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The postsynaptic density: A possible role in long-lasting effects in the central nervous system

(theory/protein modifications/structure change/synaptic strength)

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ABSTRACT **A theory is proposed that biochemical changes at the synapse that occur as a result of stimulation of specific neuronal circuits can lead to long-term changes only if alterations occur in synaptic structures in these circuits. The main synaptic structure that is thought to undergo this alteration is the postsynaptic density (PSD). There are many reports in the literature of overall structural changes at the synapse, including the PSD, resulting from various neuronal stimuli. These structural changes are here envisaged to include those of concentration and conformation of PSD proteins, changes that could alter the neural physiology of dendritic spines and even that of the presynaptic terminal.**

When a cell biologist looks at the central nervous system (CNS), he or she is immediately struck by the singular occurrence of a structure called the postsynaptic density (PSD), which is not found in other tissues. During the last decade, due to biochemical results from work on isolated preparations and due to immunological studies on tissue *in situ*, much has been learned about the protein composition and properties of this structure (cf. ref. 1). I will not enumerate all of these results, but instead focus on some which, together with published work in other neuroscience fields, could lead one to the conclusion that this structure has great meaning for changes in CNS function that last for comparatively long periods of time.

As seen in countless electron micrographs, the cerebral cortex PSD is a disc, ranging from 300 to 500 nm in diameter and from 50 to 60 mm in thickness, lying tightly apposed to the postsynaptic membrane (2, 3). In many cases it has a large perforation in the center, thus resembling a doughnut (4, 5). The PSD seems to be composed of filaments and particles, with some of the filaments apparently extending into the interior of the cell (5–7). One of the postulated functions of this structure has been as a constrictor of movement of the membrane proteins, neurotransmitter receptors, and ion channels, which undoubtedly occupy that part of the membrane to which the PSD is attached (8, 9). Of the proteins that make up the filamentous structure, actin (2, 10–12) and fodrin (13, 14) or brain spectrin, an actin- and calmodulin-binding protein, are found in the PSD, with tubulin probably also being a part of the structure (2, 10, 11, 15–18). These proteins could account for the tightness of binding of the PSD to the membrane, a tightness that can be inferred from the observations that, in a synaptosomal membrane preparation, only those portions of the presynaptic and postsynaptic membranes at the synapse are connected to each other (3), as if bound together through the proteins of the PSD linking it to the postsynaptic membrane, and probably via the synaptic cleft, linking the pre- and postsynaptic membranes. Detergent disruption of the membranes allows one to isolate PSDs (3) free of their membrane attachment. The binding of the PSD structure to the postsynaptic membrane could conceivably come about through the complexing of PSD actin and calmodulin to that fraction of the fodrin molecule that has been found (19) also on the inner surface of the membrane. This binding would be analogous to that found between actin and the erythrocyte membrane protein spectrin (20) via the intermediary erythrocyte protein ankyrin, a protein that has recently been found also in brain (21).

Other components of isolated PSD preparations recently found include γ-aminobutyric acid (GABA) and flunitrazepam receptors (22–25), glutamate receptors (ref. 26; unpublished data), one of the Ca^{2+}-activated K^+ channels (28), and various glycoproteins (29–31). It is as if these obvious membrane proteins, as well as fodrin (13) and the β-adrenergic receptor as seen by immunocytological means (32), are anchored in the PSD at the synapse, with the receptor part of the protein being on the outer surface of the postsynaptic membrane and with the anchoring parts being linked to other PSD proteins. Thus, these proteins can be alternatively considered as PSD or membrane constituents, since when the PSDs are isolated a proportion of these protein molecules come along with the PSD fraction. The PSD is also the site of phosphoproteins and their related kinases (33). Both of the nervous system second messengers, cAMP (refs. 34–37; but cf. ref. 38) and Ca^{2+} (39), are involved in activating these PSD protein kinases, the latter together with the Ca^{2+}-binding protein calmodulin, a protein also found in the PSD (40–42). In addition, a phosphoprotein phosphatase, calcineurin, a calmodulin-binding protein (41) as well as a calmodulin-activatable cyclic nucleotide phosphodiesterase (43), are present in the fraction. The presence of receptor and channel proteins, together with the protein kinases, their protein substrates and phosphatase, and the filamentous proteins, suggests that the PSD is not a static structure, but a dynamic one, perhaps having modulating influence on synaptic transmission. For example, the binding of transmitter to receptor could set in motion a series of events, such as protein phosphorylations (cf. ref. 44), resulting in conformational changes and movements of filamentous proteins, the end result of which may be changes in the open or closed times of the channel proteins anchored in the PSD. These rapid changes in protein phosphorylation and dephosphorylation could be construed as being involved only in short-term alterations in function (cf. ref. 44).

I would like to postulate that some long-term alterations in CNS function involve changes in both the general and intimate morphology of the PSD, on the basis of the axioms that the PSD acts as a strengthening anchor for the elements of the synapse and that it acts as a modulator of neuronal transmission at the synapse. In brief, this theory is based on the idea of structural adaptation to neuronal events, particularly that changes in afferent stimulation result in long-dura-

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Abbreviations: CNS, central nervous system; PSD, postsynaptic density; GABA, γ-aminobutyric acid.

tion alterations in PSD structure. Previously, other ideas have been proposed, involving either the overall PSD structure (45–47) or biochemical events unrelated to structure (48–50). With regard to the former category, it has been postulated (45), on the basis of morphological observations on changes in synapse and PSD structure, that PSDs turn over and, in doing so, may provide a nucleus and a mechanism for the formation of new synapses, a mechanism for synaptic plasticity. In a similar category, a previous proposal by Carlin and Siekevitz (46) and by others (47), based on a similar type of observation, was that synapses divide, providing two active synapses instead of one at each terminal, that the synaptic spinule and the spine apparatus as well as the PSD have a role in this division, and that the perforation in the PSD is a manifestation of the division process. This theory, which involves long-term, possibly irreversible, changes in synaptic function due to an increase in the number of synapses at terminals, is based on long-term changes in overall synapse morphology due to dynamic alterations in overall PSD structure.

In the second category, Lynch and Baudry (48) have recently proposed another theory concerning long-term potentiation in the hippocampus, on the basis of extensive experimental work by their group. The postulate is that long-term potentiation is due to an increase in glutamate binding caused by an activation of a Ca^{2+} protease, calpain, breaking down the filamentous protein fodrin, which is thought to normally occlude the glutamate receptor. Repetitive high-frequency stimulation is visualized to result in long-lasting morphological changes in the structure of the synapse produced by breakdown of a membrane fodrin–cytoplasmic filament network (48). In the same category are the biochemical results obtained during a long-duration process called "kindling." Kindling is the result of a development in which many subconvulsive electrical stimuli to various cerebral areas eventually lead to epileptic seizures after a later stimulus of similar strength, even after a lapse of one year (for review see ref. 49). The neurotransmitter pathways involved are not definitely known (50), and though there is some information on the possible involvements of the GABA pathway (51–53) and of the glutamate pathway (54, 55), the correlative neurochemical data are not clear (56, 57).

The connections between the hippocampal long-term potentiation theory and the present hypothesis concerning the PSD are the findings that fodrin is a PSD protein (13, 14), that specific glutamate receptors exist in the isolated PSD preparation (26, 27), and that a protease behaving like calpain may exist in the PSD (13). It is also relevant that tetanic stimulation of the same hippocampal perforant pathway resulted in a Ca^{2+}-stimulatable phosphorylation of a 51-kDa protein (58); this protein may be the 51-kDa major PSD protein that binds calmodulin (59) and whose phosphorylation is stimulated by Ca^{2+}/calmodulin (39). The connection between the kindling results and the PSD hypothesis is that recently it has been found that, in hippocampal membranes of the kindled animals, there occurs a decrease relevant to control, unkindled, animals in a Ca^{2+}/calmodulin-stimulated phosphorylation of proteins of 50, 58, and 60 kDa (60, 61). The molecular weights of these phosphorylated proteins are the same as those of the main proteins in isolated cerebral cortex PSD preparations whose phosphorylations are stimulated by Ca^{2+}/calmodulin (39). In addition, kindling cannot be elicited in the cerebellum (49, 61), and these phosphorylations, and even the 50- to 51-kDa protein, are much reduced in amounts in isolated cerebellar PSDs (7). Again, the 50-kDa protein described in the kindling experiments may be the 50- to 51-kDa protein that is the major protein of cerebral cortex PSDs (3, 62) and that is probably an autophosphorylatable subunit of a Ca^{2+}/calmodulin-activated protein kinase (63–65). Thus, this biochemical result, together with the one mentioned previously in the case of long-term potentiation, seems to implicate PSD proteins in long-term responses of the nervous system.

My main contention is that these biochemical responses reflect, or even antedate, long-lasting, and perhaps irreversible, changes in the structure of the PSD, structure being defined as gross anatomical structure regarding size parameters, as well as the more intimate structure relating the connections between the various proteins of the PSD, which may be as many as 30 proteins or more (3). Furthermore, I contend that for long-term effects to be manifest in the nervous system, biochemical alterations in proteins are not sufficient, but these alterations must lead to changes in structure, in this case, PSD structure. As an example, biochemical changes are manifest during the kindling process, but a structural change becomes manifest only in the final kindled state, for this state can last a long time (49, 57). I would further hold that continued stimulation of specific pathways leads to a change in the structure of the PSD and that this change in structure modifies the shape of the dendritic spine and synapse. It is thus relevant that PSDs isolated by detergent treatments still retain, by and large, their characteristic *in vivo* curvature as seen in material *in situ* (3). Thus, it could be that the characteristic shape of dendritic spines is not due to the properties of the membrane or to the presence of cytoskeletal filamentous material within the spine neck or head, as has been proposed (66), but is due to the characteristic shape of the PSD at the apex of the spine. The change in shape of the PSD could confer a change in shape to the postsynaptic membrane and to the whole dendritic spine, through the proteins attaching the PSD to the synaptic and non-synaptic membranes of the spine. Indeed, a postsynaptic cytoskeleton has been visualized (67), consisting of filaments arising from the synaptic membrane and extending into the interior of the spine; in this context, the PSD can be visualized as being a specialized elaboration of the cytoskeleton, a cytoskeletal "organelle," consisting of the tightly packed proteins mentioned earlier and other proteins presently unknown. If the volume of the spine is determined by its cytoskeletal structure, then any increase in the mass or curvature of the PSD could alter the dimensions of the spine, enlarging the head and constricting the neck.

There have been many papers on changes in synapse architecture after short or long-term conditioning of the animals; many of these are mentioned in ref. 46. Among others of relevance are the papers of Fifkova and Van Harreveld (68, 69) and of Coss and his co-workers (70, 71). On the basis of the work of these and of others, many investigators have correlated changes in the shape of dendritic spines with changes in dendritic conductance [cf. Crick (72) for a theoretical paper]. The hypothesis proposed by Lynch and Baudry (48) envisages changes in the shape of dendritic spines brought about by the biochemical alteration mentioned above, changes that have been observed after long-term stimulation and that concern dendritic spine size (68), PSD length (73), PSD concavity (74), and overall synapse, including PSD, size (75). Thus, I propose that it is changes in the PSD structure that account for the change in spine structure and in synaptic efficacy leading to long-term potentiation. To bolster this argument, there have been repeated reports that stimulation of various sorts results in a change in the structures of dendritic spines, including PSDs, such as an increase in spine number (76), increases in the PSD "web material" (77), swelling of spines (68, 78), shortening of spine stems (79), and an increase in the number of PSDs with perforations (80). Stimulation provided by changes in the animals' environments has also resulted in changes in number of dendritic spines (81–83), synapse size (83), and synapse curvature (84). Specifically, effects on the lengths and thickness of the PSD have been noted in the visual cortex of rats after visual training tasks (83, 85, 86) and in the

suprachiasmatic nucleus of rats after light or dark exposure (87, 88).

All of the above changes could have been brought about by changes in the number and connection of actin filaments in the dendritic spines (75, 89) or, as I propose, in the PSD. The change in structure not only is manifested by alterations in the external parameters of the PSD but also may be brought about by a rearrangement of the proteins within the PSD. The change in phosphorylation of these proteins, particularly the major PSD protein, mentioned above, is a reflection of this alteration, for it could result in a conformational change in this protein, as well as others, exposing a greater or lesser number of sites for phosphorylation to occur, and allowing for a different set of interactions between this protein and other PSD proteins. Changes in the efficacy of transmission at the synapse could occur in two ways: (*i*) an overall change in the length, width, and thickness of the PSD would result in a change in the diameter of the dendritic shaft or spine, leading to changes in conductance of the impulse down the dendrite, according to the cable theory (cf. ref. 90); (*ii*) the altered internal architecture of the PSD would result in a change in the open and closed times of the ion channels in the postsynaptic membranes. The latter thought is based on the idea, mentioned above, that channel and receptor proteins are anchored in the PSD and are connected to other proteins, and these latter proteins could somehow modulate the function of the channel and receptor proteins.

A corollary to this idea is that postsynaptic changes at the PSD level may also influence presynaptic events. This postulation is based on the surmise that the elements of the synapse are bound together by filaments that are anchored in the PSD, traverse the postsynaptic membrane and the synaptic cleft, and impinge on presynaptic elements such as the presynaptic membrane and closely opposed synaptic vesicles (91, 92). The basis for this surmise is the observations (93–95) that in electron micrographs thin filaments can be seen in the synaptic cleft, apparently connecting the pre- and postsynaptic membranes. Another basis is the apparent resistance to disruption of the synapse by physical forces such as shearing during cell homogenization, resulting in the isolation of the synaptic structures (cf. ref. 96). Any changes in the size and curvature of, or protein arrangement in, the PSD could have effects at the presynaptic level, via alterations in the filamentous proteins connecting the two synaptic elements. Thus, modification in PSD structure may influence the release of neurotransmitters from the presynaptic terminal and, if these modifications are long lasting, would result in a "strengthening" or a "weakening" of synaptic transmission at that terminal. A feed-back mechanism can even be envisaged here, since the neurotransmitters released presynaptically bind to postsynaptic receptors, thus influencing the interaction of these receptors with other PSD proteins and, as mentioned above, causing changes in the arrangements of proteins in the PSD. Thus, these reciprocal influences upon pre- and postsynaptic structures may be viewed as a structural correlate of Hebb's postulate (97) that the "strength" of a synapse is determined by the coincidental changes in efficiency of firing of both pre- and postsynaptic elements.

The problem then arises as to how continuous electrical stimulation, or usage, of a pathway can lead to more or less permanent changes in the conductance properties of the state of the pathway, particularly at the postsynaptic level. I have already mentioned that this change in the state of conductance could be brought about by alterations in the architecture of the synapse, particularly of the PSD, so that the problem narrows itself down as to how stimulation of any sort can lead to changes in PSD structure. Three changes that can occur, two of which involve protein synthesis, are as follows: (*i*) changes in overall size, or mass, of the PSD; (*ii*) changes in concentration of certain PSD proteins relative to one another, mass of the PSD being constant; and (*iii*) conformational changes in some PSD proteins. With regards to *i*, it has been found that protein synthesis is necessary for kindling to develop in rats (98), for long-term potentiation to manifest itself (99, 100), and for changes in dendritic spines to result from stimulation (101). It is by now well known that stimulation of certain pathways results in changes in functional activity in those pathways, as visualized by the 2-deoxyglucose method (102). The increased uptake of glucose into cells of these pathways would result in increases in ATP concentration, and, if ATP is limiting, could result in increases in protein synthesis, including that of PSD proteins. The normal turnover of brain protein is quite high, compared to other organs (103), and the increased turnover resultant from stimulation would presumably include the proteins of the PSD. It may be objected that long-lasting changes in PSD mass cannot be maintained in the face of this turnover. However, as in the case of membrane proteins (104), the structure of the PSD can be maintained in spite of the turnover of its individual protein components, particularly if these components are turning over at different rates, which is the case for membrane proteins (104). I should add that an analogous situation occurs in muscle metabolism, in which increased usage, by unknown mechanisms, leads to an increase in muscle mass. The difference between the two cases is that the increase in muscle mass is not spatially discriminate, whereas I visualize the postulated increase in mass of nervous tissue to be limited to the PSD and perhaps other synaptic elements. With regards to *ii*, the differential increased synthesis of some PSD proteins, and not of others, would presumably involve changes in differential mRNA synthesis or in the differential activation of mRNA during the translation process, possibly on polyribosomes. Differential gene activation, leading to mRNA synthesis, may possibly be brought about by the changes in the ionic milieu of the cell resultant from synaptic activity. Regarding translation, it has been found that polyribosomes occur at the base of dendritic spines (105) and their incidence increases during reinnervation of the dendate gyrus (106), and it has been speculated that these polyribosomes are involved in the synthesis of proteins of the dendritic spine or of the PSD and that the synthesis is regulated by functional activity of the synapses (107). It may be visualized that among the proteins whose synthesis rates are increased are the chief PSD protein, the 50- to 51-kDa protein kinase, and the PSD/membrane proteins such as the neurotransmitter receptors and ion channels, which, as mentioned above, seem to be anchored in the PSD. The efficiency of synaptic transmission resulting from the increased concentration of the latter proteins is obvious, while the increase in concentration of the protein kinase may lead to a modification of synaptic strength as explained below.

Finally, with regards to *iii*, it is possible that easily reversible fluctuations in the structures of proteins, brought about by, for example, phosphorylation/dephosphorylation cycles, could eventually lead to long-term irreversible changes in protein structure, particularly if those proteins are bound to others in the immediate vicinity. Such a theory, involving differential turnover of phosphorylated proteins, leading to long-term changes in synaptic strength, has recently been proposed by Crick (27). I would add that, for any long-term effect, the biochemical changes must be translated into structural changes. Thus, the finding that the major structural protein of cortical PSDs is, as mentioned above, a highly insoluble, autophosphorylatable, Ca^{2+}/calmodulin-dependent protein kinase may be quite significant. During the process of long-term continued or intermittent stimulation, the Ca^{2+} concentration within the postsynaptic terminal may remain high, keeping the calmodulin bound to the kinase and increasing its kinase activity, mainly phosphorylating itself.

The resultant highly phosphorylated protein may undergo a conformational change that would be reflected in a change in the overall structure of the PSD. As long as the Ca^{2+}/calmodulin is kept complexed to this kinase, due to the constant infusion of Ca^{2+}, allowing full phosphorylation even in the presence of dephosphorylating enzymes, then this conformational change would persist. Constant stimulation, resulting in such a fluctuation in protein structure, could then result in a long-term modification in a protein, so that it binds to a protein it did not bind to before, or the strength of binding to another protein is decreased or increased, so that its binding to one protein is loosened and it binds to another. This modification could lead to changes in synaptic strength and, if the concentration of a protein, particularly the main PSD protein, is increased, as mentioned above, then the modification could lead to a persistent change in synaptic strength. The tight structure of the PSD, particularly when it is bound to the postsynaptic membrane, contains many protein–protein complexes, such as that of the kinases to their substrates, that of calmodulin to its PSD binding proteins, at least six in number (46), and that of actin (and calmodulin) to fodrin (13, 14). It is interesting that cytosolic calmodulin-dependent protein kinase is a soluble protein (63–65) but when bound onto the PSD structure, becoming the main protein of this structure, it is highly insoluble (3, 62).

Another speculative corollary to the present thesis is the role of the synaptic structure in neuronal development, a development in which it is more and more becoming apparent that there occurs an overproduction of neurons and their connections, and the final wiring of the system is due to the pruning, the neuronal death, of unnecessary cells and their connections. It could be that there is no genetic programming that is responsible for the early death of certain cells, but these cells die because of the weakness of their synaptic structure. That is, the usage of certain pathways during development strengthens the synaptic structure, including the PSD, as postulated above, and it is these neuronal connections that survive. It is as if the very act of neuronal transmission between nerve endings is a "trophic" influence that builds up synaptic structures, and without these, the structures begin to deteriorate, leading somehow to the death of the involved cells. One of the roles of the PSD would then be as an anchor to the synapse; the strength of the synapse is a function of the state of the PSD.

In summary, then, the first thought coming to the mind of a cell biologist viewing the PSD is that it is a structure holding together the various components of the synaptic junctions. This view is strengthened by the discovery that complexing proteins, such as actin, tubulin, and fodrin, which are known to form polymeric structures together with other proteins, are found in the PSD. However, these are also proteins of "movement" and therefore should be involved in the many changes in dendritic spines and PSDs that have been observed after various stimuli have been applied to the CNS. The change in dendritic structure is ascribed to a primary change in the PSD that is a part of that structure. Another link between changes in synaptic conductance and changes in PSD structure is the occurrence in the PSD of proteins and enzymes that are either completely or somewhat singular to the CNS. I think the evidence is overwhelming that short-term changes in synaptic conductance are reflected in changes in synapse structure. The hypothesis proposed here is that these conductance changes, if kept on long enough, result in a change in dendritic, particularly PSD, structure that is probably irreversible and that leads to a change in the pattern of overall CNS conductivity. These long-lasting modifications in PSD structure may be due to changes in concentration of certain PSD proteins or to changes in the activity of these proteins, particularly the main PSD protein, due to a modification of the tertiary structure of this protein. It could be that these changes in PSD and dendritic structure are not continuous but occur by progression from one definite state to another, in "quantum" jumps. The proving of this axiom will not be easy, for neurochemical experiments on isolated PSDs will probably not reveal anything, since the PSD, in the cell, is part of a structure that includes the postsynaptic membrane, probably the spine apparatus and spinule, and possibly the presynaptic membrane as well. Nevertheless, correlative experiments, dealing with *in vivo* manipulations and an *in vitro* examination of resulting possible alterations in PSD structure and composition, can be done and may lead to a result supporting the present hypothesis.

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