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Perforated Synapses and Plasticity

A Developmental Overview

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Contents

Abstract

Against a background of existing models relating perforated synapses to synaptic plasticity, the numerical density and frequency of perforated synapses in rat neocortex have been assessed from 1 d to 22 mo of age using the disector procedure, and changes in their morphology were assessed using 3-D computer reconstructions. The data point toward perforated and nonperforated synapses being separate synaptic populations from early in development, and with perforated synapses playing a part in the maintenance of neuronal postsynaptic density surface area from mid-adulthood onwards. This suggests that they play a crucial role in synaptic plasticity, although its nature may be different fom that postulated by most recent workers.

Index Entries: Synapses; perforated synapses; synaptic development; synaptic plasticity; stereology; 3-D reconstruction; neocortex.

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Introduction

The dynamic nature of synaptic connections has presented morphologists with considerable problems, which, from a structural perspective, has frustrated the development of ideas on synaptic plasticity. The emphasis in this article is on one form of synapse, namely, perforated synapses (PSs), since over the past 20 years, these have become increasingly implicated as intermediates in synaptic remodeling and turnover (Peters and Kaiserman-Abramof, 1969; Carlin and Siekevitz, 1983; Dyson and Jones, 1984; Geinisman et al., 1989). Progress, however, has been erratic because of inadequacies in both qualitative and quantitative morphological procedures (Jones and Calverley, 1991a,b,c), with the result that a diverse array of functional models has come to the fore (*see* Calverley and Jones, 1990c).

At this juncture, it is appropriate to comment on the use of the term perforated synapses, rather than perforated postsynaptic densities (PSDs). It is true that most attention has been focused on the perforation in the PSD, since this is the most obvious feature of these junctions when viewed in transverse section. However, the presynaptic vesicular grid is also perforated, as demonstrated some years ago by Vrensen's group with their studies of E-PTA stained material (Vrensen et al., 1980; Vrensen and Nunes Cardozo, 1981). Consequently, the perforation extends through both pre- and postsynaptic paramembranous densities.

Peters and Kaiserman-Abramof (1969) demonstrated an increase in the number of perforations and also complexity in the larger PSDs, and speculated that the perforations may function to increase the perimeter length of the PSD and also the efficiency of neurotransmission. However, the early studies of this genre date from a study by Greenough et al. (1978). In an environmental complexity paradigm, they noted that rats reared in enriched and socially complex environments (EC and SC) had about 25% more PSs than did rats reared in an impoverished environment (IC); in addition, 90% of synapses with PSD profile lengths larger than 0.25 μm were perforated. Further, the frequency of PSs increased with increasing age up to 60 d postnatal. These workers discussed various interpretations of these data, all of which postulated some involvement of PSs in neuronal activity and pointed perhaps toward a new mechanism of synaptic plasticity.

The next contribution was that of Artyukhina and Ryabinina (1981), who, in demonstrating significant changes following short- and long-term reticular stimulation, noted that short-term stimulation produced a 200% increase in the frequency of PSs, but that long-term stimulation brought about a 73% decrease in frequency. They considered that the increase may have been indicative of the formation of new synaptic active zones within existing terminals by expansion of the active zone, whereas the decrease may have been a result of the merging of individual PSs into a single, but larger, active zone.

A third contribution was that of Vrensen and coworkers, who, in a series of studies in the early 1980s, described *en face* synaptic appearances, some of which had annulate or horseshoe-shaped presynaptic vesicular grids (Vrensen et al., 1980; Müller et al., 1981; Vrensen and Nunes Cardozo, 1981). These are what we would now call PSs. In particular, they noted that these synapses increased in frequency during synaptogenesis; they also noted that in the visual cortex these synapses increased in number and complexity following visual training. They concluded that these and related changes reflected enhanced synaptic efficacy following visual training. Moreover, they constitute part of a normal developmental process that is effected through the "focalization" of simple synapses and the formation of complex grids.

These initial observations were followed by what have become better-known models. The first of these, that of Nieto-Sampedro et al. (1982), is based on a series of studies and incorporates data from previous studies in Cotman's laboratory (Cotman et al., 1973; Matthews et al., 1976; Hoff et al., 1981). When unilateral lesion of the entorhinal cortex was used to induce nonde-

generative synaptic turnover in the inner molecular layer of the ipsilateral dentate gyrus, these workers noted a reciprocal response between numbers of smaller nonperforated synapses (NPSs) and numbers of the larger PSs. This suggested a precursor–product relationship between the two subpopulations. This led to the proposal that PSs were intermediate structures in an ongoing cycle of the breakdown and replacement of synapses, and that this was a normal process involved in the maintenance and adaptive capabilities of the mature CNS.

This resulted in a scheme whereby small, simple NPSs synthesized during the course of synaptogenesis increase in size with time by the addition of PSD material (Nieto-Sampedro et al., 1982). As the PSD enlarges, perforations form and subsequently enlarge, causing the PSD to weaken and distort in configuration. Perforations may also form in grooves produced by this distortion, the deepening of which will produce regions of reentry in the PSD (sometimes referred to as "tears" or "rips"). The final breakdown of the PS may give rise to several PSD fragments, one or more of which may in turn give rise to a new simple synapse. Alternatively, the disassembled PSD material may be reassembled into new PSDs. Hoff and Cotman (1982) referred to this scheme as the "conversion-disassembly" hypothesis.

The second model is that of Carlin and Siekevitz (1983). After reviewing the literature, they proposed a multi-step mechanism in which PSs appeared as intermediate structures in a process giving rise to an increase in synaptic number. They proposed that various types of repetitive stimulation caused small, simple synapses to increase in size, possibly by the addition of material from the spine apparatus. At some optimal size, a perforation developed in the paramembranous densities. As the synapse enlarged, a spinule formed at the perforation site, with membrane again being contributed by the spine apparatus. As the perforation increased in size, the paramembranous densities assumed first a horseshoe shape and then a dumbbell shape, which, as the synapse continued to increase in size, split into two discrete synaptic junctions within the same terminal. The dendritic spine then either split in two or relaxed into the dendritic shaft to produce two new, discrete small, simple synapses. Evidence was adduced for most of these steps, although none was provided in support of the hypothesis that the divided synapse can be transformed into two separate axospinous synapses.

A third model is that of Dyson and Jones (1984). After examining a range of developmental features, in both simple and multiple synapses, including changes in the morphology and size of the PSD and dense projections (DPs), synaptic curvature, and relative position of the spine apparatus, they proposed a splitting or merging model for the remodeling of synapses, in which PSs were given a prominent role. In the "splitting" proposal, the active zone of a mature NPS was initially enlarged by the addition of a less specialized, negatively curved region of paramembranous density, possibly involving the spine apparatus. With further enlargement of the synaptic contact zone (SCZ), it was proposed that a point was reached when the two PSD segments of the active zone separated, giving rise to a perforation. The spinule, which is frequently observed at the perforation, was considered to be implicated in the addition of new membranous material. In the final stage, division of the pre- or postsynaptic terminal occurred, resulting in the formation of one of the various types of multiple junction. The "merging" or coalescence model is the converse of this scheme, whereby small NPSs merge via a multifocal PS intermediate to form a single, large PS. However, the data presented tended to support the splitting concept.

More recently, Petit (1988) has presented a mechanism of synaptic plasticity to account for learning and memory, and involving the formation of PSs. His scheme includes a series of events resulting from the activation of synapses. These involve: entrance of calcium into the pre- and postsynaptic terminals; production of new synaptic proteins and enlargement of the synapse and spine head; activation of the respective

cytoskeletal networks, a change in synaptic curvature, and a widening and shortening of the spine neck; formation of a perforation; division of the synaptic active zone, changes in dendritic shape, and formation of new dendritic spines; and the addition of new dendritic material. Integral to these events is the notion that an intrinsic mechanism within the synapse induces splitting of the synaptic active zone, once a certain maximal size has been attained. Implicit within this is the further idea that the mechanism controlling this splitting involves communication between the pre- and postsynaptic terminals.

In the present series of studies, both PSs and NPSs in rat neocortex have been quantified using the disector procedure at various ages ranging from 1 d to to 22 mo, in an attempt to provide a stronger factual basis for concepts of synaptic plasticity. Considerable emphasis has also been placed on 3-D reconstructions of these synapses, in order to follow any morphological changes throughout their life history. The data obtained enable various critical comments to be made on existing plasticity models and constitute the basis of an alternative scheme.

Methods

Parietal cortex from six rats at each of the following ages: 1, 4, 7, 14, and 21 d, and 1, 2, 4, 7, 10, 12, 16, 19, and 22 mo was prepared for electron microscopy. Details of the procedures used are described in detail in Calverley and Jones (1990a,b).

Stereology

A section was randomly selected from each complete ribbon of sections and examined in the electron microscope. An area of the section was located at random, subjacent to the pial surface, and photographed by taking a series of overlapping photographs at a nominal magnification of about 3650×; the same area of tissue in an adjacent section was also photographed in the same manner. All negatives were printed to give a final magnification of 23,700× and a photomontage, with an area of about 600 μm^2, assembled for each section area

A square test grid, with an area equivalent to about 400 μm^2, was randomly positioned on each photomontage. The area that it enclosed was outlined, and the exclusion/inclusion edges indicated. All synaptic profiles lying within each test area, and not intersected by one or both of the two exclusion edges, were identified using Gundersen's unbiased counting rule.

The numerical densities of NPSs (N_{VNPS}), PSs (N_{VPS}), and all synapses (NVs) were estimated for each animal using the disector method (Sterio, 1984). The general formula used was:

$$N_v = \frac{(Q^-}{t \bullet A)}$$

where Q^- = the number of synaptic profiles (Q^-_{NPS}, Q^-_{PS}, Q^-_S) present in the test area of each section, but not in the adjacent section, A = test area studied, and t = mean section thickness. Since there is no way of directly estimating the number of NPSs (Q^-_{NPS}), this quantity was estimated by subtracting Q^-_{PS} from Q^-_S. In order to increase the efficiency of the estimate, the order of each pair of sections was reversed, so that the test section became the look-up section and vice versa, and the procedure was repeated using the new test area. The N_V estimates obtained for each animal were averaged.

The frequency of PSs (%PS) was calculated using the formula:

$$\%PS = \frac{(Q^-_{PS}}{Q^-_S)} \bullet 100$$

where Q^-_{PS} = the number of profiles of PSs present in the study area of the reference section but not in the adjacent look-up section, and Q^-_S = the number of all synaptic profiles present in the test area, but not in the adjacent look-up section. The %PS estimates obtained for each animal were averaged.

Three-Dimensional Reconstruction

The reconstruction package was based on the Apple Macintosh Plus, and consisted of two principal programs for data acquisition and image reconstruction. Suitable synaptic series were marked for reconstruction; this produced synapses for each age group.

The membrane outline of the pre- and postsynaptic terminals for each synapse in the series was traced around using a digitizing tablet and cursor. The PSD and nonPSD zones were indicated by tracing over the postsynaptic terminal outline, the dense and light labels, respectively. Together, these indicated the complete synaptic contact zone, with the PSD appearing as the black zones and the nonPSD parts of the SCZ as the stippled regions (Figs. 1–6). Only the postsynaptic terminal was reconstructed, since it contains the primary structure of interest, the PSD, and also reflects the spatial configuration of the SCZ of the presynaptic terminal. In order to produce the clearest possible reconstruction of the postsynaptic terminal and PSD, the angles of rotation were individually selected for each connection. *En face* views of the PSD and SCZ were also produced. These views are orthogonal projections of the total SCZ of the postsynaptic terminal.

Reconstruction data were collected from the reconstructions themselves and the corresponding *en face* views of the SCZ.

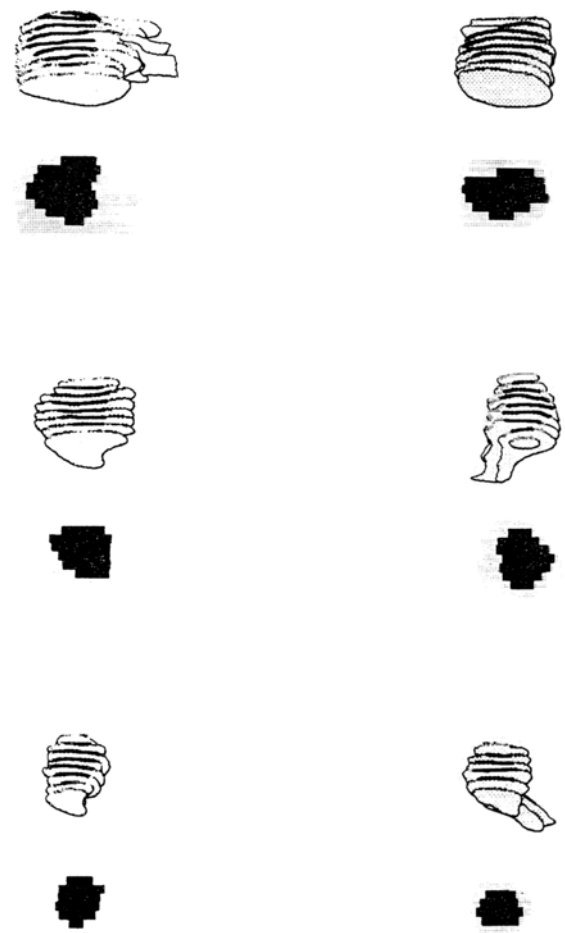

Fig. 1. Reconstructions of the postsynaptic terminals of six typical nonperforated synapses from molecular layer of 12-mo-old rat parietal cortex. An *en face* view of the postsynaptic density (dark area) and nonpostsynaptic density (light stippled area) zones of the synaptic contact zone is placed under the corresponding 3-D reconstruction of each of the synapses. The reconstructions are arranged approximately in order of decreasing size and complexity of the postsynaptic density.

Results

Perforated Synapses During Later Development and Maturation

Quantitative Stereology

Between 0.5–22 mo of age, there is a significant decrease in the numerical density of all synapses and also of NPSs, but an increase in the numerical density and frequency of PSs (Jones and Calverley, 1991a,b). These changes represent an 11% decrease in total synaptic density, a 19% decrease in that of NPSs, and a 29% increase in that of PSs. There is a large increase in the density of all synapses between 0.5–1 mo, followed by a decline to 22 mo. The NPS population shows a similar trend, its density doubling between 0.5–1 mo, followed by a halving to 4 mo, with

Fig. 2. Reconstructions of the postsynaptic terminals of perforated synapses at 1 mo.

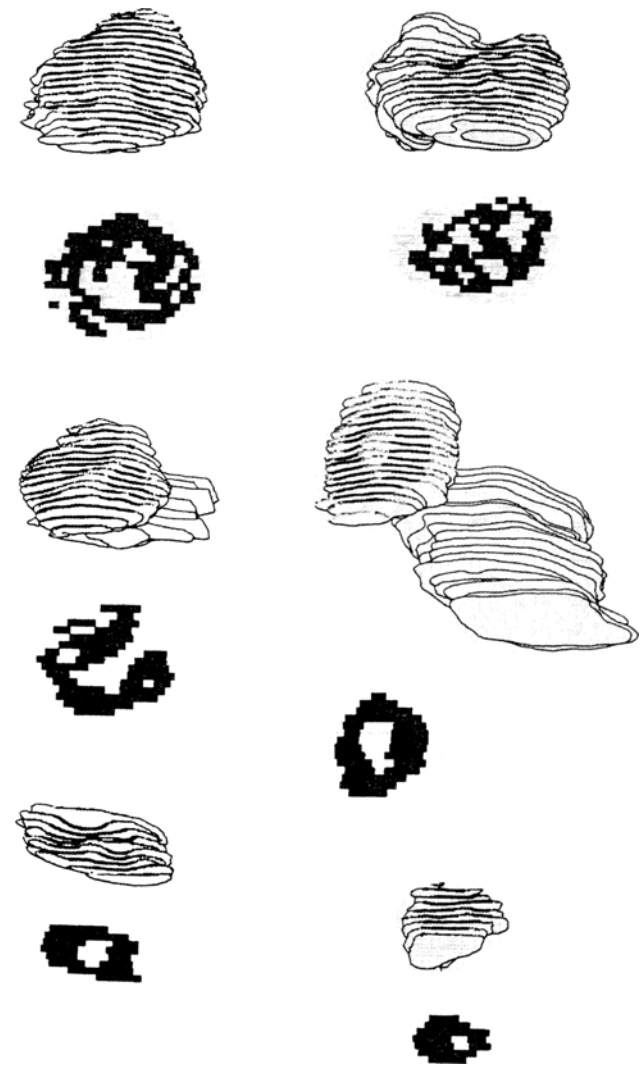

Fig. 3. Reconstructions of the postsynaptic terminals of perforated synapses at 10 mo.

some fluctuation about a gradual decline to 22 mo. In the case of the PS population, there is a steady increase in density to 12 mo, followed by a decline to 22 mo. Care has to be taken with data such as these, since because they refer to unit volume and because the reference space changes with chronological age, they cannot be legitimately compared over the whole of the age range studied (Jones and Calverley, 1991b).

Nevertheless, these fluctuations are reflected in estimates of the frequency of PSs within the total population, a parameter that is not similarly affected. There is a decrease in PSs from 18% at 0.5 mo to 13% at 1 mo, and then a tripling to 37% at 10 mo. Between 10–19 mo, the frequency is fairly steady around 34%, with a decrease to 27% at 22 mo (Jones and Calverley, 1991a,b). What is striking about these figures is the marked pres-

Fig. 4. Reconstructions of the postsynaptic terminals of perforated synapses at 19 mo.

ence of PSs relatively early in life, that is, at 0.5 mo, a level that would not have been anticipated by the splitting model of Dyson and Jones (1984).

Three-Dimensional Reconstructions

When NPSs are examined (Fig. 1), few differences are evident at the different ages; they are relatively small and have a simple configuration. They are devoid of a perforation (Jones and Calverley, 1991a,c). The NPSs from 12-mo-old material in Fig. 1 are typical of those found at 0.5 and 22 mo of age.

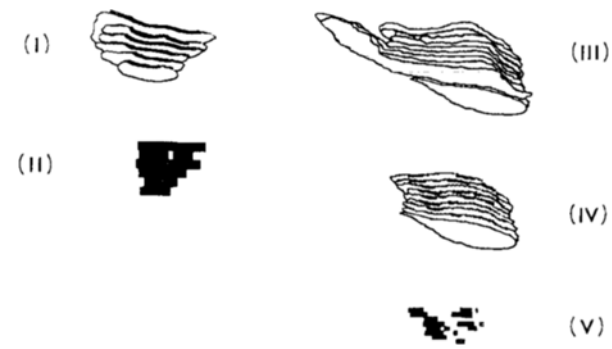

Fig. 5. Reconstructions of the postsynaptic terminals of two perforated synapses at 1 d. The presynaptic terminal of one of the synapses is also shown in (iii). *En face* views of the PSD and nonPSD zones of the SCZ are shown in (ii) and (v).

By contrast, the reconstructions of the postsynaptic terminals of PSs are markedly different and also vary among themselves, particularly with increasing age (Figs. 2–4). At 0.5 and 1 mo of age, the synapses are relatively small by PS standards and are relatively simple, the junctional region overall has a negative curvature (concave with respect to the presynaptic terminal; upper left reconstruction in Fig. 2), and there is some elevation of the postsynaptic membrane to form a small spinule at some of the perforations (especially in the upper right reconstruction in Fig. 2). *En face* views at these early ages show that the smaller PSs generally have single perforations, with the larger ones having a few perforations, some of which open to the SCZ (Fig. 2). Some of these correspond to the earlier descriptions of doughnut-shaped and horseshoe-shaped synaptic grids in the presynaptic terminals (Vrensen et al., 1980). Most of these PSs are axospinous, being located on the side of the head of dendritic spines, their most typical location. The negativity of the synaptic region is localized to the head, and does not encroach on the neck of the spines.

Between 4–12 mo of age, PSs are larger, and their organization is characterized by the apparent break-up of the PSD (Fig. 3). Although the smaller synapses have perforations that are

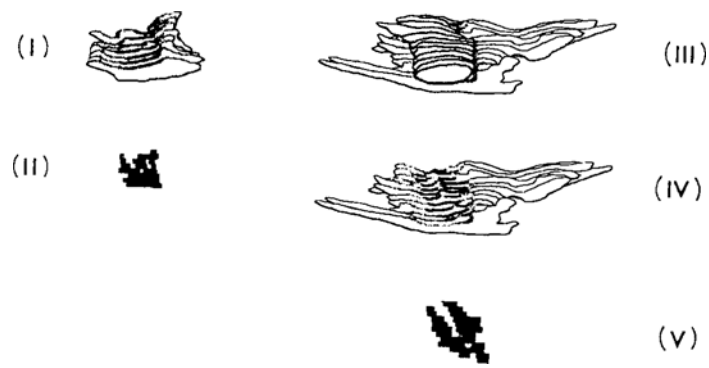

Fig. 6. Reconstructions of the postsynaptic terminals of two perforated synapses at 4 d, with the presynaptic terminal being included in

simple and discrete, the perforations themselves are fairly extensive. In the larger synapses, the perforations give the appearance of being multiple or branched. Some of the perforations are large and dominate the junctional region. At these ages, relatively large spinules can be seen at many of the perforations (lower left and upper right reconstructions in Fig. 3), indicating that the postsynaptic membrane is projecting well into the presynaptic terminal at these sites. By these ages, the junctional regions are positively curved (convex with respect to the presynaptic terminal; both middle reconstructions in Fig. 3), as opposed to the definite negative curvatures of much younger terminals (upper left reconstruction in Fig. 2). The corresponding *en face* appearances highlight the complexity of the perforations (Fig. 3). Many of the terminals have at least five branched perforations, some of which are very extensive and open to the SCZ (the stippled region around the black PSD). These are sometimes referred to as reentrant perforations.

At the older ages studied (16–22 mo), the postsynaptic junctional regions have become very large (Fig. 4). They are characterized more by SCZ areas than PSD. In other words, the PSD components appear to occupy much less of the SCZ than they did at younger ages (Fig. 2), and much less than in NPSs at any age (Fig. 1). The spinule-like appearances seen earlier are not usually as obvious, although there is one exception in Fig. 4 (right middle reconstruction). The PSD gives the impression of having been exploded, and the large perforations are frequently coextensive with the SCZ outside the PSD environs. A noticeable feature of reconstructions at the older ages is their variability. Although the largest display exploding features, a few are remarkably small (the reconstructions at the bottom) and appear to have the features typical of much younger terminals (although even here the perforations are more complex). This raises the possibility that such terminals as these may have been produced relatively recently and that they may indeed represent an earlier stage of the maturity-aging sequence of PSs.

Using the measurements option in the reconstruction program, it can be demonstrated that the PS population is characterized by increases with age in the presynaptic and postsynaptic terminal volumes, the surface areas of the PSD and nonPSD, and the surface area of the synaptic contact zone. In contrast, the corresponding NPS parameters remain relatively unchanged over this age range (Jones and Calverley, 1991a,c). In addition, the percentage of SCZ surface area occupied by the PSD decreases with increasing age in PSs, but increases in NPSs. This suggests that PSs make less use of the available SCZ area with increasing age than do NPSs. Also, the total PSD surface area of PSs/mm^3 of molecular layer increases over the age range studied, with very high

figures at 10 and 12 mo of age. The same parameter for NPSs decreases over the period studied. This suggests that PSs contribute more to the total amount of PSD surface area per unit volume of tissue in mid- to late-adulthood than do NPSs, despite the fact that NPSs outnumber PSs by between 2:1 and 3:1 at these ages (Jones and Calverley, 1991a,c).

Perforated Synapses During Early Synaptogenesis

In a study of the numerical density and frequency of PSs between 7–60 d postnatal in rat neocortex (Itarat and Jones, 1991), it emerges that PS frequency increases from 11.98% at 7 d to 33.41% at 60 d, with figures of 17.32% and 23.23% at 14 and 21 d, respectively. These figures overlap those of our previous study on older synapses (Jones and Calverley, 1991b) and suggest that there is an overshoot in the production of PSs, which occurs later than does that of NPSs.

Three-dimensional reconstructions of PSs at 1 and 4 d postnatal reveal configurations having much in common with the 0.5- and 1-mo material of the previous study (Jones and Calverley, 1991a,c). At 1 d, two types of PSs appear to be present (Fig. 5), one with a small discrete perforation (Fig. 5 i,ii), and the other with a perforation extending through the whole extent of the PSD and appearing to divide it into two or more separate components (Fig. 5 iii–v). This latter type is unlike any previously observed PSs at older ages. The negativity of these young PSs is sometimes well displayed. At 4 d, the PSD of PSs is small and compact, with discrete perforations or with perforations coextensive with the SCZ (Fig. 6).

Discussion

The rationale of the 0.5–22 mo study was to obtain data that would throw light on the splitting hypothesis, whereby NPSs enlarge and split to form two new daughter synapses (Carlin and Siekevitz, 1983; Dyson and Jones, 1984). If this splitting hypothesis is tenable, one would expect to find morphological evidence of synaptic splitting and a rapid drop-off in the frequency of PSs at younger ages, since the assumption has been that PSs are implicated in synaptic plasticity in the postdevelopmental nervous system.

Although large numbers of synapses were examined in serial sections at each of the nine ages in the study, no evidence was found in support of either the splitting or merging models. This is not conclusive evidence against these models, since intermediate forms may exist for short periods of time and may therefore be missed by such a static procedure as electron microscopy. Nevertheless, we are struck by the lack of double-headed spines, axospinous NPSs lying adjacent to one another, and spinules completely traversing the presynaptic terminals of PSs. This is not to argue that such forms do not exist; after all, it was their existence that led in part to the Carlin and Siekevitz (1983) hypothesis, whereas more recently, Geinisman et al. (1989) have described PSs on double-headed spines, in which each is contacted by different axons presynaptically. However, as in this latter study, they probably constitute a very small proportion of the population and may be of limited functional significance.

From 2 wk of age onwards, the frequency of PSs did not fall below 13.5% (Jones and Calverley, 1991b). This was a much higher frequency than had been predicted if PSs were derived entirely from NPSs and if such a transformation were a postdevelopmental phenomenon. It is true that the frequency of PSs increased with increasing age (up to 10 mo), yet at no point in the study did they become negligible in number. From this it may be concluded that few (and perhaps none) of the PSs are formed by splitting from NPSs. They may originate as PSs. It was in order to test this hypothesis that the earlier (1–60 d) study was undertaken. This confirmed the idea that PSs are relatively common (12%) as early as 7 d of age (the earliest age at which quantitation has been performed), and can readily be found at 1 d of age and even at 20 d of gestation (Itarat and Jones,

1991). Further, surprisingly high figures are obtained for PS frequency between 21–60 d postnatal, suggesting that they, too, may demonstrate a developmental overshoot phenomenon.

Taken together, these qualitative and quantitative data militate against the splitting hypothesis, at least as originally conceived. Nevertheless, this should not be taken as an argument against the involvement of PSs in synaptic plasticity. The dominant outcome of the 3-D reconstruction studies is that of the variability of the form of the PSD of PSs (Figs. 2–6). This is far more complex than suggested by such notions as fragmented, annular, notched, or horseshoe-shaped PSDs. These are little more than partial hints of its complex structure. PSs are extremely plastic structures that, with increasing age, are capable of modifying their size, number, shape, and spatial complexity (Jones and Calverley, 1991c). What still awaits resolution is whether the increasing complexity of their PSDs is age- or size-related. At each age, there is a hint that the larger PSs are more complex than the smaller ones (Figs. 2–6). Notwithstanding, this possibility, it has been clearly shown that PSs enlarge in size with increasing age (Figs. 2–4; Jones and Calverley, 1991c).

Much more work remains to be done on the young PSs. However, it is interesting that, in the 1-d synapses (Fig. 5), there are indications of two types—a typical PS with small perforation and a subdivided PS. The latter may indicate that PSs originate from two nascent PSDs, before assuming the typical PS form. Alternatively, this variation in type may be another indicator of synaptic plasticity during development.

The increase in PS frequency in response to stimulation (e.g., Müller et al., 1981; Vrensen and Nunes Cardozo, 1981) has not been investigated in the studies reported here. By themselves, the stimulation studies do not argue for a transformation of NPSs into PSs, since if PSs could be formed *ab initio*, this would not be required. The complexity of large PSs in the adult CNS should also prompt a reexamination of them as a group of interrelated, small, active zones, rather than as a single large entity.

Putting together the various strands of data on PSs, we would argue that, not only should PSs and NPSs be viewed as separate synaptic populations, but PSs are responsible for the maintenance of total synaptic efficacy. This is based on two sorts of data: the density and size of the respective synaptic populations, and the maintenance of PSD surface area. Up to 12 mo of age, the decrease in the density of NPSs is partially compensated by an increase in the density of PSs; after 12 mo, the densities of both NPSs and PSs decrease, but there is an increase in size of the remaining PSs. The total surface density of PSDs is maintained at older ages by the increase in this parameter in the PS population (Jones and Calverley, 1991a,b,c). We also wish to argue that the maintenance of synaptic efficacy by PSs is a consequence of the decrease in NPS density. This may be accomplished by an increase in the PSD surface area of existing PSs and the *de novo* synthesis of new PSs.

One proposal that may be put forward is that new synaptic contact zone material is added to the perforation site of small perforated synapses, and as this increases in amount, a spinule is formed to accommodate this excess membrane (Jones and Calverley, 1991a). As space adjacent to the enlarging synapse becomes available through changes in the size, shape, and number of adjacent cells, the spinule relaxes into the preexisting synaptic contact zone. In this manner, new synaptic material is incorporated into the contact zone, providing the opportunity for new PSD material to be added to the enlarging PS. The PSDs also become increasingly fragmented, a characteristic of PSs in older material. We suggest that this series of events is a means of maintaining the total PSD surface area of neurons available for neurotransmis-sion with increasing age and, hence, is basic to ongoing synaptic plasticity.

Summary

Against a background of existing models relating perforated synapses to synaptic plasticity, the numerical density and frequency of per-

forated synapses in rat neocortex have been assessed from 7 d to 22 mo of age, using the disector procedure. The morphology of the postsynaptic terminals of these synapses has also been analyzed in 3-D computer reconstructions from 1 d of age. Perforated synapses are relatively frequent, reaching a peak of 37% at 10 mo of age. Even at 7 d of age, 12% of synapses are perforated, and levels in excess of 30% are found at a number of other ages. With increasing age, perforated synapses increase in size and complexity, the increasing complexity being reflected in a break-up of the postsynaptic density, which is punctuated by larger, branched perforations. These and related features point toward perforated and nonperforated synapses being separate synaptic populations from early in development, and that perforated synapses play a part in the maintenance of neuronal postsynaptic density surface area from mid-adulthood onwards. This suggests that perforated synapses play a crucial role in synaptic plasticity, although the nature of this role may be different from that postulated by most recent workers.

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References

Artyukhina N. I. and Ryabinina M. A. (1981) Ultrastructural changes in synapses of the rabbit sensorimotor cortex during stimulation of the reticular formation. *Neurosci. Behav. Physiol.* **10,** 438–445.

Calverley R. K. S. and Jones D. G. (1990a) Three-dimensional computer reconstruction of perforated synapses, in *Methods in Neurosciences,* vol. 3, Conn P. M., ed., Academic, Orlando, FL, pp. 136–154.

Calverley R. K. S. and Jones D. G. (1990b) Determination of the numerical density of perforated and nonperforated synapses. *Methods in Neurosciences,* vol. 3. Conn P. M., ed., Academic, Orlando, FL, pp. 155–172.

Calverley R. K. S. and Jones D. G. (1990c) Contributions of dendritic spines and perforated synapses to synaptic plasticity. *Brain Res. Rev.* **15,** 215–249.

Carlin R. K. and Siekevitz P. (1983) Plasticity in the central nervous system: do synapses divide? *Proc. Nat. Acad. Sci. USA* **80,** 3517–3521.

Cotman C., Taylor D., and Lynch G. (1973) Ultrastructural changes in synapses in the dentate gyrus of the rat during development. *Brain Res.* **63,** 205–213.

Dyson S. E. and Jones D. G. (1984) Synaptic remodeling during development and maturation: junction differentiation and splitting as a mechanism for modifying connectivity. *Develop. Brain Res.* **13,** 125–137.

Geinisman Y., Morrell F., and De Toledo-Morrell L. (1989) Perforated synapses on doubleheaded dendritic spines: a possible structural substrate of synaptic plasticity. *Brain Res.* **480,** 326–329.

Greenough W. T., West R. W., and De Voogd T. J. (1978) Subsynaptic plate perforations: changes with age and experience in the rat. *Science* **202,** 1096–1098.

Hoff S. F. and Cotman C. W. (1982) A continuous conversion-disassembly hypothesis for synapse turnover: a quantitative serial section study. *Soc. Neurosci. Abst.* **8,** 745.

Hoff S. F., Scheff S. W., Kwan A.Y., and Cotman C.W. (1981) A new type of lesion-induced synaptogenesis: I. Synaptic turnover in non-denervated zones of the dentate gyrus in young adult rats. *Brain Res.* **222,** 1–13.

Itarat W. and Jones D. G. (1991) Perforated synapses are present during synaptogenesis in rat neocortex. *Proceedings of the Combined Meeting of Neuroscience Society and Anatomical Society,* Dunedin, pp. 26–27.

Jones D. G. and Calverley R. K. S. (1991a) Changes of synapses with age: The respective contributions of nonperforated and perforated synapses in neocortex. *Development and Involution of Neurons.* Fujisawa K. and Morimatsu Y., eds., Japan Scientific Societies Press, Tokyo.

Jones D. G. and Calverley R. K. S. (1991b) Frequency of occurrence of perforated synapses in developing rat neocortex. *Neurosci. Lett.* **129,** 189–192.

Jones D. G. and Calverley R. K.S . (1991c) Perforated and non-perforated synapses in rat neocortex: three-dimensional reconstructions. *Brain Res.* **556,** 247–258.

Matthews D. A., Cotman C.W., and Lynch G. (1976) An electron microscopic study of lesion-induced

synaptogenesis in the dentate gyrus of the adult rat. I. Magnitude and time course of degeneration. *Brain Res.* **115,** 1–21.

Müller L., Pattisellano A., and Vrensen G. (1981) The postnatal development of the presynaptic grid in the visual cortex of rabbits and the effect of dark-rearing. *Brain Res.* **205,** 39–48.

Nieto-Sampedro M., Hoff S. F., and Cotman C. W. (1982) Perforated postsynaptic densities: probable intermediates in synapse turnover. *Proc. Natl. Acad. Sci. USA* **79,** 5718–5722.

Peters A. and Kaiserman-Abramof I. R. (1969) The small pyramidal neuron of the rat cerebral cortex. The synapses upon dendritic spines, *Z. Zellforsch.* **100,** 487–506.

Petit T. L. (1988) Synaptic plasticity and the structural basis of learning and memory. *Neurology and Neurobiology. Neural Plasticity: A Lifetime Approach,* vol. 36. Petit T. L. and Ivy G. O., eds., Liss, New York, pp. 201–234.

Sterio D. C. (1984) The unbiased estimation of the number and sizes of arbitrary particles using the disector. *J. Microsc.* **134,** 127–136.

Vrensen G. and Nunes Cardozo J. (1981) Changes in size and shape of synaptic connections after visual training: an ultrastructural approach of synaptic plasticity. *Brain Res.* **218,** 79–97.

Vrensen G., Nunes Cardozo J., Müller L., and Van Der Want J. (1980) The presynaptic grid: a new approach. *Brain Res.* **184,** 23–40.