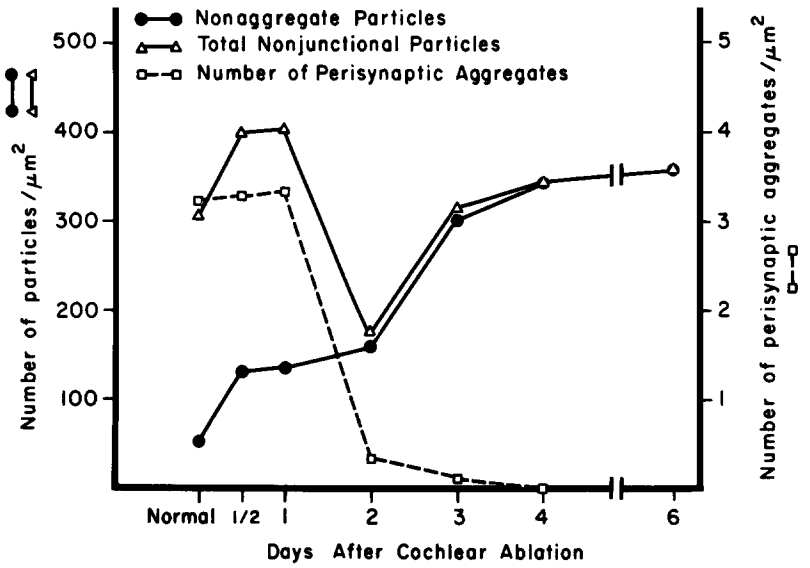


FIGURE 2 A graph showing the changes in the density of nonaggregate particles (left ordinate) and perisynaptic aggregates (right ordinate) with time after cochlear ablation. Each point is a mean obtained by counting particles from over 100 μm^2 of membrane from all animals sacrificed at each time-point. The total nonjunctional particles is the sum of the number of particles counted in the perisynaptic aggregates and the number of nonaggregate particles.

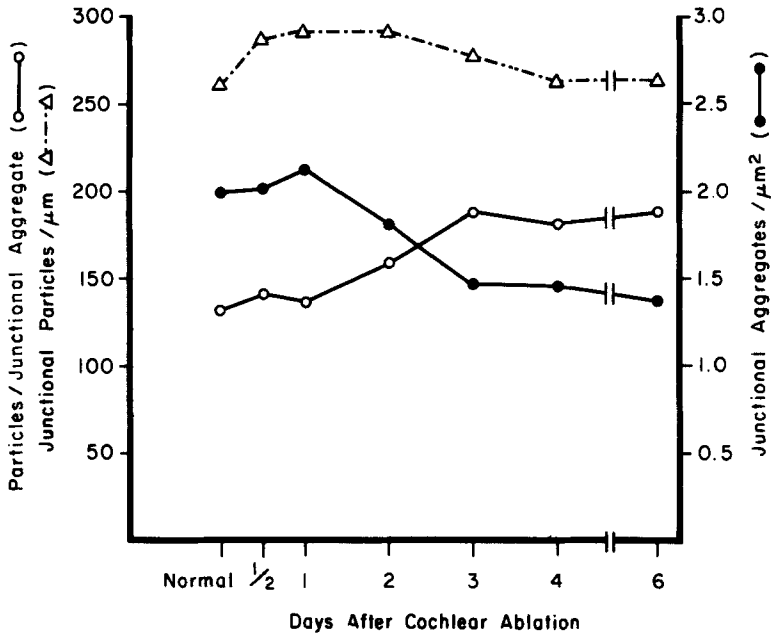
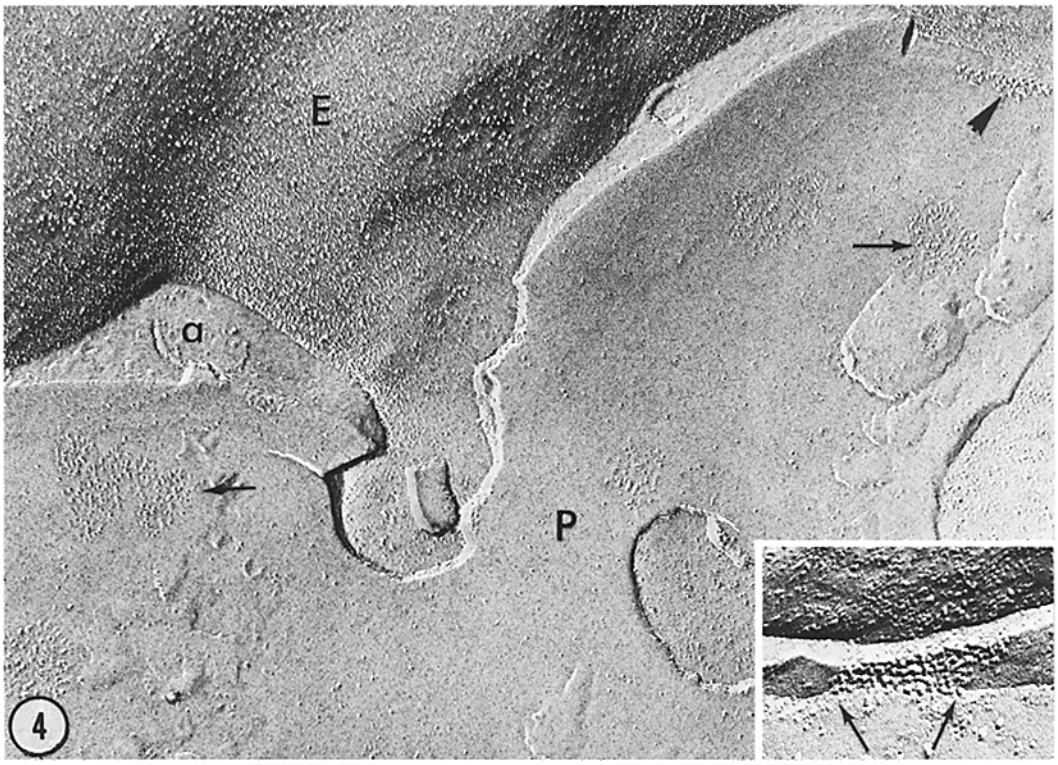


FIGURE 3 A graph illustrating changes in the junctional aggregates with time after cochlear ablation. Each point is a mean obtained by counting particles from over 100 μm^2 of membrane from all animals sacrificed at each time-point. The total junctional particles is obtained by multiplying the mean of the number of junctional aggregates/μm^2 by the number of particles/junctional aggregate.

small depressions of the external leaflet (Fig. 7). In replicas where the fracture crosses the cytoplasm of the principal cell before jumping to the external leaflet of the plasmalemma at the level of these invaginations (Fig. 8), the particle-covered depression has thin cytoplasmic striations.

FIGURE 4 The postsynaptic membrane of a principal cell 24 h after cochlear ablation. The fracture plane exposes the external membrane leaflet of the principal cell (*P*) before crossing to the cytoplasmic leaflet of an end bulb (*E*). The presynaptic active zones (asterisk) are less distinct than in normal terminals; however, the folds of the end bulb plasmalemma over the channels of extracellular space containing astrocytic processes (*a*) are unaltered. Both junctional (arrowhead) and perisynaptic aggregates (small arrows) are present on the external leaflet of the postsynaptic membrane. The number of nonaggregate particles on the external leaflet of the postsynaptic membrane of the principal cell has increased. *Inset*: Junctional aggregate seen on an evagination of the external leaflet of the principal cell membrane (between arrows) 24 h after cochlear ablation. This aggregate corresponds to junctional aggregates in normal animals. × 53,000; *inset*, × 76,000.

FIGURE 5 A fracture exposing the external leaflet of the principal cell plasmalemma (*P*), 2 days after cochlear ablation. In normal tissue, the fracture frequently jumps to the cytoplasmic leaflet of the end bulb near the synaptic junction, leaving windows in the external leaflet at the postsynaptic active zone. The preferred fracturing plane of this synapse is different 2 days after deafferentation, perhaps because most of the end bulbs have been removed from the membrane by this time. In this example, junctional aggregates (arrowheads) similar in size and shape to those in normal animals are seen on the external membrane leaflet. The number of perisynaptic aggregates (small arrows) is less than normally found on the external leaflet. *Inset*: An example, from an animal 2 days after cochlear ablation, of a junctional aggregate on the external leaflet of the principal cell at the interface with cross-fractured cytoplasm. × 68,000; *inset*, × 69,000.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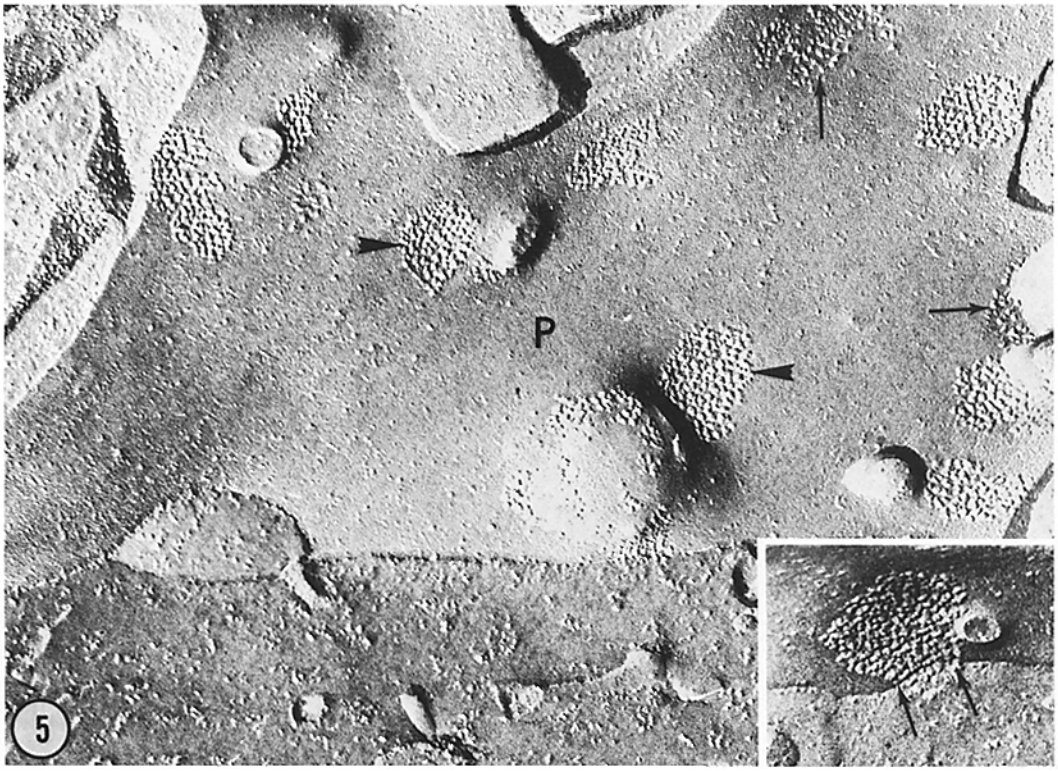
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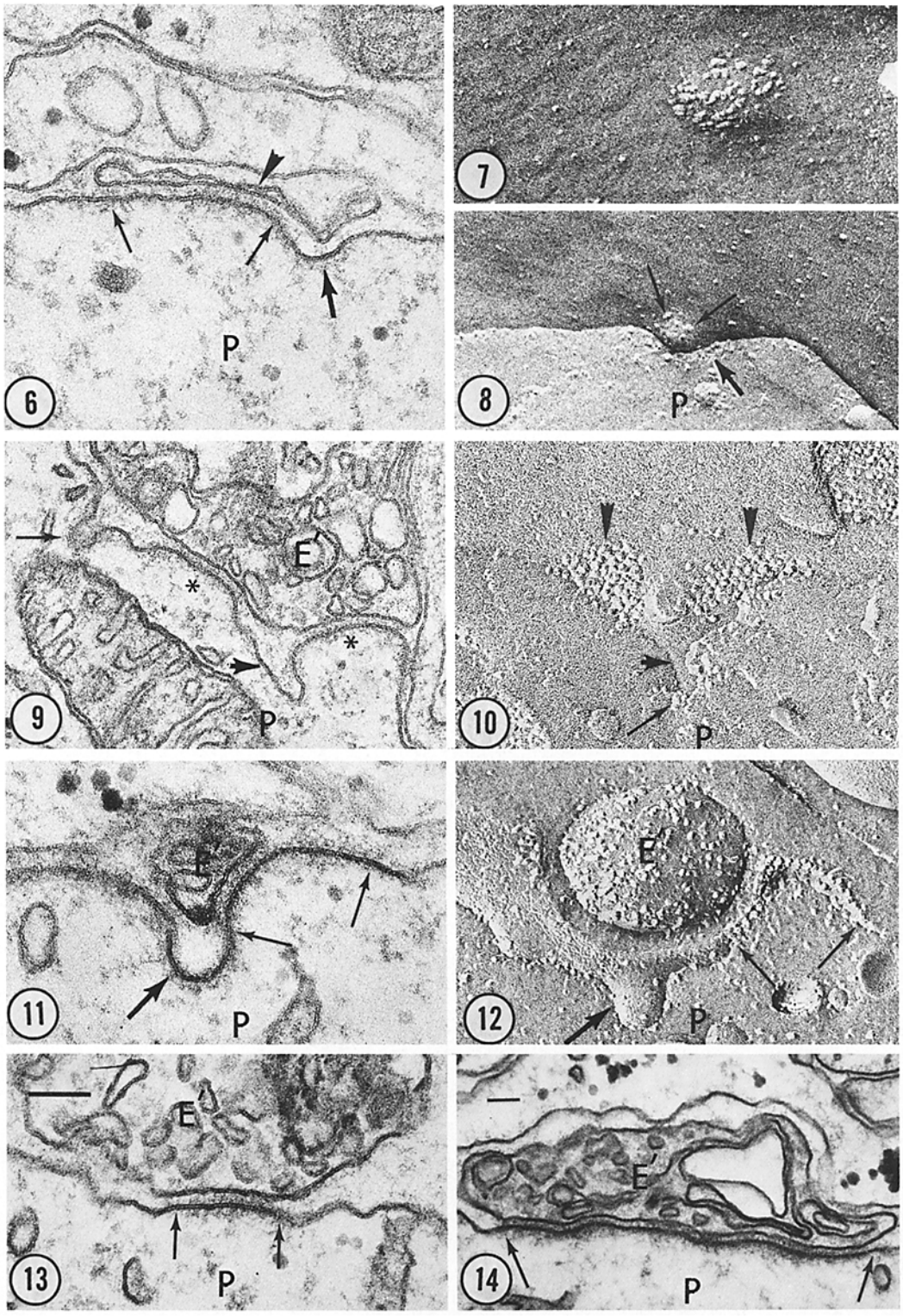
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These correspond to the coat on the membrane invaginations in thin sections (cf. Figs. 6, 7, and 8). No example was seen where the invaginations included the junctional aggregate. Between days 1 and 2, the number of nonaggregate particles increases to 159 particles per μm^2 (Table I, Figs. 2, 5). The density of junctional aggregates decreases slightly (Table I, Figs. 3, 5). However, the size of these aggregates and the number of particles in each aggregate has increased (Table I, Fig. 3). The total number of junctional particles is unchanged (Table I, Fig. 3).

FIGURES 6–12 Examples from freeze-fractured and thin-sectioned material of coated invaginations of the plasmalemma of the principal cell associated with remnants of end bulbs 2 days after cochlear ablation.

FIGURE 6 A remnant of end bulb membrane (arrowhead) is apposed to the principal cell (*P*) plasmalemma. Part of the cytoplasmic surface (between the small arrows) of the principal cell plasmalemma is lined by a postsynaptic density. A coated invagination (large arrow) of the plasmalemma is adjacent to the density. The external surface of the plasmalemma within this invagination lacks the cleft material associated with the plasmalemmae at synaptic junctions. × 84,000.

FIGURE 7 Frequent depressions on the external membrane leaflet are covered with particles of varying sizes. The range of particle sizes and the number of particles is similar to that of the perisynaptic aggregates. × 90,000.

FIGURE 8 A depression on the external membrane leaflet at the interface with the cross-fractured cytoplasm has numerous cytoplasmic striations (arrow) which correspond to the fuzzy coat of a coated invagination such as seen in Fig. 6. The external leaflet of this plasmalemmal invagination is covered with numerous particles (small arrows) of various sizes similar to those in perisynaptic aggregates. In normal tissue, perisynaptic aggregates had no associated cytoplasmic striations, nor was any paramembranous dense material found on the plasmalemma near this aggregate. × 84,000.

FIGURE 9 Opposite the remnants of the end bulb (*E'*), the plasmalemma between the former postsynaptic densities (asterisk), is deeply invaginated (arrowhead). Coated regions of the membrane (small arrow) are found at the depth of these invaginations. × 66,000.

FIGURE 10 The fracture in this replica crosses the cytoplasm of a principal cell (*P*) before exposing the external leaflet of the plasmalemma. The region illustrated is similar to that seen in Fig. 9. Between two junctional aggregates (arrowheads), the membrane has two invaginations. The upper invagination is broken off at its neck. The lower invagination (arrowhead) has fine cytoplasmic striations (small arrow) associated with the membrane at its extremity. The two junctional aggregates are spaced closer together than in normal animals. × 102,000.

FIGURE 11 A postsynaptic density (between the arrows) lines the cytoplamic surface of the principal cell (*P*) plasmalemma. Prominent cleft material is associated with the external surface of the same region of the membrane. A small remnant of an end bulb (*E'*) is apposed to part of this portion of the membrane, but much of the specialized region of the membrane is not directly apposed to any cellular process. A coated invagination of the plasmalemma (arrowhead) is immediately adjacent to but does not include any of the specialized region of the membrane. × 98,000.

FIGURE 12 Remnant of the end bulb (*E'*) indents the principal cell (*P*) plasmalemma. The remnant is apposed to part of a junctional aggregate between the arrows. Much of this aggregate is apposed to an enlarged area of extracellular space. A coated invagination of the plasmalemma is adjacent to, but does not include the junctional aggregate (cf. Fig. 11). × 96,000.

FIGURE 13 A segment of the plasmalemma of the principal cell (*P*) opposite the remnant of the end bulb is lined by a postsynaptic density, 0.22 μm in length, which is similar in size to that found at normal synapses. Bar, 0.1 μm. × 98,000.

FIGURE 14 Most, if not all, of the plasmalemma of the principal cell (*P*) beneath a remnant of an end bulb is lined by a prominent postsynaptic density (between arrows). No corresponding density is associated with the cytoplasmic surface of the end bulb plasmalemma, as might be expected if the thickening were a part of a desmosomal junction. Neither the end bulb nor other axosomatic synapses on the principal cell have a prominent postsynaptic thickening this size. Bar, 0.1 μm. × 51,000.

Three Days after Cochlear Ablation

Three days after cochlear ablation, most end bulbs have degenerated. Remnants of the terminal are commonly apposed to the principal cell. However, the frequent association of coated membrane invaginations with the remnants, seen at 2 days, is less prevalent. The length of the former postsynaptic densities is now variable, ranging from 0.25 to 0.8 μm in length (Figs. 13, 14). Prominent postsynaptic densities longer than 0.4 μm are never seen at the synaptic junctions of normal end bulbs or other axosomatic terminals on the principal cell.

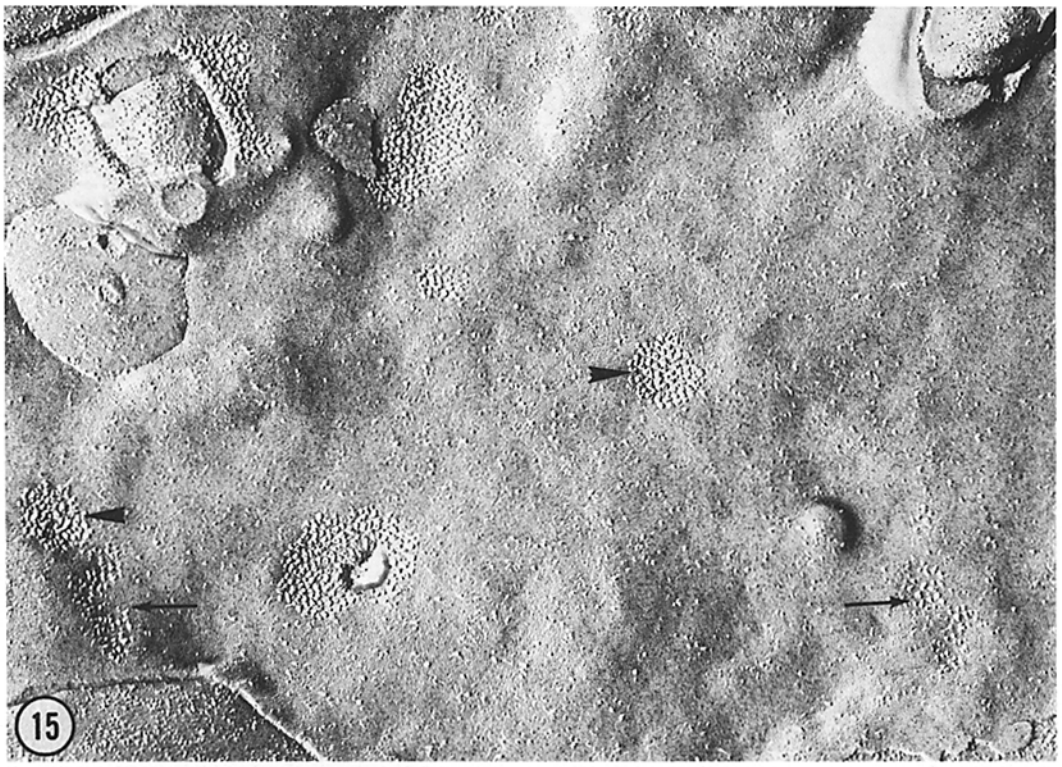
In freeze-fracture replicas 3 days after cochlear ablation, perisynaptic aggregates are infrequently found on the postsynaptic membrane (Table I, Figs. 2, 15). The number of nonaggregate particles has further increased (Table I, Figs. 2, 15). The density of junctional aggregates has decreased; however, the number of particles in each junctional aggregate has increased, and the total density of junctional particles in the membrane remains constant (Table I, Figs. 3, 15). The junctional aggregate is now irregularly shaped and variable in size (Fig. 15). The range of sizes of the aggregates corresponds to range of sizes of the postsynaptic densities.

Four to Six Days after Cochlear Ablation

Four days after cochlear ablation, no perisynaptic aggregates remain on the membrane (Table I, Figs. 2, 16). The density of nonaggregate particles increases slightly between days 3 and 4 after cochlear ablation but does not change between days 4 and 6 (Table I, Figs. 2, 16). The size and number of junctional aggregates does not change after 3 days following cochlear ablation (Table I, Fig. 3).

DISCUSSION

After cochlear ablation there are three distinct changes in the distribution of particles on the external leaflet of the principal cell: (*a*) the addition of large, nonaggregate particles, (*b*) the removal of the perisynaptic aggregates, and (*c*) the remodeling of the junctional aggregates. Because there is no change in the distribution of particles on the cytoplasmic leaflet of the membrane, these alterations do not represent differences in partitioning of the particles between the leaflets after deafferentation. Within 12 h, there is a 147% increase in the number of nonaggregate particles. This change occurs before morphological changes are seen in the end bulbs in thin sections. The increase in nonaggregate particles continues until between days 4 and 6, when there are about 350 nonaggregate particles per μm^2 of membrane. Because the nonaggregate particles are uniformly spread over the membrane and there is no shrinkage in the principal cell until about 30 days after deafferentation (31, 46), the increased density of nonaggregate particles, 12 h after cochlear ablation, must result from the addition of particles to the membrane rather than the rearrangement of particles on the membrane. This interpretation is supported by the observation that when the nonaggregate particles first increase, the number of perisynaptic and junctional aggregates are unaltered (46). When the number of perisynaptic aggregates does decrease, 2 days after cochlear ablation, there is no corresponding increase in nonaggregate particles and the total number of nonjunctional particles decreases. These data suggest that, after destruction of the spiral ganglion cells, the principal cell responds by adding particles to the membrane. The signal to initiate this response has not been identified. It is known that immediately after cochlear ablation all spontaneous activity is lost in the principal cell (18). Postsynaptic activity at the neuromuscular junction apparently has a trophic role in maintaining the postsynaptic membrane. Perhaps the changes in activity after cochlear ablation initiate the response of the postsynaptic membrane of the principal cell. The fact that this response occurs so rapidly, before there is any morphological alteration of the end bulb, supports this suggestion. The nature of these particles is not known and there have been no appropriate physiological studies; thus it is impossible to relate this change directly to a specific physiological response of the neuron. However, it is interesting to compare the response of the principal cell to the neuromuscular junction (1, 16, 27, 28), and autonomic postganglionic neuron (5, 19, 36) after denervation. In these systems, denervation results in increased sensitivity of the nonjunctional postsynaptic membrane to acetylcholine. This supersensitivity results from the addition of extrajunctional receptors to the membrane (3, 4, 16). The response is graded in time and is maintained until reinnervation occurs (29). Denervation supersensitivity is thought to be related to a loss of function in the postsynaptic element (2, 23, 24, 25), although the



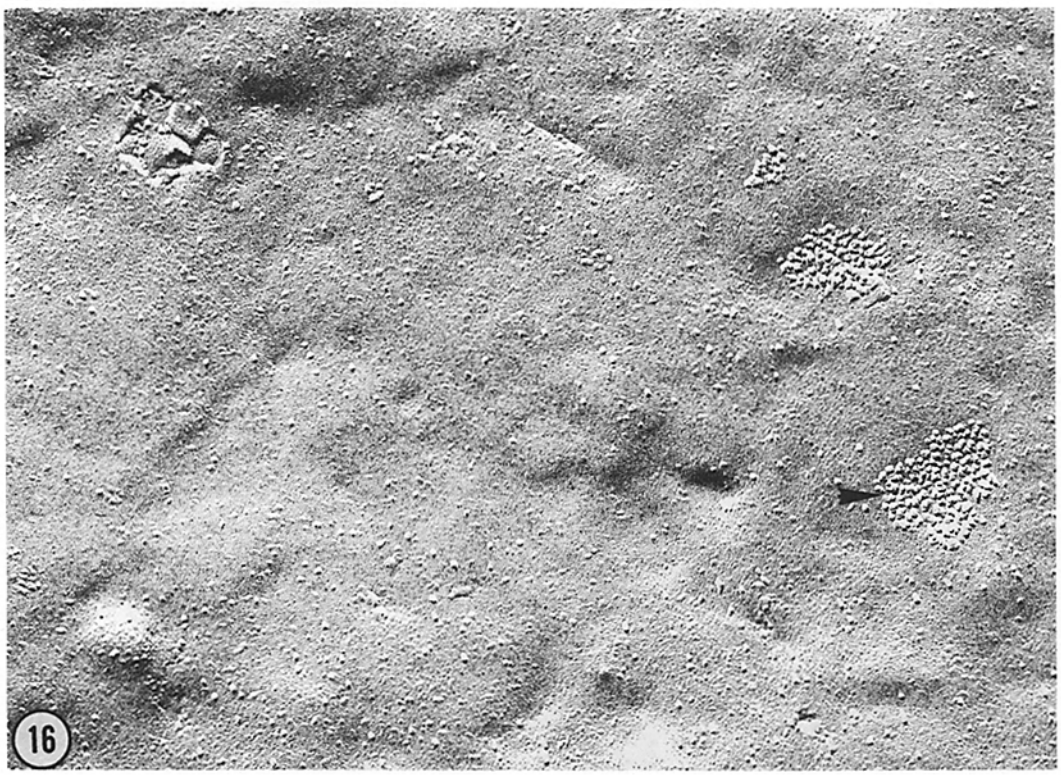


FIGURE 15 The external leaflet of the plasmalemma of a principal cell, 3 days after cochlear ablation. Junctional aggregates are present; however, their size and shape are variable. Two junctional aggregates similar in size and shape to those found in normal animals are identified by arrowheads. Only a few perisynaptic aggregates (small arrows) are found in the membrane. The number of nonaggregate particles has increased (Table II). × 64,000.

FIGURE 16 The external leaflet of a principal cell plasmalemma 4 days after cochlear ablation. Junctional aggregates (arrowhead) are present in the membrane; however, no perisynaptic aggregates are present. × 70,000.

loss of trophic influences cannot be ruled out (32). The response of the principal cell membrane occurs before changes are seen in the end bulb, but after spontaneous activity has ceased in the neurons of the rostral AVCN (18). Moreover, this graded increase in nonaggregate particles is reversed 7 days after deafferentation (15), when the former postsynaptic densities are partially reoccupied by nonprimary boutons on the soma (14, 46).

The perisynaptic aggregates are unaltered until 24–48 h after cochlear ablation when they decrease to 10% of their normal density in the membrane. Because there is no increase in nonaggregate particles or junctional aggregates at this time, it is unlikely that the decrease in perisynaptic aggregates is the result of a redistribution of their particles in the membrane. The decrease in perisynaptic aggregates occurs when all end bulbs are degenerating and large portions of many end bulbs are being removed by glial processes. At this time, the number of coated membrane invaginations and coated cytoplasmic vesicles increases near the postsynaptic membrane associated with the remnants of the end bulb. Coated invaginations are a normal feature of postsynaptic membranes which may function in the turnover of components of the membrane (45). On the principal cell membrane in normal animals, these coated vesicles typically have only random particles on their external leaflet. The increased frequency of coated invaginations when the number of perisynaptic aggregates in the membrane is decreasing and the presence of these aggregates on shallow depressions and coated invaginations of the membrane suggests that coated vesicles may remove the perisynaptic aggregates from the membrane.

Three days after cochlear ablation there has been extensive remodeling of the junctional aggregate. The number of junctional aggregates has decreased, but many of those present are larger and the total number of junctional particles in the membrane remains constant. As a consequence of the focal loss of the plasmalemma between synaptic junctions, the junctional aggregates could be drawn together and ultimately fuse. This would explain the decrease in the number of junctional aggregates and the fact that some of the junctional aggregates are larger than those in normal membranes. The variation in the length of the postsynaptic density also could reflect the fusion of the junctional aggregates associated with the density. It has been suggested that after deafferentation the postsynaptic densities associated with the synaptic remnants are removed from the membrane by coated vesicles, whereas the bared active zones are reoccupied by nonprimary synapses (14, 43, 44). The freeze-fracture data presented here show that the junctional aggregates, which are coextensive with the postsynaptic density, are not removed from the membrane after deafferentation. Instead, the aggregates coalesce to form fewer but larger aggregates.

In the cholinergic electroplaque, where membrane particles at the postsynaptic active zone have been identified as receptors (6, 7, 12, 26), there is no change in the density of particles on the junctional postsynaptic membrane after denervation (9). At the neuromuscular junction, denervation does not affect the sensitivity of the junctional membrane (1, 27, 28) and there is no change in the number of junctional particles (13). In contrast, the junctional particles on the principal cell are on the external membrane leaflet; however, like the cholinergic synapses, they are confined to a region of the membrane directly beneath the presynaptic active zone. And, although there is some redistribution of junctional particles, the density of particles in the membrane remains constant. Despite these favorable comparisons, the identification of the particles as a receptor protein remains to be proved.

The neuronal membrane in the central nervous system exhibits a remarkably specific response to deafferentation. The response includes the addition of one class of membrane particle, the loss of an aggregate of particles, and the remodeling of another aggregate. Many nonneuronal membranes, in response to changes in functional state, are reorganized by altering the state of aggregation of their membrane proteins (8, 30, 41, 42). The variety and selectivity of the response of the postsynaptic membrane is unique. This response is separated into three discrete changes occurring at different time intervals. The signal to initiate the response occurs while the presynaptic element is structurally normal. Later changes mediated by coated vesicles occur after the presynaptic element has been altered or removed from the membrane. In terms of the organization of the postsynaptic membrane, the three different responses suggest the existence of at least three different types of membrane particles, each dependent upon different cues for its maintenance in the membrane. Further studies to identify the factors involved in

the maintenance of the different components of the postsynaptic membrane and their relationship to synaptic function are in progress.

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Please address all reprint requests to Dr. R. L. Gulley, Department of Anatomy, Case Western Reserve University, Cleveland, Ohio 44106.

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