Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 8335–8339, November 1988
Neurobiology

Polarity orientation of microtubules in hippocampal neurons: Uniformity in the axon and nonuniformity in the dendrite

(compartmentation/ribosomes/Golgi)

PETER W. BAAS*†, JEFFREY S. DEITCH‡, MARK M. BLACK*, AND GARY A. BANKER‡

*Department of Anatomy, Temple University School of Medicine, 3420 North Broad Street, Philadelphia, PA 19140; and ‡Department of Anatomy, Cell Biology and Neurobiology, Albany Medical College, Albany, NY 12208

Communicated by James M. Sprague, August 1, 1988 (received for review June 9, 1988)

ABSTRACT **We have analyzed the polarity orientation of microtubules in the axons and dendrites of cultured rat hippocampal neurons. As previously reported of axons from other neurons, microtubules in these axons are uniform with respect to polarity; (+)-ends are directed away from the cell body toward the growth cone. In sharp contrast, microtubules in the mid-region of the dendrite, ≈75 μm from the cell body, are not of uniform polarity orientation. Roughly equal proportions of these microtubules are oriented with (+)-ends directed toward the growth cone and (+)-ends directed toward the cell body. At distances within 15 μm of the growth cone, however, microtubule polarity orientation in dendrites is similar to that in axons; (+)-ends are uniformly directed toward the growth cone. These findings indicate a clear difference between axons and dendrites with respect to microtubule organization, a difference that may underlie the differential distribution of organelles within the neuron.**

Vertebrate neurons generate and maintain two morphologically and functionally distinct types of neurites, axons and dendrites (1–6). It has long been recognized that axons and dendrites differ in their complements of cytoplasmic organelles (1, 6). Most notable in this regard, ribosomes and Golgi elements are present in dendrites but are absent from axons. What is the basis for the nonuniform distribution of organelles in neurons? Several lines of evidence indicate that the distribution of organelles in a cell reflects active transport processes that selectively convey organelles from their sites of synthesis and assembly to other locations in the cell (7, 8). These observations raise the possibility that many of the differences between the organelle composition of axons and dendrites are produced by differences in the organization of the transport systems that convey materials from the cell body into the axon or dendrite.

The transport of organelles is a microtubule-based process; microtubules provide the substrate for organelle translocation and, by virtue of their intrinsic polarity, influence the directionality of transport (7–9). The intrinsic polarity of microtubules is based on the asymmetry of the tubulin subunit and its self-assembly characteristics; the (+)-end is preferred for subunit addition over the (−)-end (10, 11). Microtubule-based translocators convey organelles specifically toward either the (+)- or the (−)-end of the microtubule (7–9). In the axon, microtubules are uniform with respect to polarity, with the (+)-ends directed away from the cell body (12–15). Thus, only those organelles that translocate toward (+)-ends of microtubules will be conveyed from the cell body into the axon.

Do microtubules in dendrites have the same polarity orientation as those in axons? To date, information concerning the polarity orientation of dendritic microtubules derives from a few atypical cell types. In the dendrite-like processes of teleost retinal cone cells (16) and frog primary olfactory neurons (17), microtubules are uniform with respect to polarity, but unlike in axons, the (+)-ends are directed toward the cell body. This result is predicted, however, by the presence of centrosome-like organizing structures in the distal terminals of these processes. Because typical dendrites do not contain such organizing structures in their terminals, it is unclear whether these observations can be extended to dendrites in general.

Here we describe studies that compare the polarity orientation of microtubules in the axons and dendrites of cultured rat hippocampal neurons. We report that dendrites, unlike axons, contain microtubules of nonuniform polarity orientation. Burton, in a preliminary report (18), has described comparable findings for the dendrites of frog mitral cells. This difference in microtubule organization between axons and dendrites may underlie the establishment of compartmentation and polarity in the neuron.

MATERIALS AND METHODS

Rat hippocampal neurons were cultured on coverslips as described (3), and maintained for 2 weeks, time sufficient for them to extend well-differentiated axons and dendrites.

The polarity orientation of microtubules was determined by the "hook" method (19), as modified for cultured neurons (see ref. 15 for details). This method involves lysing the neurons with 0.6–0.8% Brij 58 in a microtubule assembly buffer containing exogenous brain tubulin. The exogenous tubulin adds onto existing microtubules as lateral sheets that appear as "hooks" in cross-section. The handedness of the hooks reveals the polarity orientation of the microtubule. A clockwise hook indicates that the (+)-end of the microtubule is directed toward the observer, while a counterclockwise hook indicates the opposite (19). Because consecutive sections showed identical hooking patterns, we scored one representative section from each axon or dendrite.

RESULTS

Fig. 1 is a phase-contrast micrograph of neurons treated by the hooking procedure and embedded in Epon. Even after lysis, axons and dendrites are readily distinguishable; axons are thinner and uniform in diameter, whereas dendrites are thicker and taper with distance from the cell body. Also, dendrites generally grow no longer than 300 μm, whereas axons grow much longer, weaving a complex network by 2 weeks in culture (3).

Axons and dendrites were distinguished electron microscopically by careful distance measurements matching their locations observed by phase-contrast microscopy of the Epon block with their locations in thin sections. Axons and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

†To whom reprint requests should be addressed.

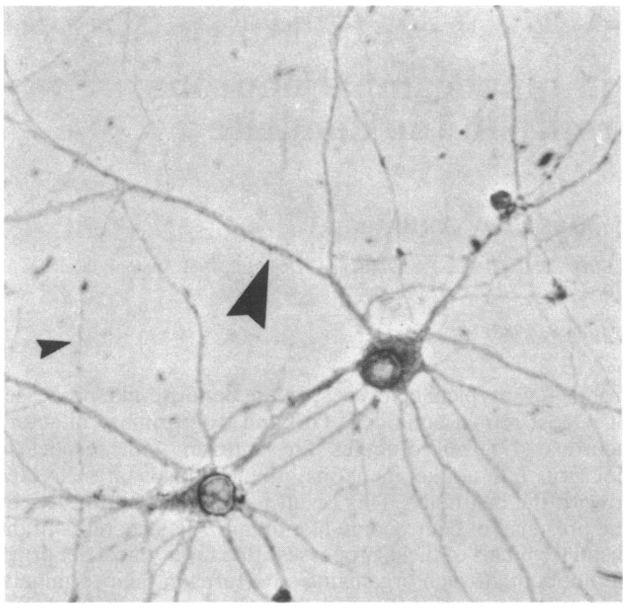

FIG. 1. Phase-contrast micrograph of cultured rat hippocampal neurons embedded in Epon after "hook" treatment. Axons (small arrowhead) and dendrites (large arrowhead) are readily distinguishable. (×450.)

dendrites were also distinguished on the basis of their ultrastructural features. Dendrites are several times larger in diameter than axons, more irregular in shape, and contain microtubules that are spaced farther apart than those in axons (see ref. 3 for details).

Polarity Orientation of Microtubules in the Axon. Because our principal concern in the present study was to compare microtubule organization in axons and dendrites, we first sought to confirm in cultured hippocampal neurons previous observations on axons of other neurons. In 50 axons analyzed, microtubule orientation was uniform, with 95% ± 8% (mean ± SD) of the hooks turning in a common direction (Fig. 2*a*; Table 1). In the majority of these cases, however, we could not unambiguously determine the origin of the axons observed. Thus, we could not label the hooks as clockwise or counterclockwise from a common vantage point. In three cases, however, we were able to section axons that were clearly growing toward the edge of the coverslip, thus enabling us to unambiguously identify the handedness of the hooks. In these cases, hooks were oriented clockwise as viewed from the growth cone looking toward the cell body (Fig. 2*b*; Table 1). Thus, the axons of cultured hippocampal neurons, like those of other neurons (12–15), contain microtubules that are uniformly oriented with their (+)-ends directed away from the cell body toward the growth cone.

Polarity Orientation of Microtubules in the Dendrite. We next examined the polarity orientation of microtubules in dendrites. Identification of the neuron that gave origin to each dendrite chosen for analysis was unambiguous in all cases. In one set of

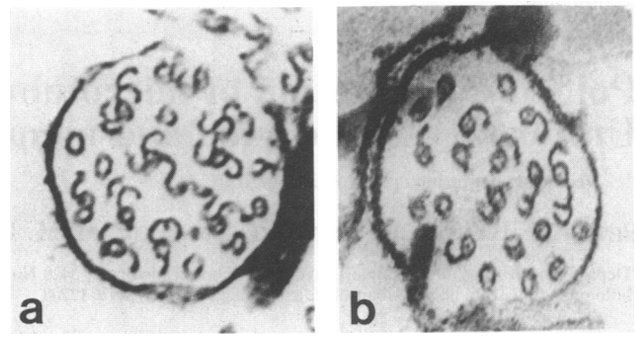

FIG. 2. Electron micrographs of cross-sections through typical axons treated to reveal microtubule polarity orientation. (*a*) Axon of unknown origin. Hooks are oriented predominantly in one direction, indicating uniform polarity orientation. (*b*) Axon of known origin. Hooks are predominantly clockwise (as viewed from the growth cone), indicating uniform polarity orientation, (+)-ends directed toward the growth cone. (×100,000.)

experiments, 16 dendrites were sectioned in their mid-region, ≈75 μm from the cell body. In sharp contrast to the axon, mid-regions of the dendrite contained microtubules that were clearly nonuniform in their polarity orientation (Fig. 3). In every dendrite examined, roughly equal numbers of microtubules were oriented in each direction. As shown in Tables 2 and 3, 57% ± 6% of the hooks were clockwise as viewed from the growth cone, indicating (+)-ends directed toward the growth cone, while 43% ± 6% of the hooks were counterclockwise, indicating (+)-ends directed toward the cell body. In every sample, the number of microtubules with (+)-ends distal equaled or slightly exceeded the number with (+)-ends proximal to the cell body.

There was no apparent restriction of microtubules with a particular orientation to any particular region of the dendrite. For example, there was no indication that microtubules with (+)-ends distal were concentrated in either the central or peripheral regions. Indeed, it was not uncommon for two microtubules of opposite polarity orientation to exist side by side (Fig. 3). This lack of spatial organization is not surprising in that serial section analyses show that dendritic microtubules are not parallel to one another along their lengths, but rather weave complex paths through the dendrite (20). This is also apparent in our micrographs; some microtubules appear in perfect cross-section while others are skewed (see Fig. 3).

It should be mentioned that the proportion of microtubules hooked was somewhat less in the dendrite than in the axon (51% vs. 70%). This may reflect a difference between the properties of axonal and dendritic microtubules but is perhaps more likely due to differences in the penetration of the exogenous tubulin into dendrites versus axons; dendrites are larger in diameter and tend to be surrounded by axons. The degree of hooking in dendrites was, however, comparable to or higher than that achieved in previous studies on neuronal tissue (12–15).

Polarity Orientation of Microtubules in Distal Regions of the Dendrite. To better understand microtubule organization in

Table 1. Polarity orientation of microtubules in axons

Combined data from 50 axons of unknown origin*				Axons of known origin (hooks viewed from growth cone looking toward cell body)				
Majority	Minority	Ambiguous	Unhooked	Sample	Clockwise	Counterclockwise	Ambiguous	Unhooked
231	15	34	116	1	12	0	2	5
				2	3	0	0	6
				3	7	0	3	8

*In these cases we could not determine whether hooks were clockwise or counterclockwise from a known vantage point. Therefore, hooks were identified as turning in one direction or the other, after which the higher numbers from all the samples were combined (majority), as were the lower numbers from all the samples (minority).

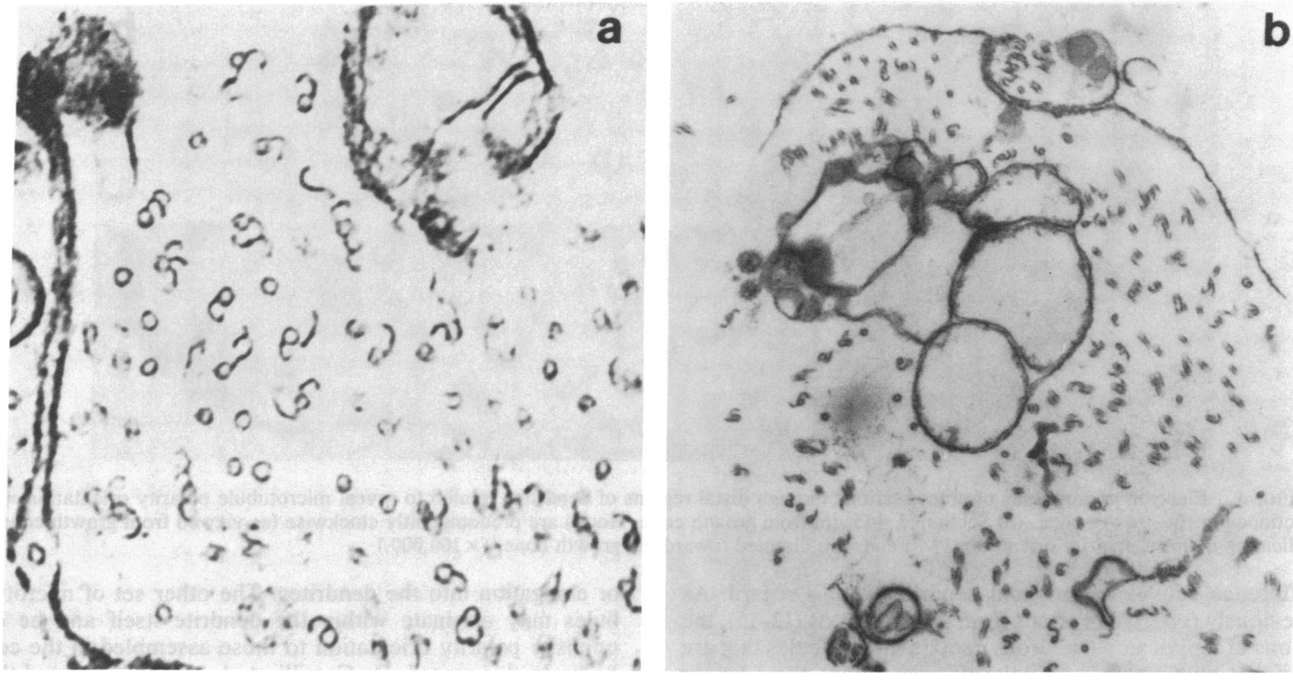

FIG. 3. Electron micrographs of cross-sections through the mid-regions of dendrites (≈75 μm from the cell body), treated to reveal microtubule polarity orientation. (*a*) Portion of a dendrite in cross-section. (*b*) Cross-section of an entire dendrite. Approximately equal numbers of hooks are clockwise and counterclockwise (as viewed from the growth cone), indicating nonuniform microtubule polarity orientation. Some microtubules appear in perfect cross-section, while others are skewed, reflecting the fact that dendritic microtubules are not parallel to one another. (*a*, ×100,000; *b*, ×40,000.)

dendrites, we wanted to determine whether the proportion of microtubules of each polarity orientation was constant throughout the dendrite. To begin examining this issue, we analyzed microtubule polarity orientation in distal regions of the dendrite. Because distal dendrites were similar in diameter to axons, we relied on distance measurements in the Epon block to identify the distal dendrites in our thin sections. Consistent with the characteristics of the mid-region of the dendrite, we found distal dendrites to be clearly less round than axons and to contain microtubules spaced farther apart than those in axons (compare Figs. 4*a* and 2). Similar to axons, however, distal regions of the dendrite contained microtubules of uniform polarity. This was the case in three dendrites sectioned at the growth cone (Fig. 4*a*; Tables 2 and 3), and in three additional dendrites sectioned ≈15 μm from the growth cone, where the dendrite was somewhat larger in diameter (Fig. 4*b*; Tables 2 and 3).

Table 2. Polarity orientation of microtubules in dendrites (hooks viewed from growth cone looking toward cell body)

Sample	Clockwise	Counter-clockwise	Ambiguous	Unhooked
Sectioned in the mid-region				
1	20	19	6	59
2	11	6	0	10
3	10	6	8	10
4	7	5	0	22
5	21	14	7	66
6	17	15	9	27
7	13	10	4	12
8	8	8	2	12
9	10	4	2	55
10	8	5	6	17
11	15	11	3	53
12	11	10	9	22
13	21	15	9	17
14	12	10	9	17
15	14	12	5	11
16	29	28	3	41
Sectioned at the growth cone				
1	5	0	1	3
2	6	1	2	3
3	3	0	0	3
Sectioned ≈15 μm from the growth cone				
1	18	3	3	19
2	21	1	4	11
3	12	1	5	10

Microtubule Organization in the Cell Body. In a final set of experiments, we examined the orientation of microtubules in the cell body. In five neurons examined in multiple regions of the cell body, we detected no uniformity or pattern with respect to microtubule polarity orientation (data not shown). This finding is consistent with evidence suggesting that axonal and dendritic microtubules are not continuous with one discrete organizing structure, such as the centrosome, in the neuron cell body (21).

DISCUSSION

We have analyzed the polarity orientation of microtubules in cultured rat hippocampal neurons. Our data show a clear

Table 3. Microtubule polarity orientation in axons and dendrites (hooks viewed from growth cone looking toward cell body)

	% clockwise*	% counter-clockwise*	% hooking	Polarity orientation
Axons†	95 ± 8	5 ± 8	70	Uniform (+)-end distal
Dendrites				
Mid-region	57 ± 6	43 ± 6	51	Nonuniform
Distal region	94 ± 6	6 ± 6	64	Uniform (+)-end distal

*Mean ± SD of the % for each sample.
†Assuming that hooks in axons of unknown origin reflect the same polarity observed in axons of known origin.

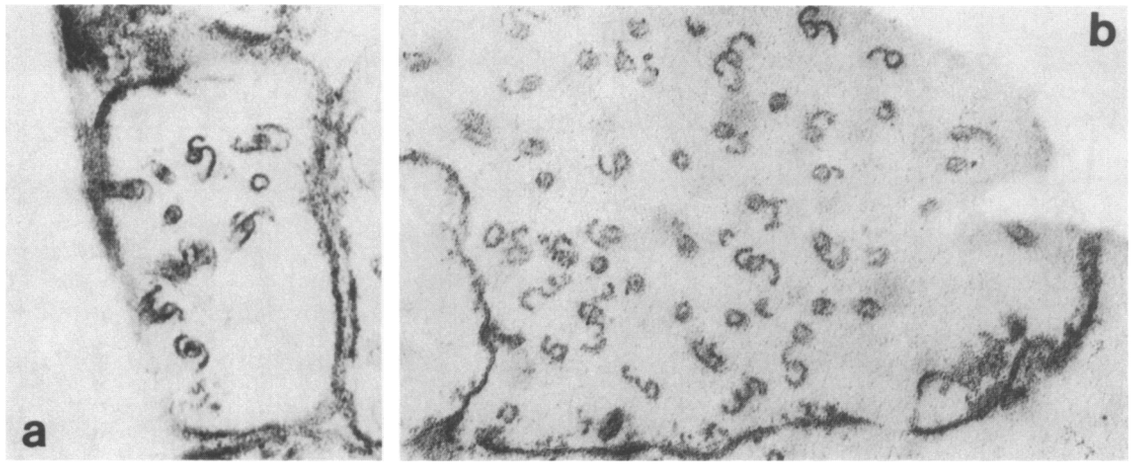

FIG. 4. Electron micrographs of cross-sections through distal regions of dendrites treated to reveal microtubule polarity orientation. (*a*) Sectioned at the growth cone. (*b*) Sectioned ≈15 μm from growth cone. Hooks are predominantly clockwise (as viewed from growth cone), indicating uniform polarity orientation. (+)-ends are directed toward the growth cone. (×100,000.)

difference between axons and dendrites in this regard. As previously reported of axons from other neurons (12–15), the axons of hippocampal neurons contain microtubules that are uniform with respect to polarity orientation; the (+)- or "fast-growing" ends of the microtubules are directed away from the cell body toward the growth cone. In contrast, the polarity orientation of microtubules in dendrites is not uniform; roughly equal proportions of microtubules are oriented with (+)-ends directed toward the growth cone and (+)-ends directed toward the cell body. We believe that our finding of a nonuniform polarity orientation of microtubules in the dendrites of cultured hippocampal neurons will prove to be indicative of dendrites generally because these dendrites so closely resemble dendrites *in situ* (3, 5, 22). In addition, Burton (18) has provided preliminary evidence that microtubules in frog mitral dendrites are also nonuniform in polarity orientation.

Although these considerations argue that nonuniform microtubule polarity orientation is a general feature of dendrites, the details of this organization may vary from case to case. For example, the relative proportions of microtubules of each orientation may vary among different types of neurons or at different stages of neuronal development. In addition, the uniform microtubule polarity orientation observed in the distal regions of these dendrites, which are still growing, may or may not apply to fully mature dendrites.

The nonuniform polarity orientation of microtubules observed in the mid-region of the dendrite may reflect a single population of microtubules that is random with respect to polarity, or two separate populations that are organized with respect to polarity. We favor the latter possibility because previous work on other systems indicates that microtubule arrays of nonuniform polarity orientation generally arise due to the overlap of separate arrays of microtubules that are uniform with respect to polarity (23). Also, the uniformly oriented microtubules found distally in the dendrite presumably originate more proximally in that dendritic microtubules can be quite long relative to the length of the dendrite (24). Thus, all regions of the dendrite may contain an ordered array of microtubules with (+)-ends directed distally. If this is correct, then superimposed upon these microtubules is a second population of microtubules with a uniform and opposite polarity orientation.

One possibility is that the two populations of microtubules in the dendrite arise at different locations in the neuron. For example, the microtubules with (+)-ends distal may be assembled in the cell body and then undergo either transport or elongation into the dendrites. The other set of microtubules may originate within the dendrite itself and be of opposite polarity orientation to those assembled in the cell body. In this regard, De Camilli *et al.* (6) have reported the selective migration of centrosome-associated components into the dendrites, but not the axon. These components may permit the nucleation and assembly of a distinct subset of microtubules within the dendrite itself.

Compartmentation in the Neuron. The presence of microtubule populations of opposite polarity orientation in dendrites, but not axons, has significant implications for the establishment of compartmentation and polarity in the neuron. Axons and dendrites contain different complements of the various cytoplasmic organelles. In particular, ribosomes and Golgi elements are present in the dendrite, but are absent from the axon (1, 6). Microtubules contribute to the distribution of organelles in cells by providing directional tracks for their transport. While individual microtubules can support transport toward either their (+)- or (−)-ends, certain organelles appear to move preferentially toward only one end of the microtubule (7–9). For example, in many nonneuronal cells, Golgi elements are localized in the region of the centrosome (25), perhaps reflecting their preferential transport toward the (−)-ends of microtubules concentrated within the centrosome (26). Also, in insect ovarioles, ribosomes are transported along microtubules in the nutritive tubules to developing oocytes (27). Comparison of the polarity orientation of these microtubules with the directionality of ribosome transport indicates that the translocation of ribosomes occurs specifically toward the (−)-ends of microtubules (28).

The basis for the directional specificity of organelle transport toward one or the other end of the microtubule may lie in the nature of the interactions between particular organelles and specific translocator molecules. At least two motors for microtubule-associated transport have been identified in neurons, one that transports organelles toward (+)-ends of microtubules and another that transports organelles toward (−)-ends of microtubules (7, 29). The various types of transported organelles may differ in their affinities for these transport motors, with some types of organelles having a preferential affinity for (+)-end-specific transport motors, and other types of organelles having a preferential affinity for (−)-end-specific transport motors. Such preferential affinities may constitute the basis for the transport of different types of organelles toward either (+)- or (−)-ends of microtubules. In this regard, the observations that ribosomes and Golgi elements appear to move selectively toward (−)-ends

of microtubules suggest that these organelles may have a preferential affinity for (−)-end-specific translocators.

The above considerations, together with the differences in the polarity orientation of axonal and dendritic microtubules, can account for certain features of neuronal compartmentation. From the time of their discovery, dendrites have been viewed as extensions of the somatic cytoplasm, whereas the axon has been considered a separate process with a distinct origin (30). While it has long been recognized that certain organelles such as ribosomes and Golgi elements enter dendrites but are excluded from the axon (1, 6), these observations have never been satisfactorily explained. Because of the polarity orientation of axonal microtubules, only those somatic organelles that are transported toward the (+)-ends of microtubules will enter the axon. In contrast, because the polarity orientation of dendritic microtubules is nonuniform, with both (+)- and (−)-ends directed distally, organelles that are transported toward (+)-ends as well as organelles that are transported toward (−)-ends of microtubules will enter the dendrite. If ribosomes and Golgi elements are translocated toward the (−)-ends of microtubules in neurons, as they appear to be in other cell types, then the polarity orientation of axonal and dendritic microtubules can account for the differential distribution of these organelles within the neuron. In this view, organelle compartmentation in the neuron is secondary to the generation of microtubule arrays of different polarity orientation in the axon and the dendrite. Thus, the establishment of these distinct microtubule arrays may provide a structural basis for many of the differences that distinguish the dendrite from the axon.

We thank Dr. Ray Lasek for many helpful comments. This work was supported by National Institutes of Health Grants NS 17681 and NS 23530 to M.M.B., and NS 23094 and NS 17112 to G.A.B.

1. Peters, A., Palay, S. L. & de Webster, H. (1979) *The Fine Structure of the Nervous System* (Saunders, Philadelphia).
2. Wuerker, R. B. & Kirkpatrick, J. B. (1972) *Int. Rev. Cytol.* **33,** 45–75.
3. Bartlett, W. P. & Banker, G. A. (1984) *J. Neurosci.* **4,** 1954–1965.
4. Black, M. M. & Smith, W. (1988) in *Intrinsic Determinants of Neuronal Form and Function,* eds. Lasek, R. J. & Black, M. M. (Liss, New York), pp. 463–486.
5. Banker, G. A. & Waxman, A. B. (1988) in *Intrinsic Determinants of Neuronal Form and Function,* eds. Lasek, R. J. & Black, M. M. (Liss, New York), pp. 61–82.
6. De Camilli, P., Solimena, M., Moretti, M. & Navone, F. (1988) in *Intrinsic Determinants of Neuronal Form and Function,* eds. Lasek, R. J. & Black, M. M. (Liss, New York), pp. 487–520.
7. Vale, R. D. (1987) *Annu. Rev. Cell Biol.* **3,** 347–378.
8. Lasek, R. J. (1988) in *Intrinsic Determinants of Neuronal Form and Function,* eds. Lasek, R. J. & Black, M. M. (Liss, New York), pp. 1–60.
9. Miller, R. H. & Lasek, R. J. (1985) *J. Cell Biol.* **101,** 2181–2193.
10. Binder, L. I., Dentler, W. & Rosenbaum, J. L. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1122–1126.
11. Bergen, L. & Borisy, G. G. (1980) *J. Cell Biol.* **84,** 141–150.
12. Heidemann, S. R., Landers, J. M. & Hamborg, M. A. (1981) *J. Cell Biol.* **91,** 661–665.
13. Burton, P. R. & Paige, J. L. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3269–3273.
14. Filliatreau, G. & DiGiamberdino, L. (1981) *Biol. Cell* **42,** 69–72.
15. Baas, P. W., White, L. A. & Heidemann, S. R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5272–5276.
16. Troutt, L. L. & Burnside, B. (1988) *J. Neurosci.* **8,** 2371–2380.
17. Burton, P. R. (1985) *J. Comp. Neurol.* **242,** 147–160.
18. Burton, P. R. (1987) *J. Cell Biol.* **105,** 31a (abstr.).
19. Heidemann, S. R. & Euteneuer, U. (1982) *Methods Cell Biol.* **24,** 207–216.
20. Sasaki, S., Stevens, J. K. & Bodick, N. (1983) *Brain Res.* **259,** 193–206.
21. Sharp, G. A., Weber, K. & Osborn, M. (1982) *Eur. J. Cell Biol.* **29,** 97–103.
22. Caceres, A., Banker, G. A. & Binder, L. (1986) *J. Neurosci.* **6,** 714–722.
23. Euteneuer, U., Ris, H. & Borisy, G. G. (1985) *Eur. J. Cell Biol.* **37,** 149–155.
24. Hillman, D. E. (1988) in *Intrinsic Determinants of Neuronal Form and Function,* eds. Lasek, R. J. & Black, M. M. (Liss, New York), pp. 83–114.
25. Kupfer, A., Louvard, D. & Singer, S. J. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2603–2607.
26. Brinkley, B. R. (1985) *Annu. Rev. Cell Biol.* **1,** 145–172.
27. Stebbings, H. (1988) in *Nature and Function of Cytoskeletal Proteins in Motility and Transport,* ed. Wohlfarth-Bottermann, K. E. (VCH, Deerfield Beach, FL), pp. 145–156.
28. Stebbings, H. & Hunt, C. (1983) *Cell Tissue Res.* **233,** 133–142.
29. Paschal, B. M. & Vallee, R. B. (1987) *Nature (London)* **330,** 181–183.
30. Deiters, O. (1865) *Untersuchungen über Gehirn und Ruckenmark* (Vieweg, Braunschweig, F.R.G.).