Luminal Material in Microtubules of Frog Olfactory Axons: Structure and Distribution

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ABSTRACT   The substructure and distribution of luminal material in microtubules of olfactory axons were studied in the bullfrog, *Rana catesbeiana*. By using numerous fixation methods, with and without osmium tetroxide, the luminal component was shown not to be an artifact of fixation. The material consists of globular elements 4–5 nm in diameter loosely arranged within the lumen in a discontinuous column. Counts of microtubules showing luminal material were obtained for axons in the proximal and distal ends of the olfactory nerve, and it was found that 16–18% more of the microtubules in the distal regions showed the luminal component. This raises the possibility that the material might be translocated within the microtubule lumen and tends to accumulate as it moves distally toward the axon terminal. In contrast to those of the olfactory axons, microtubules assembled in vitro from frog brain tubulin did not show luminal material. When microtubules in olfactory axons were depolymerized in situ by cold and calcium treatment and then induced to reassemble, most of those that were formed de novo showed empty lumina. Such evidence suggests that the luminal material is not an integral component of the microtubule. The hypothesis is discussed that material may be translocated within the lumina of microtubules. Furthermore, in the case of neuronal microtubules, the possibility is raised that they may serve as conduits for their own wall subunits.

Microtubules are the most prominent cytoskeletal elements of axons and, along with neurofilaments, they provide a linear armature or framework to which the other axoplasmic matrix components are bridged. A role for microtubules in fast axoplasmic transport is supported by many studies (7, 12, 15, 17, 20, 22, 23, 32, 33), even though it is likely that the mechanisms involved are related to those providing for intracellular transport of various components in other eucaryotic cells (see reference 27 for comprehensive review).

Even though there is little reason to believe that neuron microtubules are structurally different from those found in the cytoplasm of other cell types, the existence of dense material in their lumina (9) sets them apart from many kinds of microtubules. Luminal densities are sometimes seen in other kinds of singlet microtubules, such as those of blood platelets (2, 26) and in certain epithelial cells of insects (1, 5, 21). Peters et al. (24) examined cross sections of microtubules of pyramidal neurons of the rat cerebrum and noted a dense dot-like structure in their lumina measuring 3–4 nm in diameter. The luminal density was considered to be a cross-sectional profile of a central filament, as deduced from unpublished micrographs of longitudinal views of these microtubules. Echandia et al. (9), however, published longitudinal views of neuronal microtubules from the toad which were interpreted as showing that the luminal material is made up of granules irregularly disposed inside the microtubule. It was further suggested that the luminal granules may be a structural component of neuronal microtubules or ". . . a visible expression of an endotubular flow of materials" (9). Dustin (8) also notes that this material may represent molecules migrating in the microtubule lumen.

The work of Stanley et al. (34) provides one of the best pieces of evidence that (*a*) dense material in microtubules is not an artifact of fixation, and (*b*) the luminal material may be transported within the microtubules. During spermiogenesis in *Drosophila*, there are several kinds of microtubules that form the differentiating sperm tail. In addition to the usual doublet elements and the central pair microtubules of the axoneme, a set of nine accessory singlet microtubules are formed in close proximity to the doublet elements. Initially, the lumina of all these microtubules are lucent, but late in spermiogenesis dense luminal material appears in accessory microtubules and in the pair of central microtubules of the axoneme, but not in the A and B members of the doublet

elements. Because the microtubules of flagella of nearby sperm which are at an earlier stage of spermiogenesis do not show such luminal densities, it is unlikely that the luminal material represents a fixation artifact. Furthermore, the temporal appearance of luminal material in the preexisting microtubules is consistent with the hypothesis that such material is transported within the lumina of microtubules.

Even though there are a dozen or so published articles that include mention of "central densities," " axial densities," or "central dense dots" in singlet microtubules, no one has attempted to better characterize such elements. The present paper represents the initial report of a focused effort to learn more about the luminal material in microtubules of frog olfactory axons.

MATERIALS AND METHODS

The adult bullfrogs used in this study belonged to two species, *Rana catesbeiana* (the "northern" bullfrog) and *R. sepentrionalis* (the "southern" bullfrog). In a few studies, the smaller leopard frog (*R. pipiens*) was used. Isolation of olfactory nerves was accomplished by decapitating the frog and quickly using scissors to open the cranial chamber from the ventral aspect while working under a dissecting microscope. During isolation, a portion of the olfactory lobe of the brain was left on one end of the nerve (the distal end) and some of the anastomosing nerve bundles from the olfactory epithelium were left on the nerve's other (proximal) end. Nerves were transferred into drops of cold frog nerve saline (9) and cut transversely into three to four pieces.

A number of fixative combinations were used in this study, although the one of choice for stabilizing and fixing microtubules consisted of 0.1 M PIPES at pH 6.9, 10 mM EGTA, 2 mM MgCl, 0.5 mM guanosine 5'-triphosphate (GTP), and 2% glutaraldehyde. To visualize wall protofilaments of microtubules, 2% tannic acid was added to the above fixative. Fixation was usually for 1 h at room temperature with continuous agitation. Material was then secondarily fixed for 1 h in 1% osmium tetroxide buffered with 0.1 M cacodylate (pH 7.2-7.4), dehydrated in acetone, and embedded in Araldite 502 resin. Silver to gray sections were obtained with a diamond knife and examined in a Philips 300 transmission electron microscope. Serial sections were obtained using a simple micromanipulator as a "third hand," following the methods described in detail by Rieder (25). Ribbons of 12-20 wrinkle-free sections could be placed on a grid in this manner, with such sections being selected for a uniform silver interference color.

Frog brain tubulin was isolated from the whole brains of 7-10 bullfrogs using a modification (18, 19) of the method of Shelanski et al. (28), where 4 M glycerol is used to enhance the yield and assembly of tubulin. The tubulin was carried through two cycles of disassembly-assembly using PIPES buffer, and microtubules were assembled at 37°C and collected by centrifugation at 100,000 g. A fairly uniform pellet of microtubules could thus be obtained which was shown by SDS slab gel electrophoresis to mainly consist of tubulin.

Calcium ionophore (A-23187) used in this study was obtained from Calbiochem-Behring (La Jolla, CA) and dissolved as completely as possible in absolute ethanol at a concentration of 10 mg/ml. It was used at a concentration of 10 μg/ml by adding 2 μl of the stock solution to 2 ml of calcium-free saline, providing an ethanol concentration of 0.1% in the medium. Control studies showed that this concentration of ethanol alone had no effect on olfactory nerve microtubules under the conditions cited. Several additional methods were used in this research, and these are presented more appropriately in the Results section.

RESULTS

For purposes of study, one can isolate and divide the bullfrog olfactory nerve into proximal and distal halves, with reference to the perikarya of the axons comprising the nerve (Fig. 1). The easiest way to identify the two halves is to leave a portion of the olfactory lobe attached to the distal nerve end during its isolation and, in the case of the proximal end, to leave intact some of the small anastomosing nerves from the olfactory epithelium. Figs. 2 and 3 show cross sections of axons in the proximal and distal regions of the olfactory nerve. Axons from the proximal portion of the nerve often show a more granular axoplasm and a slightly smaller diameter than those

in the distal region (cf. Figs. 2 and 3). Axons usually show 2-3 microtubules (mt) which appear as circular profiles, and within many of these profiles a central structure can be seen, which appears dot-like. These dot-like structures represent the particulate luminal material characteristic of these and other neuronal microtubules. In the frog olfactory nerve, luminal material was seen in microtubules in axons throughout the length of the nerve. In addition to microtubules, olfactory axons contain a variable number of neurofilaments (nf), often grouped together, smooth endoplasmic reticulum (ser), and mitochondria (mi). A single profile of reticulum is usually seen in a cross section of an axon, and sometimes a mitochondrial profile is present (Fig. 3).

To test whether luminal particles of microtubules of olfactory axons are artifacts of fixation, pieces of olfactory nerve were fixed in nine different fixative and buffer and buffer combinations. In all cases, even when glutaraldehyde-fixed samples were processed without secondary fixation in osmium tetroxide (Fig. 41), luminal particles could be seen in these microtubules. Such evidence indicates that luminal particles do indeed exist as stable structural entities and are not merely artifacts of fixation. To fix olfactory axonal microtubules on a routine basis, glutaraldehyde was used in a microtubule-
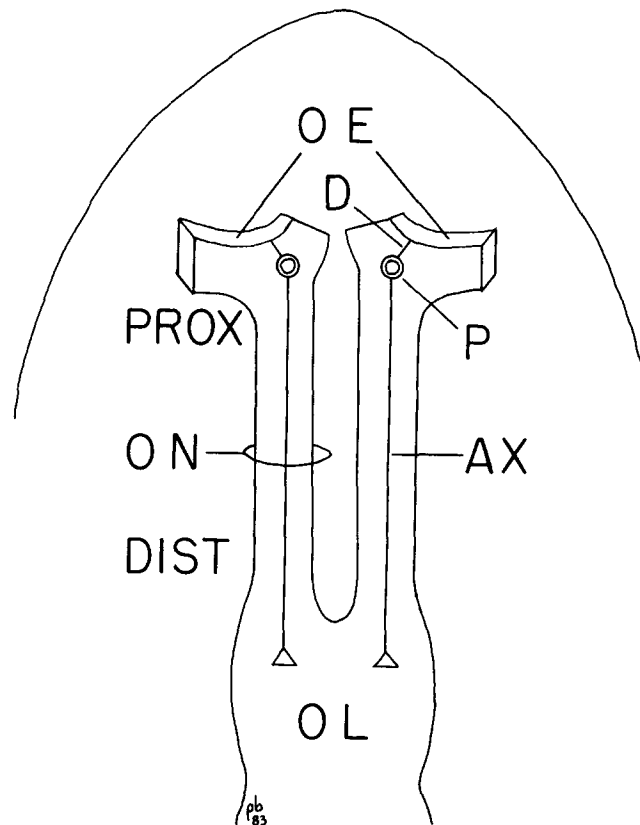

FIGURE 1 A diagrammatic representation of the olfactory pathway of the bullfrog is shown within an outline of the anterior portion of the skull. The perikarya (P) of the olfactory neurons are located in the olfactory epithelium (OE); each neuron sends a short dendrite (D) to the surface of the epithelium and tapers basally to form a tiny axon (AX) which extends, through the olfactory nerve (ON), to the olfactory lobe (OL) of the brain. In the brain the axon is expanded and forms a vesicle-filled terminal. The two olfactory nerves of the frog, each containing about 20 million axons, can be isolated and divided into proximal (PROX) and distal (DIST) regions with respect to the perikarya.
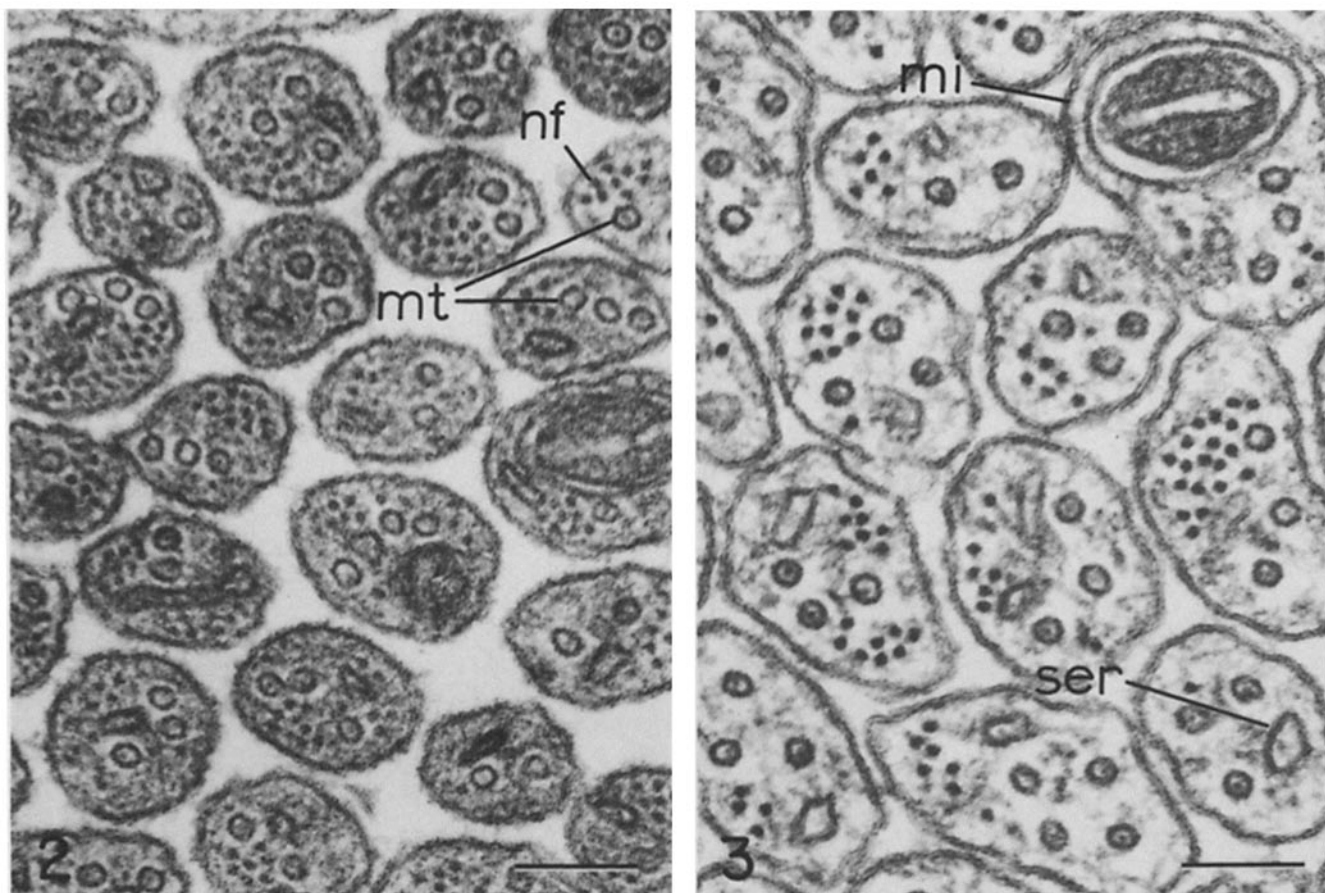
FIGURES 2 and 3   Cross sections of olfactory axons are shown from the proximal (Fig. 2) and distal (Fig. 3) regions of the olfactory nerve. Each axon usually contains 2–3 microtubules (*mt*), a variable number of neurofilaments (*nf*), an occasional profile of a mitochondrion (*mi*), and a profile of smooth endoplasmic reticulum (*ser*). Note that axons in the proximal region of the nerve (Fig. 2) are slightly smaller in diameter and have a denser axoplasmic matrix than axons in the distal region (Fig. 3). It should also be noted that neurofilaments tend to occur in groups and that many microtubules have dense structures in their lumina. Bar, 0.1 μm. × 168,500.

stabilizing buffer, as described above under Materials and Methods. Fixation of pieces of nerve after 1–2 h at room temperature in a microtubule-stabilizing buffer containing 1% Triton X-100 showed that luminal particles were still present in axonal microtubules, even though the rest of the axon, often including neurofilaments, had disappeared.

In cross sections of axonal microtubules seen at moderate magnification, the luminal material generally shows a round profile that is roughly centered in the lumen (Fig. 4*a*), even though in some cases it appears eccentrically located or transversely elongate (Fig. 4, *c*, *h*, and *i*). The luminal profiles vary somewhat in size, ranging in diameter from 4.0–6.0 nm. At high instrument magnification (> × 50,000), the structure of this dense material is more variable; in some instances the material appears bipartite (Fig. 4, *d*, *e*, and *g*) and many of the luminal masses show a substructure that suggests that they are made up of spherical particles (Fig. 4, *d*, *e*, *g*, and *i*). In some cross-sectioned microtubules, wispy material is seen to extend radially from the luminal material to the wall of the microtubule (Fig. 4, *b* and *f*). In Fig. 4*i*, where the microtubule is cut slightly obliquely, three distinct spherical elements are apparent. Measurements of a number of such subunits indicate that they are ~4 nm in diameter. When olfactory axonal microtubules are seen in longitudinal profile, the density of the luminal material is usually such that it cannot be

identified with certainty, since it is seen against the wall of a microtubule. In oblique sections of microtubules luminal particles are more easily seen, often appearing as a short chain of beads (Fig. 4*i*). Luminal material is seen in axonal microtubules fixed without secondary fixation in osmium tetroxide, as shown in Fig. 4*l*, even though the luminal elements are not as electron-dense as they would be with secondary fixation. No evidence of luminal particles could be seen in negatively stained microtubules of olfactory axons. Such preparations were obtained by incubating pieces of olfactory nerve in a microtubule-stabilizing medium containing detergent (4) for 15–30 min, rinsing in detergent-free buffer, macerating the pieces, and adsorbing the macerate to grids for negative-staining in 2% uranyl acetate.

To compare the distribution of luminal particles between microtubules in the proximal and distal ends of axons, olfactory nerves from at least three bullfrogs and three leopard frogs (*R. pipiens*) were divided into three regions: proximal, middle, and distal. Cross sections were obtained of the proximal and distal regions such that counts could be obtained of the number of microtubules in which particles could be seen in their lumina. The results are shown in Table I. In the bullfrog, 81.9% of the profiles of distal microtubules showed luminal particles, whereas particles were seen in only 63.3% of the proximal microtubules. Similar results (76.5% distal
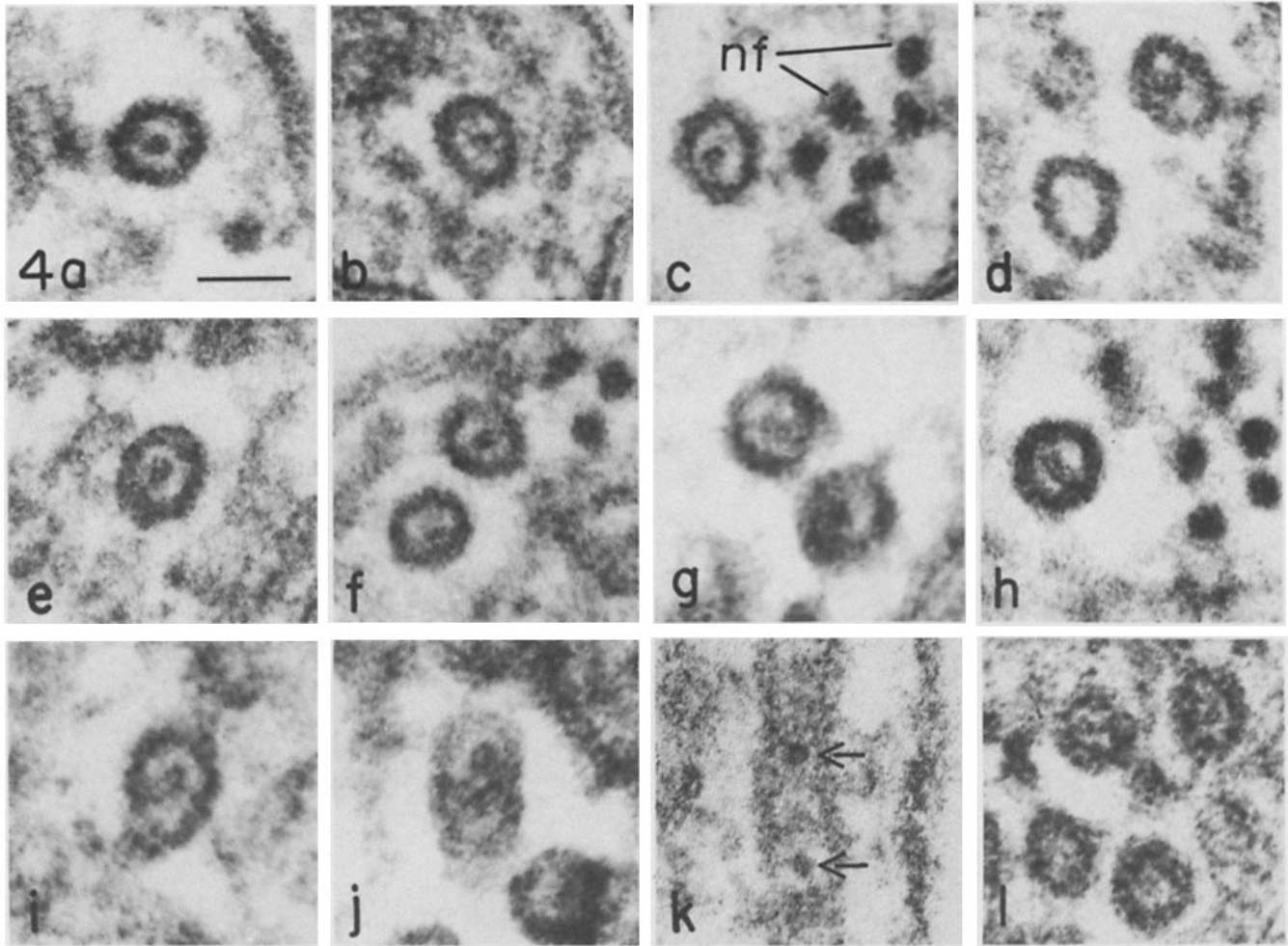
Figure 4 (a–l) High-magnification views of olfactory axonal microtubules are illustrated in this series, and most of the profiles show transverse sections of microtubules containing dense luminal material. a shows a rather typical luminal mass, and as shown in b and f, in some cases the mass appears to be connected to the wall of the microtubule by wispy bridges. Substructure can be seen in the luminal material in Fig. d and e and h–j, and in some instances the material appears bipartite (Figs. d, e, and g); in i, which is a slightly oblique section through a microtubule, a short chain of three apparently spherical particles is clearly seen. Another oblique section through a microtubule is seen in j, where the luminal material appears as a clumped mass of several roughly spherical particles. k shows what appears to be particulate elements (arrows) in a longitudinal profile of a microtubule, and l shows luminal material in microtubules for nerve fixed in the absence of osmium tetroxide. Bar, 25 nm; × 518,000.

and 59.9% proximal) were obtained with olfactory nerves from the leopard frog. Another way of comparing the number of luminal particles in profiles of proximal and distal microtubles is to note that 18.6 and 16.6% more of the distal microtubules showed particulate material in the bullfrog and leopard frog, respectively. Obviously, these data suggest that luminal material in these microtubules becomes more abundant with increasing distance from the perikarya of these axons (i.e., as the microtubules approach their termination in the olfactory lobe of the brain), assuming a uniform number of microtubules from one end of the axon to the other.

To further evaluate the distribution of luminal material in microtubules, sets of serial cross sections of pieces of olfactory nerve were examined. Profiles of luminal particles were often seen to be continuous in a given microtubule in a number of consecutive sections, although the particles usually varied in outline and apparent density (Fig. 5, a–l). In Fig. 5, a–g, luminal material is seen in the microtubule denoted by the arrow in sections a and b, it is apparently missing in c (as indicated by the o-arrow), and then the material appears again

in the next four sections. In Fig. 5, h–l, the microtubule indicated by the arrow shows distinct luminal material in the second, third, and fifth sections of this five-section series. In both sets of serial sections, the luminal particles seen in a given microtubule vary in appearance from section to section. Information to date from studies of serial sections suggests that the luminal material may be a loosely filamentous form, since the same microtubules in a number of consecutive cross sections show a luminal component. The evidence for this is weak, however, when one considers that the sections are probably 50–70 nm thick. For the sake of discussion, assume that the luminal material is made up of globular elements 4–5 nm in diameter. If a number of the globular particles were distributed along the lumen of a microtubule, when this microtubule was viewed in cross section it would be impossible to determine whether one was observing a beaded filament end-on or the collective densities of a string of superimposed, but separate, beads. There are two observations that suggest that material may follow a zigzag path through the microtubule lumen: (a) the profiles of luminal material may

Distribution of Microtubules with Luminal Particles in Frog Olfactory Axons in the Proximal and Distal Ends of the Olfactory Nerve

	Frog. No.	Proximal		Frog no.	Distal	
		No. of MTs counted	MTs with particles		No. of MTs counted	MTs with particles
			%			%
Rana catesbeiana	1	659	78.0	1	618	91.6
	2	608	57.1	2	738	76.4
	3	795	54.8	3	818	80.1
				4	530	79.5
		Mean % = 63.3			Mean % = 81.9	
Rana pipiens	1	1,016	64.5	1	913	79.5
	2	840	59.3	2	1,017	70.8
	3	841	56.0	3	900	79.2
		Mean % = 59.9			Mean % = 76.5	

Microtubules were examined and counted only in fields where they, and the axons in which they were seen, appeared in precise cross-section. Care was taken to evaluate and count microtubules in different regions of the same section. Counts were obtained from micrographs enlarged to × 114,000 using a negative taken at an instrument magnification of × 42,000.
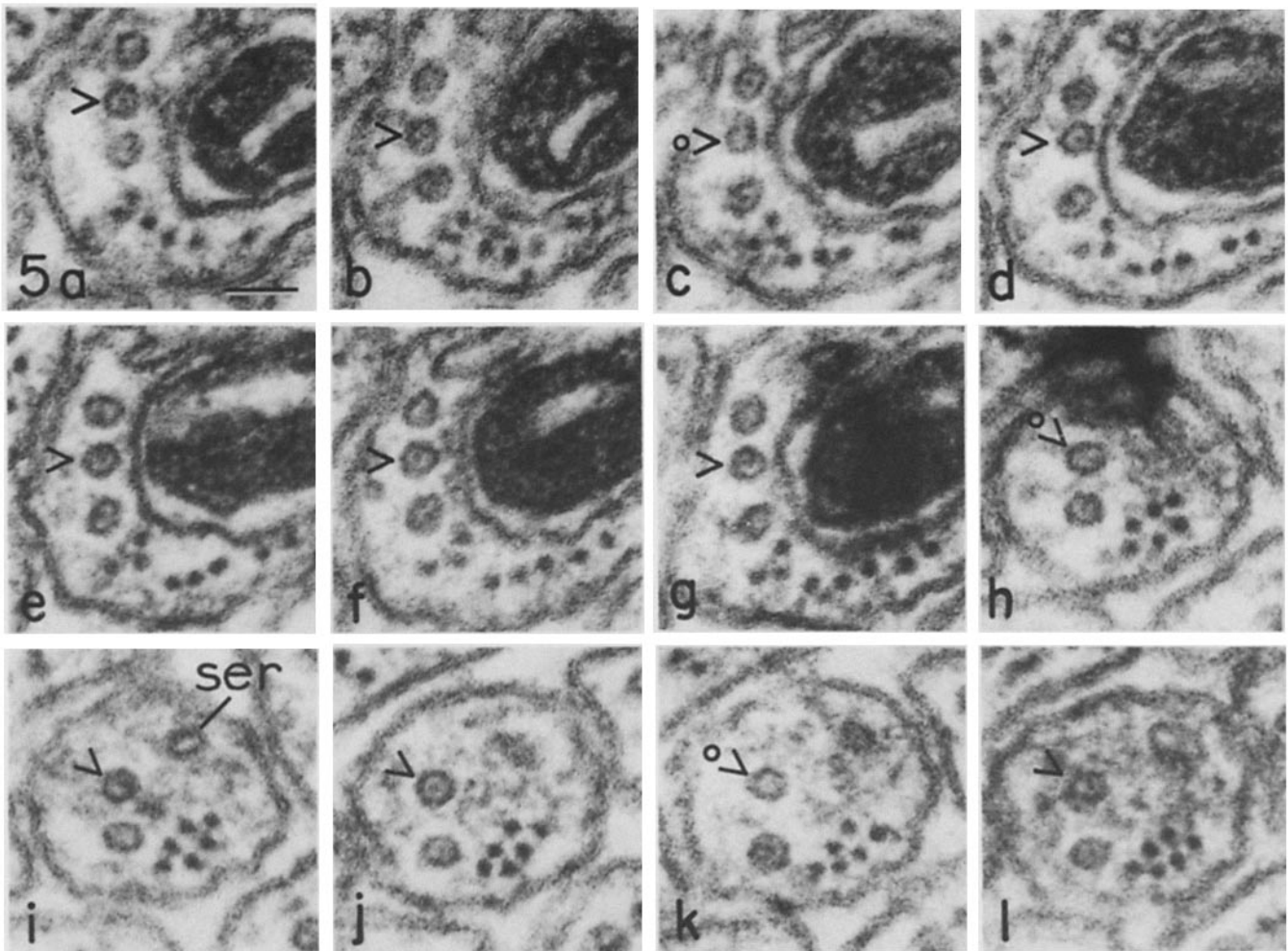

FIGURE 5 (a–l) Two sequences of serial cross sections of olfactory axons are shown which illustrate the discontinuous distribution of luminal material in microtubules. In a–g, the microtubule shown by the arrow is followed through the seven-section series. The microtubule shows luminal material in Fig. a and b, but in c, it is not observed (o-arrow), and it again appears in d–g. In the second series, h–l, the microtubule indicated in h (o-arrow) shows little evidence of luminal material, in i and j it is present, it is not seen in the microtubule in k (o-arrow), and it is again clearly seen in l. Examination of serial sections in this manner indicates that luminal material is distributed in a discontinuous manner in the microtubules. Note the variable appearance of profiles of smooth endoplasmic reticulum (ser) in h–l. Bar, 50 nm. × 210,600.

be eccentrically located, from one side of the lumen to the other, in consecutive serial sections, and (b) the luminal material sometimes shows an elongate profile (Fig. 4h), as if its axis were diverging in a plane perpendicular to the long axis of the microtubule.

Axonal microtubules fixed in the presence of 2% tannic acid provided relatively little information about the nature of luminal material, although the lumina of such microtubules are usually filled with amorphous material of moderate electron density, as shown in Fig. 6. The 13 wall protofilaments of the microtubules are well delineated, although the luminal material is less clearly visualized. An obvious question to ask is whether microtubules polymerized in vitro from isolated olfactory nerve tubulin would show luminal material. Several attempts were made to isolate olfactory nerve tubulin. However, these were unsuccessful, probably because we were not willing to destroy enough frogs to provide the volume of material required. Tubulin from whole bullfrog brains was thereafter isolated and carried through two cycles of purification. Microtubules assembled from frog brain tubulin are shown in Fig. 7, and in this case they were fixed in the absence of tannic acid. The lumina of such in vitro assembled microtubules are clearly empty. If these same in vitro polymerized microtubules were fixed in the presence of tannic acid, most of them showed the expected 13 wall protofilaments and all of them showed empty lumina (Fig. 8). This suggests that the material in the lumina of the brain microtubules from which the purified tubulin was derived is displaced during purification.

Even though microtubules assembled in vitro from frog brain tubulin were devoid of luminal material, it is likely that much of this tubulin was from cells other than those associated with the olfactory tract. Also, considering that neuronal microtubules are somewhat resistant to cold depolymerization,

it is possible that much of the tubulin was derived from cells other than neurons, even though microtubules of glial cells of amphibians are known to contain luminal material (12). To determine whether microtubules polymerized in situ in olfactory axons showed luminal material, pieces of isolated olfactory nerve were incubated in cold (0-4°C) nerve saline containing 10 mM CaCl and 10 μg/ml calcium ionophore. After 30-90 min of cold incubation, nerve pieces were rinsed three times (1 min each) in calcium-free saline containing 10 mM EGTA to chelate calcium ions. Thereafter, nerve pieces were incubated 15-30 min at 37°C in calcium-free saline with 10 mM EGTA and 0.5 mM GTP. Material was then fixed in microtubule stabilizing fixative and prepared for electron microscopy. The results are shown in Figs. 9 and 10. Examination of axons from control material shows a reduced number of profiles of microtubules, even though some are present and their distribution is somewhat variable (e.g., in one 400-mesh grid square, axons may show no microtubules at all, whereas in a nearby grid square, several adjacent axons will show a microtubule or two). It should be noted that olfactory axonal microtubules are very stable, and even with exposure to cold (0-4°C) or high concentrations of calcium (to 75 mM) for 1-2 h, variable numbers of microtubule profiles persist. With combined cold and calcium treatment, however, and in the presence of calcium ionophore, the number of microtubules could be reduced in a fairly predictable manner over a time span short enough to minimize osmotic damage. In experimental nerve pieces rinsed with EGTA and incubated at 37°C, axonal microtubules were abundantly seen (Fig. 10). In this material there were groups of axons that appeared slightly larger in diameter than neighboring axons and whose axoplasm was usually less dense. The microtubules in these axons were usually empty and they appeared to be slightly larger in diameter than those in neighboring axons showing
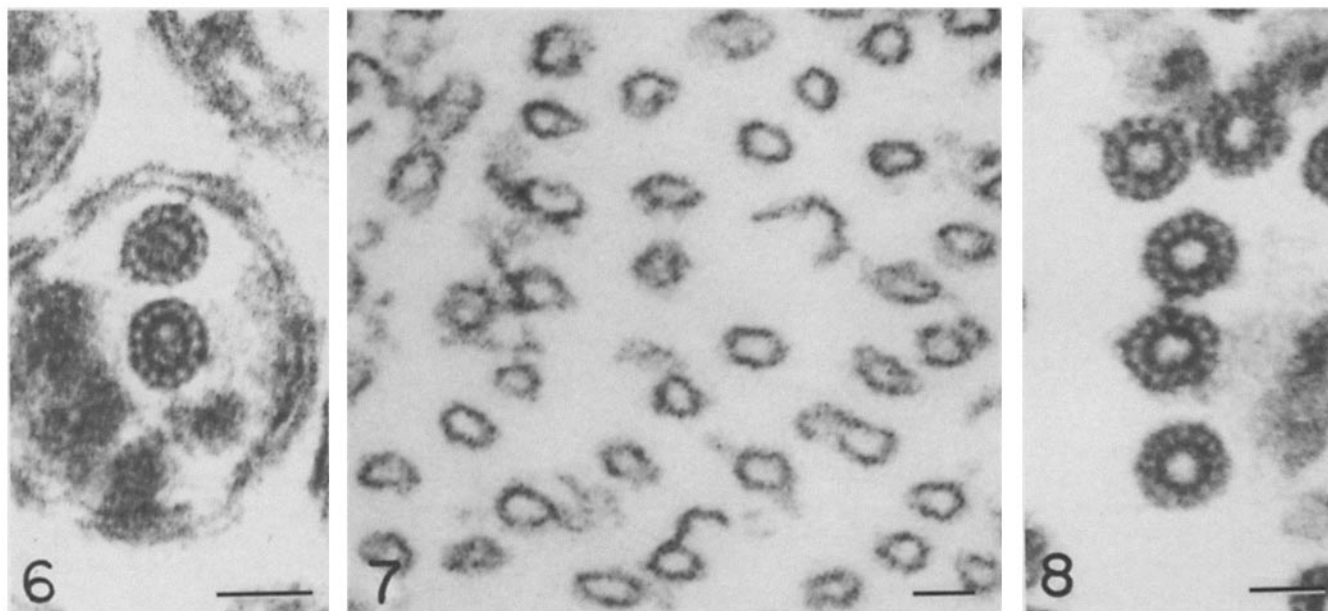
FIGURES 6-8   Fig. 6 shows a cross section of an olfactory axon fixed in the presence of tannic acid to delineate wall protofilaments of microtubules, of which there are 13. Many microtubules in such axons show amorphous dense material in their lumina, as in the two microtubules shown, even though little evidence of substructure can be seen. Fig. 7 shows microtubules assembled in vitro from frog brain tubulin and fixed in the absence of tannic acid. Note that these microtubules show no evidence of luminal material, indicating its loss during isolation of the tubulin. If these microtubules are fixed in the presence of tannic acid (Fig. 8), most show 13 protofilaments and all of them are devoid of luminal material (compare Fig. 8 with Fig. 6). Bars, 25 nm. Fig. 6, × 518,000; Fig. 7, × 345,000; Fig. 8, × 520,000.
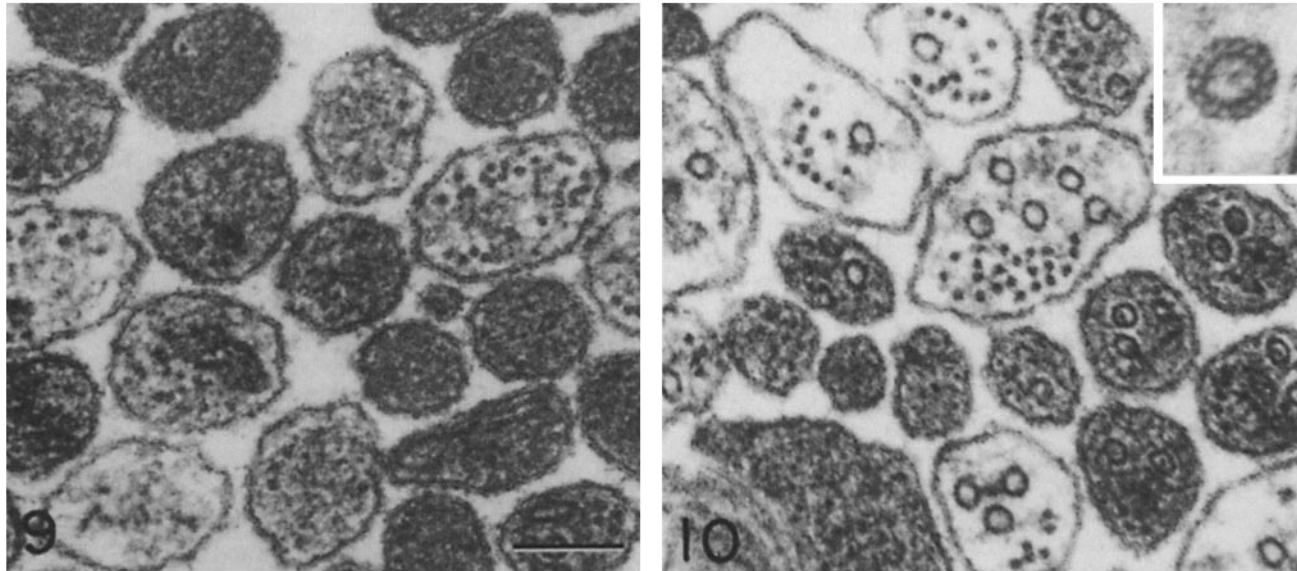
Figures 9 and 10   Fig. 9 shows cross sections of axons from a nerve piece incubated in cold saline containing calcium and ionophore for 1 h, then fixed. Note the loss of microtubules as a result of such treatment; some microtubule profiles can always be found in such material, however, and one is seen beneath the scale bar. Fig. 10 shows cross sections of axons in a nerve piece treated as above, but then rinsed in EGTA and incubated at 37°C for 30 min to induce reappearance of microtubules. After warming, microtubules are abundantly seen and many show lumina devoid of dense material, particularly in axons with less dense axoplasm. The empty microtubules often appear larger in diameter than normal, and these have 14 (as shown in the inset) or even 15 wall protofilaments. As discussed in the text, the evidence suggests that the in situ disassembly and induced reassembly of microtubules results in the appearance of a significant number of empty microtubules, and these probably assembled de novo rather than by addition of soluble tubulin to cold-resistant fragments of preexisting microtubules. Bar, 100 nm. Figs. 9 and 10, × 14,700; inset, × 435,500.

microtubules with luminal particles. In Fig. 10, for example, 23 microtubules can be seen, 14 of which are empty and 9 of which contain luminal material; 12 of the 14 empty microtubules are seen in axons having a "clear" axoplasm. Fixation in the presence of tannic acid revealed that the larger-appearing microtubules actually contain 14 (inset, Fig. 10) or even 15 wall protofilaments instead of the normal 13, and it is concluded that these microtubules were assembled de novo. Further, in these microtubules it is obvious that material was not usually incorporated into their lumina during assembly.

DISCUSSION

Echandia et al. (9) first focused attention on the existence of dense material in the lumina of microtubules of neural origin, and such microtubules, as seen in neurons and glia of the toad, were said to contain a "dense core" of granules irregularly distributed in the microtubule lumen. The present work generally confirms this observation. Other workers have made passing reference to the occurrence of dense luminal material in various kinds of microtubules (1, 2, 5, 11, 13, 14, 21, 24, 26, 29, 31, 34). As to the detailed morphology of luminal material, only Echandia et al. (9) attempted to visualize substructural detail at high instrument magnifications. It was noted that the luminal material appeared to be made up of granules and that in some cross sections of microtubules two or even three granules could be seen. Studies of microtubules of frog olfactory axons suggest that the luminal material is made up of globular elements 4–5 nm in diameter which are loosely strung together to form a discontinuous column. Several authors have suggested the possibility that the luminal material may represent molecules being transported within microtubules (8, 9). In this regard, Slautterback (30), who

coined the name "microtubule" and who directed attention to these linear elements as ubiquitous organelles of cells, first suggested that the lumen of microtubules might serve as a conduit for ions and small molecules.

Luminal material is missing from microtubules assembled in vitro from frog brain tubulin, and when microtubules are depolymerized in situ in olfactory axons, then induced to reassemble, most of the microtubules formed de novo are empty. Furthermore, in sectioned material luminal particles appear not to be intimately associated with the inner surface of the microtubule, and the distribution of the material in the lumen of the microtubule is highly variable. These observations suggest that the luminal material is not an integral structural component of the microtubule itself and probably is not tightly bound to tubulin. The volume of luminal material is small compared with the volume occupied by the microtubule wall. For a given segment of microtubule 25 nm in diameter with a wall 5 nm thick, where a core of luminal material 4.5 nm in diameter occupies half the length of the segment, one can calculate the volume of the core cylinder and relate it to the volume occupied by the microtubule wall. Calculations indicate that, considering the total volume occupied by the microtubule wall and a core of luminal material half the length of the segment, the volume of luminal material would be ~2% that of the total (disregarding any associated proteins bound to the surface of the microtubule).

In experiments involving in situ depolymerization of axonal microtubules using combined cold and calcium treatment, followed by induced reassembly of microtubules, it was noted that many of the microtubules that "reappeared" were empty; however, there were more microtubules that showed luminal material. An explanation for this may be related to the observation that, even after prolonged cold and calcium treatment,

many microtubule profiles could be found in axons of control material. Thus, the microtubules that showed luminal material after treated pieces of nerve were incubated at 37°C may have been assembled by addition of tubulin to segments of preexisting microtubules that resisted the depolymerization treatment, with luminal material once again trapped within microtubules. An alternative possibility is that the cold-calcium treatment loosened up the wall lattice of many microtubules such that they could not be readily seen in sectioned material; when axons were then exposed to an environment favoring assembly, the wall lattice assumed its original configuration as a discrete tubular element with luminal material retaining its position within the tubule.

Chalfie and Thomson (6) studied the oversized (29 nm) microtubules in mechanoreceptor neurons of a nematode worm. Serial sections indicated that such microtubules may show a darkly stained core as they terminate, and that the filled endings are correlated with the proximal ends of the microtubules lying within the anterior projections of the neuronal processes. The distal ends of these discontinuous microtubules, on the other hand, appeared diffuse. While terminations of microtubules are frequently seen in processes of nematode neurons, endings are rarely seen in olfactory axonal microtubules. Preliminary studies of serial sections involving tracing 1,001 microtubules having a total length of 868,560 nm revealed only eight discontinuities, and some of these may have been due to fixation. In the case of the aforementioned nematode neurons, the mean of the calculated "average microtubule lengths" (3, 16) is 13.5 μm, whereas the average microtubule length of frog olfactory microtubules is at least 100–200 μm (based on conservative preliminary data). The dense luminal material in olfactory axonal microtubules does not resemble that associated with termination points of nematode neurons, which could represent organizing centers for these microtubules (6). However, some of the nematode microtubules show discontinuous luminal material identical in appearance to that seen in olfactory axonal microtubules, and Chalfie and Thomson (6) note that "... stain is also seen in the core at intervals along the length of the microtubule, but it is fainter than the core-staining at the ends."

Even though the proximal and distal ends of microtubules of nematode neurons showed a characteristic structure, being filled or diffuse (6), Bray and Bunge (3) noted that microtubules followed in serial sections of axons of cultured dorsal root ganglia ended abruptly without "... obvious ultrastructual specialization." Central densities, or luminal material, was followed in serial sections of microtubules and found to be discontinuous, even though material could be followed for a number of sections. The average length of the axonal microtubules followed by Bray and Bunge (3) was 108 μm, and preliminary estimates of the average length of microtubules of frog olfactory axons are in keeping with this value.

Significantly more luminal material is found in microtubules in axons of the distal portion of the olfactory nerve, and this observation is consistent with the hypothesis that the material is translocated in an anterograde direction and accumulates distally, even though to date I have been unable to experimentally demonstrate such transport. Two important questions related to the existence of luminal material in microtubules are, first, what is its chemical composition and, second, how does it gain access to the interior of the microtubule? Answers to these questions will represent important

steps toward establishing its function, which probably is related to the great length of axons and the role microtubules play in maintaining axoplasmic integrity. As to how the material gains access to the interior of the microtubule, there are three obvious possibilities: (a) it represents material in transit from one or the other of the two ends of a microtubule, (b) it becomes enclosed within the microtubule during the tubule's assembly, and (c) it represents tubulin or a tubulin-associated component released from the wall of the microtubule into the lumen. The first two possibilities appear to be the most viable of the three and the indirect evidence presented in this paper would seem to favor the first alternative. It is worth considering whether the luminal material could be tubulin, and whether axonal microtubules may function as conduits for their own building-block subunits.

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