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LESION-INDUCED SYNAPTOGENESIS IN BRAIN: A Study of Dynamic Changes in Neuronal Membrane Specializations

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When incoming fibers to a given brain region are damaged and degenerate, the remaining undamaged fibers can, in some cases, form new synapses, and restore physiologically functional circuitry. Synaptic membrane events underlie this reconstruction: the connection between membranes is broken and reformed. In order to understand these membrane events, it is necessary to know the molecular composition of the synapse and the nature of the interaction between pre- and postsynaptic membranes. The synaptic membranes are probably joined by proteins extending from their surfaces. The postsynaptic membrane has on its outer surface an array of lectin receptors, probably glycoproteins. On its inner surface, juxtaposed to the bilayer, the membrane has an electron-dense structure called the postsynaptic density which, from studies on the isolated structure, is composed of a few polypeptides. On the basis of the molecular composition and structure of CNS synapses and ultrastructural studies of the lesion-induced synaptogenesis, some of the underlying dynamic events at synaptic membranes are inferred. New synapses are formed either by reutilization of the old contact sites or by generation of new ones. The protein and carbohydrates in the cleft are enzymatically degraded and a new synapse is generated in response to ingrowing fibers by the addition or reutilization of the specialized proteins of postsynaptic membrane, which differentiate a small segment of the postsynaptic membrane.

INTRODUCTION

The synapse, a term introduced in 1897 by Foster and Sherrington to describe "the mode of nexus between neuron and neuron" (1), is a connection between specialized pre- and postsynaptic neuronal plasma membranes. In the mammalian brain, most excitatory synapses have a highly stereotyped structure (Fig. 1). The presynaptic (axonal) membrane is distinguished by the presence of a set of dense projections on its cytoplasmic side and the postsynaptic (dendritic) membrane, which is joined to the presynaptic, is distinguished by a postsynaptic density on its cytoplasmic surface. The total length of the synaptic junction is about 0.5 μm, and the membrane specializations extend about 400 Å into the cytoplasm. A brain neuron has over its surface up to 80,000 synapses. The exact size, number, and position of the synapses on a cell determines the operational properties of the neuron in its circuit.

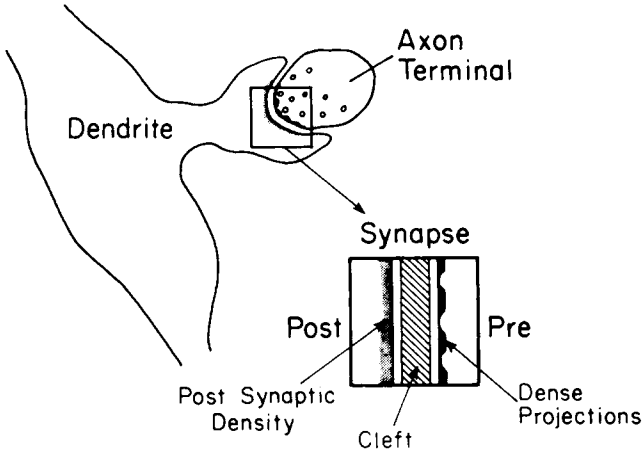

Fig. 1. Diagram of a typical central nervous system excitatory synapse.

It is now evident that damage to the adult brain does not simply result in the loss of neurons and their connections as previously suspected. Rather, in some cases the remaining undamaged neurons are induced to sprout new axonal branches and form new synapses in place of those which were lost. In this way a denervated neuron regains a large proportion of its lost synaptic input. The significance of this restoration of synaptic input is unclear, but it may aid or hinder recovery of function in neuronal circuits, depending on the exact functional consequence of the circuit alteration (2–6).

Over the past several years, in collaboration with Dr. Gary Lynch, I have been studying the phenomena and underlying mechanisms in the recovery of function after brain damage. Our studies, carried out in the rat hippocampus, have shown that removal of an input to a neuron causes other inputs to the same cell to establish more connections and thus restore the lost synaptic input. We have shown that this process is rapid, occurring in some case in less than 2 weeks, and the new synapses appear functional (7–10).

Changes in pre- and postsynaptic membranes play a central role in determining the nature of this degeneration-reinnervation process. In lesion-induced synaptogenesis, old connections are broken and new ones formed. The reinnervation process may follow either of two courses. In one, terminals of damaged neurons die and the connections between neurons are broken, so that a vacant postsynaptic site is created (Fig. 2 a). Over time, this vacant site is reoccupied by new presynaptic terminals (11–13). Alternatively, it seems as if, in some cases, old postsynaptic sites are lost and new ones generated in the process of reinnervation (Fig. 2 b) (Matthews et al., manuscript in preparation). Thus, a sequence of changes in the post- and presynaptic membranes is involved in lesion-induced synaptogenesis.

In order to understand the process of the formation of new connections, and the breaking of old ones at a molecular level, it is necessary to know: (a) What are the constituents of a CNS synapse? and (b) what molecules or intramolecular bonds join the pre- and postsynaptic membranes?

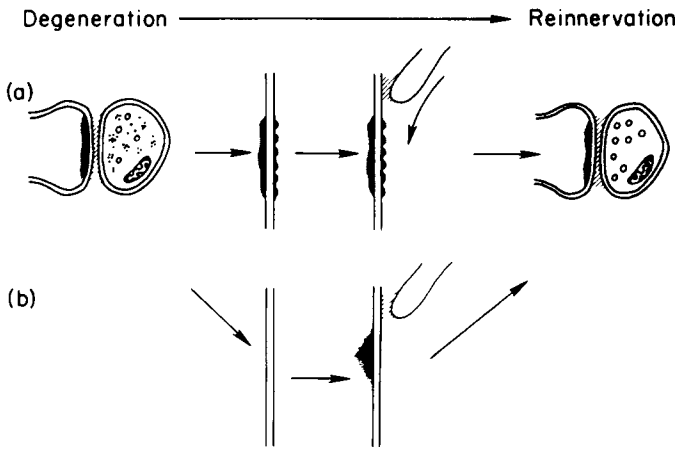

Fig. 2. Model outlining the two types of changes which appear to occur in synaptic membranes during the degeneration-reinnervation process. In one case (a), a vacant postsynaptic site remains after the synaptic bouton degenerates. This site can be recognized by a growing sprout, and a new synapse can be formed there. In the other case (b), the postsynaptic site is lost and a new site created.

CONSTITUENTS OF CNS SYNAPSES

Until very recently, when isolation techniques opened the way to direct analysis, the composition of CNS synapses was studied indirectly by cytochemical methods. These methods suggest that the synaptic membrane specializations such as the postsynaptic density (PSD) are mainly proteins and that the material within the synaptic cleft is protein and carbohydrate. Treatment of synaptic junctions with proteolytic enzymes destroys the PSD and ultimately dissociates the synaptic cleft (14–17). Other enzymes do not have such a drastic effect (15, 16). Carbohydrate stains such as ruthenium red and periodic acid-silver methenamine are associated with the intracellular spaces at synaptic junctions (18, 19), neuraminidase decreases the uranyl acetate staining of the synaptic cleft (20), and ferritin-lectin conjugates (Con A or RCA) bind to the external surface of the postsynaptic membrane overlying the PSD (21, 22). These studies suggest that the cleft and the external surface of the postsynaptic membrane are rich in carbohydrates, and that the internal surface specialization, the PSD, is primarily protein.

Recently, we developed methods to isolate synaptic junctions and postsynaptic densities from homogenates of rat brain. These fractions made it possible to determine the composition of the synaptic region directly. In order to isolate synaptic junctions, synaptic membranes (SM) (Fig. 3 a) are isolated by a combination of differential and density-gradient centrifugation and treated with Triton X-100. The synaptic junctions (Fig. 3 b) which remain insoluble are purified from other membranes on a density gradient (17, 23, 24). The resulting fraction consists of about 70% intact synaptic junctions and postsynaptic membranes from which the presynaptic membrane has been removed. PSD (Fig. 3 c) are isolated by treatment of SM fractions with sodium-N-lauroyl sarcosinate followed by density-gradient centrifugation. This fraction consists of about 80% PSDs (25). Both preparations retain many of their known in vivo characteristics, such as their unique

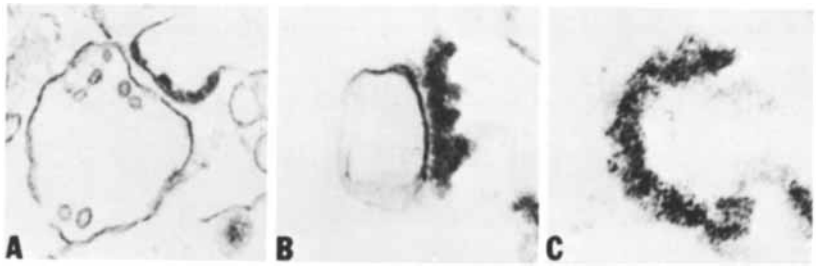

Fig. 3. Subfractions from brain synapses which can be isolated. A synaptic membrane (a), a synaptic junction (b), and a postsynaptic density (c).

staining with ethanolic phosphotungstic acid and bismuth iodide uranyl lead, and are of sufficient purity to make direct biochemical analysis meaningful.

Protein makes up about 80% of the synaptic junctional complex (SJC) fraction. Carbohydrates account for 4%, and lipids and bound detergent, the remainder (Churchill et al., manuscript in preparation). On sodium dodecyl sulfate (SDS)-polyacrylamide gels, the SJC fraction is seen to consist of one prominent polypeptide with a molecular weight of 53,000 and several less prominent ones of higher molecular weight. This pattern is simpler than that of the SM fraction, which contains a greater heterogeneity of polypeptides. The high molecular weight glycopeptides are more concentrated in the SJC fraction. The individual carbohydrates of the SJC glycopeptides measured by gas chromatographic analysis are similar in type to those seen in SM, except that sialic acid is reduced (Table I).

In order to identify the location of the carbohydrates in the SJC fractions, we studied the binding of Con A-ferritin conjugates to SJC. We found that the Con A binding sites are essentially exclusively localized to the external surface of the synaptic membrane directly overlying the PSD (Fig. 4). The PSD and the membrane outside the cleft area bind little, if any, Con A conjugate. We attempted to determine if the Con A binding sites in the SJC fraction are glycoproteins by extracting the fraction with chloroform-methanol to remove glycolipids. The insoluble residue binds Con A at nearly the quantities one would expect if all the Con A binding sites are glycoproteins. These results indicate that the lectin receptors on the surface of the postsynaptic membrane within the synaptic cleft are primarily glycoproteins (21).

We examined the structure of the isolated SJC in the electron microscope. In tissue, Akert and co-workers (26) have observed globular elements, and de Robertis (27) identified fibers as parts of the PSD. A set of knobs appears to extend from the postsynaptic membrane into the cleft (28). In our studies on isolated SJC, the external surface of the synaptic membrane overlying the PSD is found to be decorated with a set of knobs or bristles; these are absent on the matching face of the presynaptic membrane (17). It is likely that the bristles are the structures responsible for binding Con A. The PSD appears to consist of a matrix of fibers and globules, 80 Å in diameter.

Proteins appear to be the principal macromolecular constituent of the PSD, as indicated by its sensitivity to proteolytic enzymes, distinctive staining properties (14–16), and the presence of a prominent phosphodiesterase activity (29). An analysis was made of the protein composition of a fraction of PSD prepared from rat brain (30). Protein

TABLE I. Carbohydrate Composition of Synaptic Membrane (SM) and Synaptic Junctional Complex (SJC) Proteins

	SM	SJC
Total	207	202
N-acetyl neuraminic acid	47 ± 2 (4)	19 ± 2 (3)
fucose	18,18	44,19
manno	38 ± 2 (3)	43 ± 4 (3)
galactose	28 ± 1 (3)	44 ± 4 (3)
glucosamine	63 ± 8 (3)	45 ± 11 (3)
galactosamine or mannosamine	13 ± 4 (3)	20 ± 1 (3)

Fractions were extracted with chloroform-methanol to remove lipids (35). N-acetyl neuraminic acid was measured by the Warren method (36). All other carbohydrates were measured by gas-liquid chromatography (37, 38). Means ± SE (nmoles/mg protein) are for three or more preparations. Glucose values are not included because SJC fractions appear to bind sucrose during isolation, so values of glucose are abnormally high (144 ± 2 (3)) (Churchill et al., manuscript in preparation).

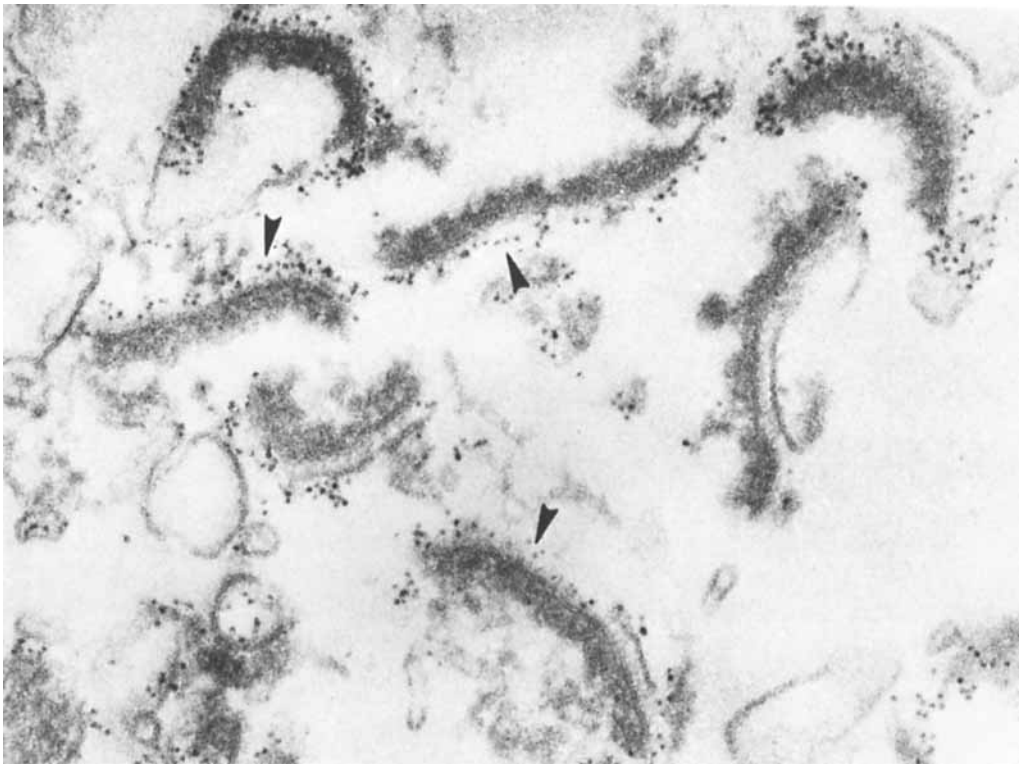

Fig. 4. Isolated synaptic junctional complexes. Con A-ferritin conjugates bind to the external surface of the postsynaptic membrane (arrows).

makes up 90% of the material in the PSD fraction. Two major polypeptide bands are present on the basis of SDS-polyacrylamide gel electrophoresis. The major polypeptide fraction has a molecular weight of 53,000, comprises about 45% of the PSD protein, and comigrates on gels with a major polypeptide of the synaptic plasma membrane. The other polypeptide band has a molecular weight of 97,000, accounts for 17% of the PSD protein, and is not a prominent constituent of other fractions. Six other polypeptides of higher molecular weight (100,000–180,000) are consistently present in small amounts (3–9% each). The PSD fraction contains slightly greater amounts of polar amino acids and proline than the synaptic plasma membrane fraction, but no amino acid is usually prominent. The PSD apparently consists of a structural matrix formed primarily by a single polypeptide or class of polypeptides of 53,000 molecular weight. Small amounts of other specialized proteins are contained within this matrix.

Thus, the postsynaptic membrane is specialized in such a way that it displays on its external surface an array of bristles (probably glycoproteins) and on its internal surface a proteinaceous matrix consisting of a few distinct polypeptides.

One of the roles of the PSD may be to restrict the lateral diffusion of specialized postsynaptic molecules from the synaptic area into outlying membrane. PSD proteins may provide a fixed cytoarchitectural base which interacts with integral and peripheral proteins of the membrane and lowers their mobility (17, 30). It is known that the postsynaptic membrane has a closely packed array of intramembraneous particles at the synapse and that these particles, unlike those away from the PSD, are immobile (31). Similarly, it appears as if the surface lectin receptors at the synapse are relatively less free to diffuse in the membrane, compared to those outside the membrane (Cotman et al., manuscript in preparation). The idea that a protein on the internal surface of the membrane can restrict the mobility of overlying components has been put forth previously as a role of spectrin in the erthrocyte membrane (32).

The surface lectin receptors may participate in the adhesion of the pre- and postsynaptic membranes, or they may subserve a role in synaptic transmission as, for example, receptors for neurotransmitters. Considering the ubiquitous presence of these receptors at excitatory synapses, a more general role, e.g., as neuronal recognition factors, is implied. However, it is also possible that lectin-binding molecules contain similar carbohydrates, but serve different specialized functions.

NATURE OF THE SYNAPTIC CONNECTION

At present the molecular basis of the connection between nerve cells at the synapse is unknown. Several models have been suggested, including a direct covalent attachment, ionic interactions, and other unspecified noncovalent interactions (27, 33, 34). In order to analyze the nature of the junction between pre- and postsynaptic membranes, we have studied the effects of agents known to disrupt covalent and noncovalent bonds on the integrity of the synaptic junction.

We have found that the synaptic junction is remarkably durable to a variety of treatments which break ionic, coordination, and some hydrophobic bonds. It is not dissociated by high salt, EDTA, high pH, urea, or detergents at moderate concentrations. The synaptic junction is, however, very sensitive to mild proteolysis (Table II).

TABLE II. Effects of Various Agents on the Structural Integrity of Synaptic Junctions

	Treatment		% clefts attached
NaCl	4 M	pH 7.3, 0.5 – 1.5 hr	110
			95
			109
EDTA	5 mM	0.1 M NaCl, 0.01 Tris, pH 8.3	95
			94
	10 mM	"	95
pH	10.0	0.1 M NaCl, 0.01 Tris, 1/3 hr	79
			90
			101
	11.1	"	98
urea	1 M	pH 7.3	85
			87
Triton X-100	1%	0.1 M NaCl, 0.01 Tris, pH 7.3, 0.5 hr	83
			66
trypsin	20 μgm/ml	0.1 M NaCl, 0.05 M Bicine, pH 7.0, 0.5 hr, 37°C	24
			18
	40 μgm/ml	0.1 M NaCl, 0.05 M Bicine, pH 7.0, 2 hr, 37°C	<1

Samples consisting of either 200-μm slices of rat dentate gyrus or synaptosomal fractions were incubated as described and prepared for electron microscope analysis (21). From a series of micrographs, the number of intact and dissociated synaptic junctions was determined (Cotman et al., manuscript in preparation).

These data suggest that proteins are involved in the union of pre- and postsynaptic membranes and that the interaction is probably covalent. It is unlikely to depend solely on ionic, coordination, or weak hydrophobic bonds. In view of the resistance of the synaptic junction to disruptive treatments, it is tempting to consider that proteins or glycoproteins extend from pre- to postsynaptic membrane and covalently join the synaptic membranes. However, other strong noncovalent interactions between membranes cannot be entirely excluded since none of the treatments we have used singly or in combination are sufficient to disrupt all noncovalent interactions.

CONCLUSION

On the basis of the structural and compositional data on the CNS synapse, it is possible to propose a tentative sequence of membrane events involved in the process of synaptogenesis induced by lesions. As terminals originating from damaged neurons degenerate, they are removed by the action of degradative enzymes supplied by phagocytic glial or other cells, or by the dying terminal itself. Mild proteolysis is probably sufficient to break old connections. In some cases, the postsynaptic site is retained and serves as a site for an ingrowing process. The concentration of immobile lectin receptors, which are probably glycoproteins, may aid in providing a surface where a sprouting nerve process can attach. Alternatively, new postsynaptic sites may be generated by the attachment of specific pro-

teins to the internal surface of the dendritic membrane. Once an afferent is juxtaposed to a competent postsynaptic site, a synapse is formed. The nature of the connection is unclear, but it may involve the formation of peptide bonds.

Thus, a series of dynamic changes in pre- and postsynaptic neuronal membrane specializations appears to regulate the breaking of old synapses and the making of new. The further elucidation of these membrane events may increase our understanding of the brain's repair process and our capacity to treat brain damage.

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