0270-6474/82/0203-0284$02.00/0

The Journal of Neuroscience
Vol. 2, No. 3, pp. 284-291
March 1982

PREFERENTIAL LOCALIZATION OF POLYRIBOSOMES UNDER THE BASE OF DENDRITIC SPINES IN GRANULE CELLS OF THE DENTATE GYRUS[1]

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Received August 10, 1981; Revised October 29, 1981; Accepted November 6, 1981

Abstract

Electron microscopic studies of the dentate gyrus of the rat have revealed an apparent association between polyribosomes and dendritic spines. The present study was designed to elucidate the nature of this association. Our qualitative observations revealed that polyribosomes appeared primarily in two locations within the dendrite: (*1*) beneath the base of identified spines just subjacent to the intersection of the spine neck with the main dendritic shaft and (*2*) beneath mounds in the dendritic membrane which had the appearance of the base of a spine which extended out of the plane of section. To quantitatively define the nature of the apparent association, we attempted to determine (*1*) the proportion of spines with associated polyribosomes and (*2*) the proportion of the polyribosomes within dendrites which are associated with spine bases. Evaluation of profiles which were identifiable as spine neck-dendritic shaft intersections in a single section revealed that an average of 12.2% had associated polyribosomes. A serial section analysis revealed a somewhat higher incidence, however. Of a collection of 34 through-sectioned spines, 29% had polyribosomes which were revealed in one or more of the sections comprising the series. To evaluate what proportion of polyribosomes within the dendrite was associated with spines, we evaluated a series of photographs covering approximately 1250 μm^2 of the dentate molecular layer from five animals, identifying all polyribosomes within dendrites and scoring their location as being (*1*) under spines, (*2*) under mounds, or (*3*) other. An average of 9.6% of the polyribosomes were found under processes identifiable as spine neck-dendritic shaft intersections, while 71.4% of the polyribosomes were found under mounds. Only 19% were not obviously associated with spines or mounds. Spine bases and mounds comprise only 3 and 35%, respectively, of the outline of dendritic profiles, however, indicating that the high incidence of polyribosomes under these elements cannot be accounted for by chance. To attempt to determine whether the mounds represent the base of dendritic spines, 68 mounds in 21 dendritic profiles were selected from the middle of the series of 20 serial sections. Nineteen of these mounds (28%) were continuous with an identified spine, and an additional 31% were continuous with thin processes of the size and appearance of spine necks. Thus, most of the mounds probably do represent the base of spines which extend out of the plane of a single section.

One of the major tenets of modern neurobiology is that the bulk of the protein synthetic machinery of a neuron (polyribosomes and rough endoplasmic reticulum) is concentrated in the neuronal cell body (see Peters et al., 1976). The intricate and detailed morphological specializations of the neuron (axons, dendrites, and synapses) are thought to depend on the specific and highly targeted transport of already synthesized protein from the neuronal soma (Grafstein and Forman, 1980). This concept is difficult to reconcile with some of the known and supposed properties of CNS neurons. For example, most neurons of the mammalian CNS maintain thousands of specialized receptive sites on their surface. These sites are contacted by different classes of synapses, which presumably often utilize different transmitter types. Yet neurons maintain specialized zones at considerable distances from their cell body and are apparently capable of selectively modifying connections in response to a variety of signals including denervation and patterns of activity. It would seem likely that both maintenance and modifi-

[1] We thank Ms. Sharon L. Vinsant for her excellent technical help. This work was supported by National Institutes of Health Grants 5 R01 NS12333 (to O. S.) and 5 R01 NS15488 (to W. B. L.). O. S. is the recipient of Research Career Development Award 1 K04 NS00325.

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cation would require differing amounts of precursor proteins, depending upon local requirements at the synapse. How such local regulation could occur is currently a matter of speculation. For example, synaptic constituents could be derived from a generally available pool of proteins which was available throughout the neuron. This would seem very inefficient, however, since it would require the synthesis and distribution of excess protein in order to provide sufficient quantities at a local level when these proteins were required. A more efficient mechanism would involve a synthesis regulated by need. However, if all protein synthesis occurred at the cell body, it is difficult to envision how distant synaptic sites could signal their needs and how necessary synaptic constituents could be transported back to the appropriate sites. A regulation of synthesis of synaptic constituents by individual synapses could be envisioned, however, if the machinery for protein synthesis was actually located at or near the synapse.

Because of these considerations, we were particularly interested when our studies of cellular events during post-lesion reinnervation of the granule cells of the dentate gyrus revealed dramatic increases in incorporation of protein precursors in regions of the neuropil distant from neuronal somata (Fass and Steward, 1981; B. Fass and O. Steward, manuscript in preparation). Electron microscopic studies investigating what elements might be responsible for the incorporation revealed polyribosomal rosettes associated with dendritic spines. These clusters of polyribosomes were found in a stereotyped location at the base of spines, lying just subjacent to the intersection of the spine neck with the main dendritic shaft. The present paper provides the initial description of this association and seeks to determine how close the association actually is between spines and polyribosomes. Our results suggest that (*1*) a relatively large proportion of spines has associated polyribosomal clusters and (*2*) the vast majority of the polyribosomes in dendrites appear to be associated with dendritic spines rather than being randomly distributed within the dendrite.

Materials and Methods

All animals were male Sprague-Dawley-derived rats. Five of the six animals were young adults, ranging in weight from 300 to 450 gm, and the sixth was approximately 10 months old. Our discovery of the association between polyribosomes and spines arose as a consequence of our electron microscopic studies of the reinnervation of the dentate gyrus following unilateral entorhinal lesions. Thus, the material analyzed in the present study was control material (the dentate gyrus contralateral to the lesion) from animals which had survived unilateral destruction of the entorhinal cortex for 4, 10, 12, 14, and approximately 200 days prior to sacrifice. One of the five young adult rats was prepared for a serial section analysis of changes in spine shape following the induction of long term potentiation (W. B. Levy, research in progress). The animal was prepared for acute electrophysiological recording (see Levy and Steward, 1979) and was perfused for electron microscopy after inducing long term potentiation in the entorhinal-dentate system. The serial sections analyzed in the present study were derived from the control side of this animal, contralateral to the potentiating stimulation.

It should be noted that there is a very sparse crossed projection from one entorhinal cortex to the contralateral dentate gyrus (Goldowitz et al., 1975). While our electron microscopic studies suggest that this crossed projection contributes no more than 5% of the synapses in the entorhinal terminal zone, the control material analyzed in the present study may not be entirely normal. However, the sparseness of the projection and the consistency of our results in all animals of the present study despite considerable differences in post-lesion survival give us confidence that the material which we have analyzed differs little, if at all, from normal material.

Animals were perfused while deeply anesthetized with sodium pentobarbital with a solution of 2% paraformaldehyde, 2% glutaraldehyde in 0.13 M sodium cacodylate buffer (pH 7.2). The brains were removed, postfixed in the perfusion solution for several hours, and then sectioned coronally at 150 to 300 μm either with a hand-held razor blade or with the aid of an Oxford Vibratome. From the rostral portion of the dentate gyrus, a segment of the ventral blade extending from the layer of granule cell bodies to the distal tips of the dendrites was dissected from both sides of the brain. These blocks were osmicated for 45 min and embedded in Epon/Araldite with routine procedures. Thin (approximately 600-Å) sections were cut with the aid of an LKB ultramicrotome, stained with uranyl acetate, and photographed with a Hitachi HU12A electron microscope. The sampling procedures are described more fully below.

Results

Qualitative observations. As is the case with most neurons of the mammalian CNS, the highest concentration of ribosomes is found in the somata of dentate granule cells. In comparison with many other types of neurons, the cytoplasm of the granule cells is not complex but still contains large numbers of ribosomes collected into classical rosette formations (polyribosomes, see Fig. 1*A*). Relatively little rough endoplasmic reticulum is evident in these somata compared to the number of polyribosomal rosettes.

In contrast to the relatively high concentrations in cell bodies, dendrites contain relatively few ribosomes. Nevertheless, clusters of polyribosomes can be found in granule cell dendrites as is the case with most other neurons (Peters et al., 1976), and these tend to decrease in frequency with distance from the soma. What caught our attention about the polyribosomes in granule cell dendrites was their frequent association with the necks of dendritic spines. For example, when the intersection of a spine neck with a main dendritic shaft was visible in a section, polyribosomal rosettes frequently could be seen just subjacent to the intersection (see Fig. 1, *B* and *C*). In dendritic zones relatively proximal to the soma, the collection of polyribosomes was often quite dramatic (Fig. 1*B*). Further distally, the polyribosomes appeared as single rosettes, containing about six ribosomes in any single section. Figure 1*C* illustrates a typical profile from a mid proximodistal location along the dendrite. These collections of polyribosomes were all the more striking

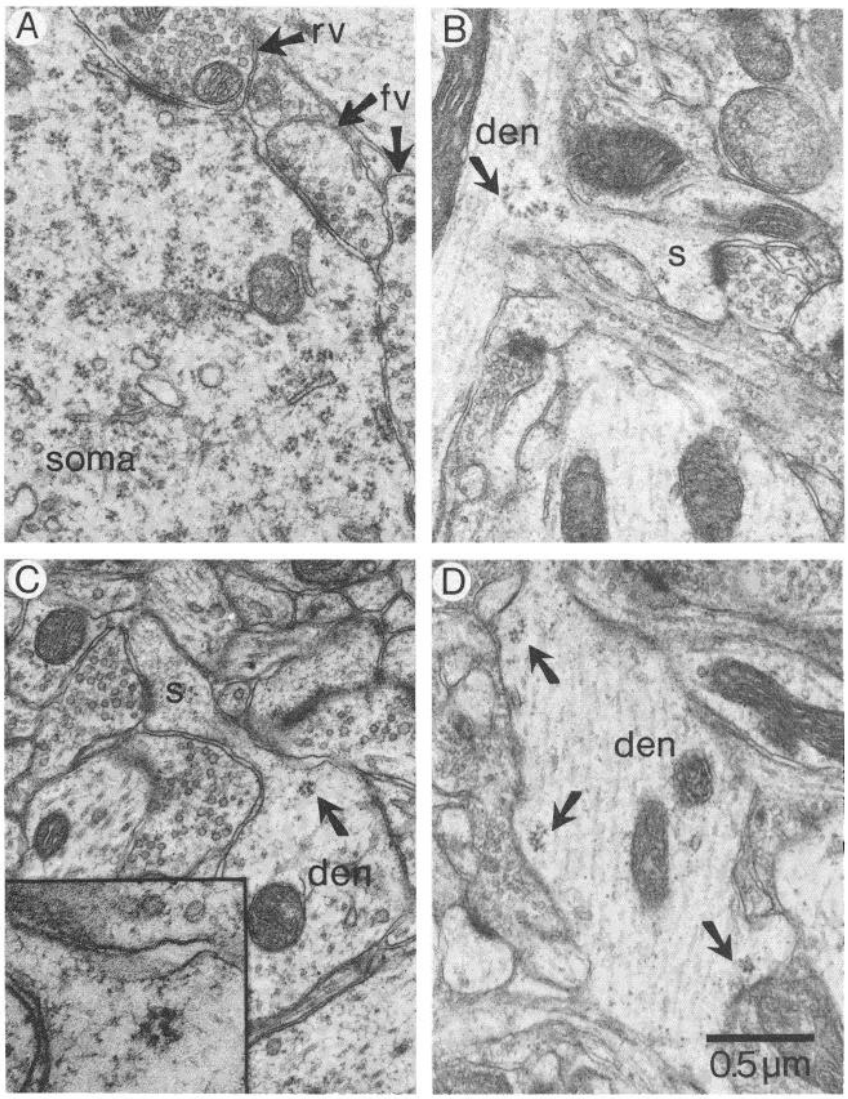

Figure 1. Distribution of polyribosomes in dentate granule cells. *A*, The soma of a dentate granule cell contains polyribosomes throughout the cytoplasm. Note the asymmetric contact with round vesicles (*rv*) and the symmetric contacts with flattened vesicles (*fv*). *B*, Polyribosome clusters at the spine neck-dendritic (*den*) shaft intersection from a location relatively proximal to the granule cell soma. The polyribosomes are indicated by the *arrow*, and the spine head is indicated by the *s*. *C*, A representative profile from mid proximodistal locations along the dendrite is illustrated. Note that there are fewer ribosomes in the cluster than more proximally (compare with *B*). *D*, Clusters of polyribosomes appear under mounds in the dendritic shaft, which resemble the base of dendritic spines (compare with *C*). The *inset* in *C* illustrates the polyribosome cluster magnified three times.

since, even in proximal dendrites where the polyribosomal clusters under spine necks were quite dramatic, few polyribosomes were evident throughout most of the dendritic cross-section.

As illustrated in Figure 1*D*, polyribosomal clusters also were found under mounds jutting from the dendritic shaft. These mounds contain no microtubules, which are characteristic of dendritic shafts, but do contain a flocculent material identical in appearance to that found in spines (see Fig. 1). We will present evidence below that most of these mounds represent the base of dendritic spines which extend out of the plane of section. Very rarely, elements which appeared to be polyribosomes were observed within spine necks and, even more rarely, within spine heads. In most cases, the ribosomes in spine heads were single or at most two or three ribosomes (see, for example, Fig. 2*B*), and convincing profiles with five or six ribosomes in the cluster were quite rare in control material (for one example, see Fig. 2*C*).

The qualitative observations thus suggest that polyribosomes occur most frequently under mounds in the dendritic membrane or under spine neck-dendritic shaft intersections. This localization suggests that there may be some special association between polyribosomes in the dendrites and spines. The quantitative analyses were designed to elucidate the nature of this association, focusing on the questions of (*1*) how many spines have polyribosomes at their base and (*2*) what proportion of the polyribosomes in dendrites are associated with spines.

Quantitative analysis. The quantitative analyses focused on mid proximodistal locations along the granule cell dendrite, which represent the middle portions of the molecular layer of the dentate gyrus. In material pre-

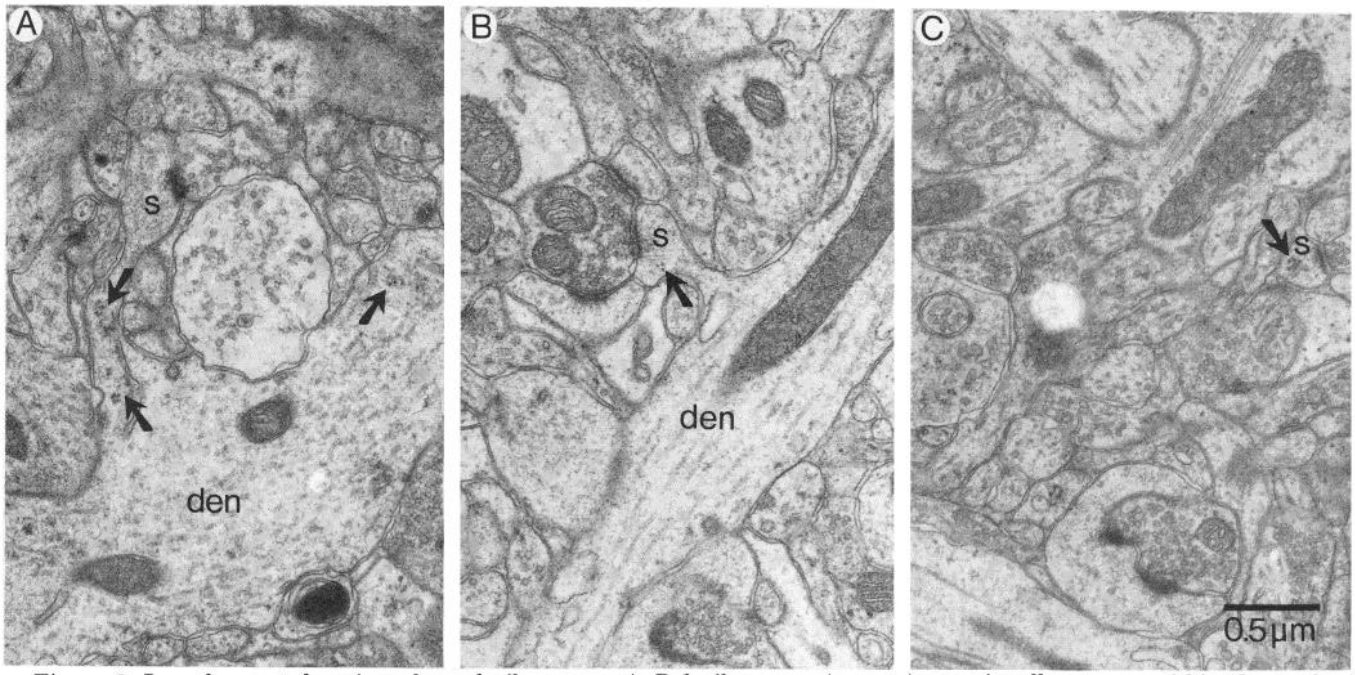

Figure 2. Less frequent locations for polyribosomes. *A*, Polyribosomes (*arrows*) occasionally appear within the neck of a dendritic (*den*) spine, and one example from the middle molecular layer of the dentate gyrus is illustrated. *B*, Even more rarely, a few electron-dense granules of the size of ribosomes may be observed in spine heads (*s*). Usually, these are single elements, but occasionally two or three are collected in a cluster, and very rarely, clusters with multiple ribosomes are found (*C*).

pared for electron microscopy, the middle molecular layer lies approximately 75 to 150 μm from the layer of granule cell somata. The first two analyses address the question of what proportion of spines has associated polyribosomes. As a first approach, we analyzed the incidence of polyribosomes in profiles identified as spine neck-dendritic shaft intersections from the control side of the five animals sacrificed at various times after unilateral entorhinal cortical lesions. For this purpose, an individual who was not involved in data analysis (Ms. S. L. Vinsant) was asked to photograph 21–22 spine neck-dendritic shaft intersections from mid proximodistal locations along the dendrite. A spine neck-dendritic shaft intersection was identified as a dendritic shaft (distinguished by the presence of mitochondria and parallel arrays of microtubules) with a protruding thin process which either was actually contacted by a presynaptic terminal or which contained a spine apparatus. If there was any doubt about whether a given spine neck was, in fact, connected to a dendrite, the intersection was not scored. While only one intersection was singled out for photography, others meeting the selection criteria were often visible in the × 10,000 field of approximately 58 μm^2, and these were included in the sample. For this reason, the average number of intersections scored in each animal was greater than the 21–22 which were singled out for photography, ranging from 40 to 73 with a mean of 56. The intersection was considered positive for polyribosomes if more than two ribosomes (identified on the basis of size and electron density) lay under the base of the spine. In fact, however, the average number of polyribosomes in positive profiles was 6.4 ± 1.2 (mean ± standard deviation). An average of 12.2 ± 1.5% of the identified spine neck-dendritic shaft intersections had underlying polyribosomes. Most of these lay immediately subjacent to the portal into the spine neck (see Fig. 1, *B* and *C*), and only a few were displaced to one side of the portal. Even the displaced clusters lay within 0.1 to 0.2 μm of the center line of the spine neck, however, and, on qualitative grounds, were judged to lie within or subjacent to the base of the spine.

Obviously, the analysis above provides only a relative indication of the frequency of polyribosomes in spines, since a single section does not reveal all of the area subjacent to a spine base. This relative value may be quite useful in revealing relative changes in the incidence of polyribosomes, such as those which occur during the reinnervation of the dendrites (Steward and Fass, 1981, 1982). Furthermore, a measure derived from single photographs hopefully will stimulate investigations in other brain regions even though that tissue may not have been prepared expressly for the analysis of polyribosomes. Clearly, however, a better estimate of the actual incidence can be obtained by serial section analysis. For this purpose, we made use of a serial section collection which was prepared in order to analyze spine size, shape, and neck diameter (W. B. Levy, research in progress). Twenty serial sections were collected from the control side of the dentate gyrus, and an area of approximately 210 μm^2 was photographed on each section from a mid proximodistal location along the dendrite. This 210-μm^2 sample area from each section was reconstructed as a montage of four photographs taken at × 10,000, covering an area approximately 7 × 30 μm (the size varied somewhat from montage-to-montage due to differences in the overlap of the composite photographs). From this serial montage, a total of 34 through-sectioned spines were identified and reconstructed. It should be noted that the selection and reconstruction of the spines took place approximately 1 year *before* the initiation of the polyribosome study, and

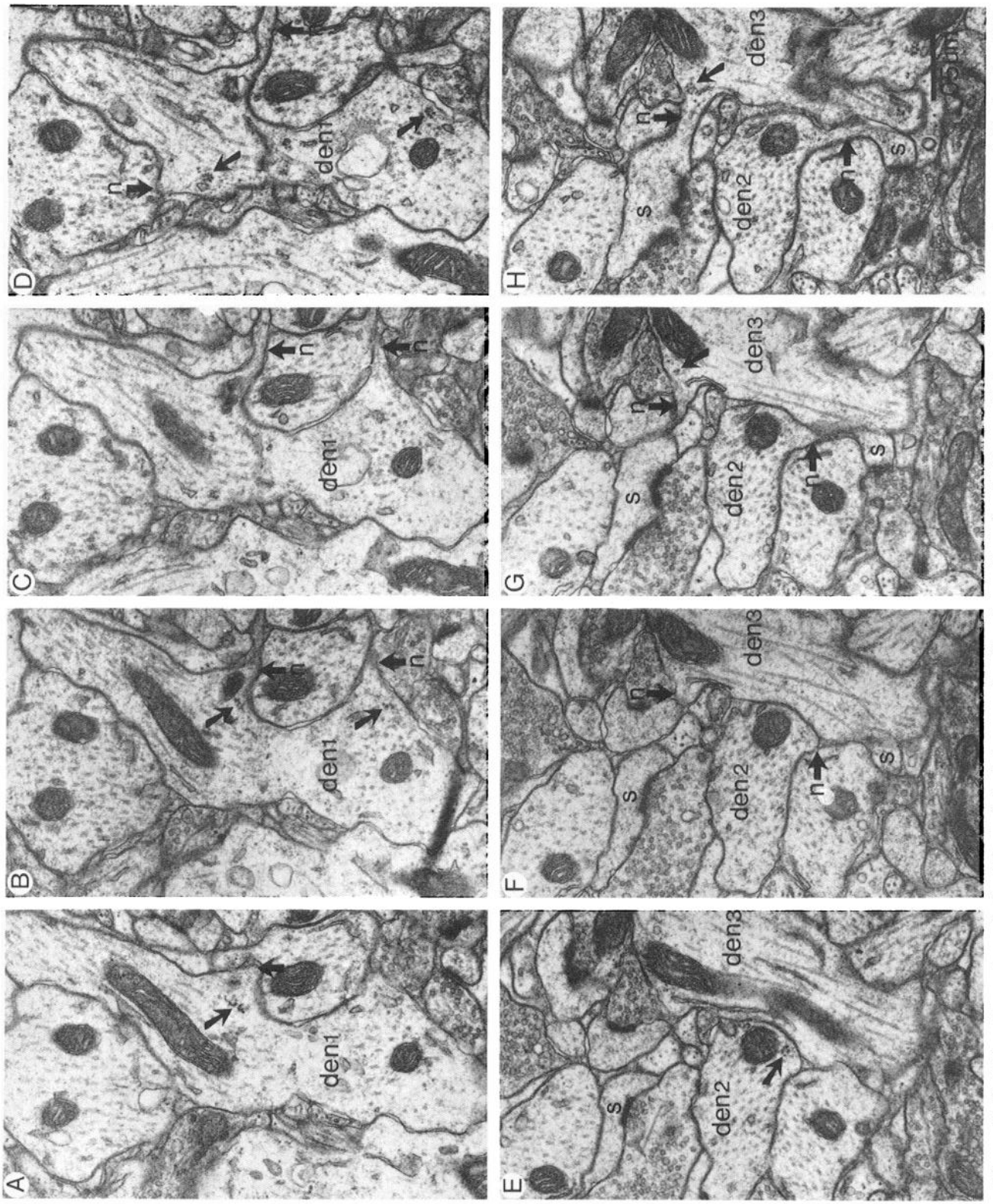

Figure 3. Serial sections through dendrites of dentate granule cells. A number of polyribosomal clusters appear under mounds in the dendritic shaft (*unlabeled arrows*). The serial sections reveal these mounds to be the base of dendritic spines, identified on the basis of a spine head with a contact region (*s*), or a spine neck. The dendrite in *A* to *D* (*den 1*) has three such clusters. The cluster in the middle portions of the section (*arrows* in *A* and *B*) lies under a spine neck (*n*) with a spine apparatus. The cluster in dendrite 2 (*den 2*; *E* to *H*) lies under a mound which represents a spine base (the head of the spine may be seen in *G* and *H*). The cluster indicated by the *unlabeled arrows* in *G* and *H* in dendrite 3 (*den 3*) also lies under a spine base, identified by its continuity with a spine head with a contact region.

thus there is no possibility of any conscious bias in the selection of the population of spines with regard to the presence of polyribosomes. From the population of 34 spines, 10, or 29%, contained polyribosomes subjacent to the base of the spine in one or more of the photographs comprising the through-section series. Examples of through-sectioned spines which contained polyribosomes are illustrated in Figure 3. As is evident, the polyribosomes are found within a few tenths of micrometers from the center line of the spine neck as is also the case in the fortuitous sections which contain both a spine neck and the associated polyribosomes (see above). Thus, both analyses reveal that polyribosomes are associated with many, but not all, spine bases within mid proximodistal dendritic zones. What remains to be determined is whether this is a special association or whether the polyribosomes are simply distributed randomly within the dendrites, coincidentally coming to lie under the base of a spine.

To attempt to determine how many of the polyribosomes within dendrites were associated with spines, we made use of the series of 21–22 photographs from each of five animals taken to analyze identified spine neck-dendritic shaft intersections (see the first analysis above). From this collection of photographs, all polyribosomes within dendrites were identified. For this analysis, our criteria for polyribosomes were slightly more stringent, requiring the presence of three or more particles of the size and electron density of ribosomes in a group with the spacing of typical polyribosome rosettes. The location of this population of polyribosomes then was determined based on whether they appeared (*1*) under spine necks or synaptic specializations on dendrites or spine heads, (*2*) under mounds on the dendrite of the sort which often represent the base of spines, or (*3*) in other locations within the dendrite without an obvious association with spine necks or mounds.

As illustrated in Table I, the series of photographs (covering approximately 1250 μm^2 of the molecular layer in each of the five animals) contained relatively few polyribosomes (an average of 104 across animals). Of this population, an average of 9.6% was found under identified spine necks or synaptic specializations on dendritic shafts or spine heads. Of this 9.6%, 34% (3% of the total) were found in spine heads or within spine necks. Some of these profiles are illustrated in Figure 2. The majority (71.4%) of the polyribosomes appeared under the mounds in the dendritic membrane which serial sections reveal are often the base of dendritic spines which extend out of the plane of the section (see below). Only 19% on the average had no obvious association with spine necks or mounds, and most of these were found very close to the periphery of the dendritic profile, in the same position relative to the dendritic membrane as the polyribosomes under mounds.

TABLE I

Polyribosomes in dendrites

The average number of polyribosomal clusters identified per animal was 104 ± 15. The proportion of these which were found under spines, mounds, and other elements is compared with the proportion of the dendritic circumference which spines and mounds comprise. Values are expressed as the mean ± standard deviation.

	Percent under Spines	Percent under Mounds	Percent Other
Distribution of polyribosomes within dendrites	9.6 ± 1.1	71.4 ± 4.4	18.8 ± 5.2
	Percent Spines	**Percent Mounds**	**Percent Other**
Proportion of dendritic membrane comprised of spines and mounds	3 ± 0.7	35 ± 5.2	62 ± 5.2

While the above analysis suggests a preferential localization of polyribosomes under spines and mounds, the significance of the proportions depends on how much of the dendritic circumference is occupied by spines and mounds. Indeed, the proportions could reflect a random distribution of polyribosomes if spine bases and mounds represent a high proportion of the dendritic circumference. To address this question, we identified all dendritic profiles which contained polyribosomes from the series of photographs analyzed above. The outline of the dendrite was traced, marking the areas which represented the bases of identified spines and the mounds. The proportion of the dendritic outline occupied by spine bases and mounds then was determined planimetrically. As illustrated in Table I, an average of 3% of the dendritic outline is comprised of the bases of identified spines and 35% is comprised of mounds. Most of the dendritic outline (an average of 62%) is *not* occupied by either of these elements. If the distribution of polyribosomes within the dendrite is random, one would expect them to be localized under spine bases, mounds, and other regions in the same proportion as the proportion of the dendritic outline occupied by each of these. As is evident in Table I, the incidence of polyribosomes under spine bases and mounds is considerably higher than would be predicted based on the proportion of the dendritic outline which spine bases and mounds comprise. The difference between the observed distribution and that predicted by chance is highly significant ($\chi^2 = 87.6$; $p < 0.0001$).

On the basis of qualitative evidence, we have argued that many of the mounds represent the base of spines which extend out of the plane of section. This conclusion is based on their appearance, the fact that the cytoplasmic composition of mounds is similar to spines, and the fact that serial reconstructions reveal that many mounds are continuous with spines. For example, each of the bases of the through-sectioned spines of Figure 3 appear as mounds in one or more sections. The serial sections provide an opportunity to evaluate the issue in more detail, however, enabling a determination of how many of the elements identified as mounds actually extend into spines. To address this question, 21 dendritic profiles were selected from a section midway through our 20-section series. The dendritic outlines were traced, and a total of 68 patches were identified as mounds using the same criteria as the analysis of Table I. The serial sections then were used to determine how many of these mounds could be traced into spines. Of the total 68 mounds, 19 (28%) continued into spines (identified on the basis of a contact region or the presence of a spine apparatus), 21 (31%) continued into thin processes of the

size and appearance of spine necks, and 28 (41%) were not traceable into any process resembling a spine. Since granule cell dendrites do not branch extensively and since the branches which do emerge are robust and not thin spine-like processes, it is probable that most of the thin processes in fact represent spine necks. Thus, we feel confident that at least 59% of the elements scored as mounds represent the base of dendritic spines. The percentage is probably considerably higher, since those which could not be traced may extend perpendicular to the plane of section, making it impossible to follow the process to its termination. Thus, the combined evidence strongly suggests that the polyribosomes in dendrites have a very close association with the base of dendritic spines and, indeed, are rarely found elsewhere.

Discussion

The presence of polyribosomes within dendrites and even subjacent to synaptic specializations is certainly not unknown (see, for example, Bodian, 1972). Previous authors have not, however, reported the type of close association between polyribosomes and spines at sites relatively distant from the soma that we have described here. While the dendrites of granule cells are not rich in polyribosomes, a high proportion of the polyribosomes which are present are found under the base of dendritic spines or under the mounds which usually represent the base of spines which extend out of the plane of section. From the opposite perspective, a high proportion of the spines have associated polyribosomes (about 30% in mid proximodistal dendritic regions). While each of our quantitative analyses can be faulted on methodological grounds, we feel that we have consistently made conservative assumptions and that our analyses underestimate rather than overestimate the degree of association between polyribosomes and spines. While it is conceivable that this association could reflect a functionally insignificant accumulation of the polyribosomes in eddies out of the main stream of dendritic flow, the stereotyped localization at the portal of the spine seems so consistent that we feel it is likely to be of functional significance. What the functional significance might be is a matter for speculation.

It is well recognized that polyribosomes represent the machinery for protein synthesis, and if all other elements required for synthesis are present (mRNA and tRNA, free amino acids, etc.), one would expect these elements to be manufacturing some protein. Indeed, the fact that the ribosomes are collected in characteristic rosettes suggests that they are bound to a messenger RNA and actively engaged in protein synthesis (see Warner et al., 1962). The selective localization of polyribosomes invites the speculation that the proteins being synthesized are related to the synaptic specialization or to the spine. Furthermore, the localization also would seem ideal if synthesis were regulated in part by activity over the synapse. At the same time, the fact that they usually are not localized within the spine suggests that their activity also might be regulated by electrical, ionic, or other events within the main dendritic shaft. It is difficult to conceive of a situation which is intuitively more appealing as a mechanism for protein synthesis-dependent maintenance or modification of a synapse as a function of the activity over that synapse operating in conjunction with other regulatory mechanisms within the postsynaptic cell.

Such a mechanism fits well with prevailing notions of the nature of synapse maintenance and modification. For example, spines appear quite sensitive to the presence and/or activity of presynaptic fibers (for a review, see Globus, 1975). The kinds of changes in spine size or number which apparently come about as a consequence of activity seem likely to require modulation of the cytoskeletal composition of the spine. If the proteins of the neuronal cytoskeleton are synthesized by free polyribosomes (see Lasek, 1981, for a discussion of evidence for this possibility), then perhaps the polyribosomes in spine bases are involved in adjusting spine size or shape through local regulation of the synthesis of some cytoskeletal protein(s).

Protein metabolism also has been implicated in other types of synaptic modulation. For example, information storage by neurons has long been assumed to involve a selective modification of synaptic connections (see Eccles, 1972, for a review), and most hypotheses suggest that the modification occurs as a consequence of the history of activity over the synapse. There is also a considerable body of evidence which suggests that the mechanisms of information storage involve the synthesis of proteins (see Rose et al., 1975, for a recent review). Thus, activity over synapses may lead to protein synthesis-dependent modification of those synapses. These types of modification could be quantitative rather than qualitative (more receptor proteins in the postsynaptic specialization or changes in other synaptic constituents which would modify synaptic efficacy). Again, the polyribosomes at spine bases seem ideally situated to provide protein constituents for these types of synaptic modification.

While these hypotheses are entirely speculative at this time, other evidence supports at least some of the suggestions. First, studies of incorporation of protein precursors in the dentate gyrus reveal an incorporation in the neuropil, and several lines of evidence suggest that this incorporation is taking place in dendrites. Furthermore, incorporation within the neuropil increases dramatically during the reinnervation of these dendrites after lesions of the entorhinal cortex (Fass and Steward, 1981; B. Fass and O. Steward, manuscript in preparation). The increase during lesion-induced synaptogenesis suggests that alterations in protein metabolism within dendrites might be associated with synaptic modification. In parallel with these increases in incorporation of protein precursors, there is a dramatic increase in the incidence of polyribosomes under dendritic spines (Steward and Fass, 1981, 1982). All of this evidence, although circumstantial, suggests that protein synthesis does occur in dendrites and may be related to the synaptic remodeling induced by lesions.

Other lines of evidence reveal that structural and functional synaptic modifications also occur as a consequence of physiological activation. For example, the projection system from the entorhinal cortex to the dentate granule cells is well known for its ability to exhibit long term

potentiation of synaptic efficacy (Bliss and Lømo, 1973; Douglas and Goddard, 1975). The changes in efficacy are accompanied by rather dramatic structural alterations in the postsynaptic element (Fifkova and Van Harreveld, 1977; Desmond and Levy, 1981). Moreover, recent evidence suggests that long term potentiation is accompanied by changes in protein metabolism (Duffy et al., 1981). Thus, a number of converging lines of evidence are consistent with the hypothesis that synaptic remodeling induced by physiological activation also may be related to local regulation of protein metabolism.

We have chosen to interpret our observations within the context of synapse maintenance and modification, but clearly, there could be other roles for the polyribosomes. Whatever their role, the available evidence suggests that they are likely to be actively manufacturing some protein or proteins. It seems virtually certain that they could not carry out their synthetic activities without being regulated by the local environment of the dendrite and the spine. There are a number of critical questions which remain to be resolved, including (*1*) whether these elements are engaged in the synthesis of one species of protein or many, (*2*) what type or types of protein are being made and for what purpose, (*3*) whether the synthetic activity of the polyribosomes is regulated by the physiological activity of either the spine with which these elements are associated or the dendritic shaft, and (*4*) how the position of the polyribosomes with respect to the spine is maintained. Studies are currently in progress to explore these issues.

References

Bliss, T. V. P., and T. Lømo (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of perforant path. J. Physiol. (Lond.) *232:* 334–356.

Bodian, D. (1972) Synaptic diversity and characterization by electron microscopy. In *Structure and Function of Synapses*, G. D. Pappas and D. P. Purpura, eds., pp. 45–65, Raven Press, New York.

Desmond, N. L., and W. B. Levy (1981) Ultrastructural and numerical alterations in dendritic spines as a consequence of long-term potentiation. Anat. Rec. *199:* 68A.

Douglas, R., and G. Goddard (1975) Long-term potentiation of the perforant path-granule cell synapse in the rat hippocampus. Brain Res. *86:* 205–215.

Duffy, C., T. J. Teyler, and V. E. Shashoua (1981) Long-term potentiation in the hippocampal slice: Evidence for stimulated secretion of newly synthesized proteins. Science *212:* 1148–1151.

Eccles, J. C. (1972) Possible synaptic mechanisms subserving learning. In *Brain and Human Behavior*, A. G. Karczmar and J. C. Eccles, eds., pp. 39–61, Springer-Verlag, New York.

Fass, B., and O. Steward (1981) Increases of protein-precursor incorporation in the denervated neuropil of rat dentate gyrus during sprouting. Anat. Rec. *199:* 80A.

Fifkova, E., and A. Van Harreveld (1977) Long-lasting morphological changes in dendritic spines of dentate granule cells following stimulation of entorhinal area. J. Neurocytol. *6:* 211–230.

Globus, A. (1975) Brain morphology as a function of presynaptic morphology and activity. In *The Developmental Neuropsychology of Sensory Deprivation*, A. H. Riesen, ed., pp. 9–91, Academic Press, New York.

Goldowitz, D., W. F. White, O. Steward, G. Lynch, and C. W. Cotman (1975) Anatomical evidence for a projection from the entorhinal cortex to the contralateral dentate gyrus of the rat. Exp. Neurol. *47:* 433–441.

Grafstein, B., and D. S. Forman (1980) Intracellular transport in neurons. Physiol. Rev. *60:* 1167–1283.

Lasek, R. J. (1981) The dynamic ordering of neuronal cytoskeletons. Neurosci. Res. Program Bull. *19:* 7–32.

Levy, W. B., and O. Steward (1979) Synapses as associative memory elements in the hippocampal formation. Brain Res. *175:* 233–245.

Peters, A., S. L. Palay, and H. deF. Webster (1976) *The Fine Structure of the Nervous System: The Neurons and Supporting Cells*, W. B. Saunders Co., Philadelphia, PA.

Rose, S. P. R., J. Hambley, and J. Haywood (1975) Neurochemical approaches to developmental plasticity and learning. In *Neural Mechanisms of Learning and Memory*, M. R. Rosenzweig and E. Bennett, eds., pp. 293–309, MIT Press, Cambridge.

Steward, O., and B. Fass (1981) Increases in incorporation of protein precursors during reinnervation of rat dentate gyrus are paralleled by increases in the incidence of polyribosomes associated with dendritic spines. Soc. Neurosci. Abstr. *7:* 474.

Steward, O., and B. Fass (1982) Polyribosomes associated with dendritic spines in the denervated dentate gyrus: Evidence for local regulation of protein synthesis during reinnervation. Prog. Brain Res., in press.

Warner, J. R., A. Rich, and C. E. Hall (1962) Electron microscope studies of ribosomal clusters synthesizing hemoglobin. Science *138:* 1399–1403.