The Cytoskeleton of Nerve Cells in Historic Perspective

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Introduction

Neurons can be usually distinguished at once from most other cells by their peculiar multi-branched shapes, which allow them to maximize their spatial reach and surface-to-volume ratios while keeping metabolically manageable individual sizes. Each of them is capable of departing so much from the basic round form of cells, and of growing and sustaining their characteristic ramified morphology, mainly because of the development of an intracellular framework constituted of filamentous structures collectively known as the "cytoskeleton". Nowhere else are the advantages of possessing this highly complex and versatile scaffolding, common to nearly all eukaryotic cells, more apparent than in neurons. It is hardly surprising, therefore, that its existence, chief components and functions were commonly found or first studied in nerve tissue. The history of how the biologically important concept of a cytoskeleton emerged is, therefore, from the very beginning, to a large extent a history of the neurocytoskeleton (for a survey of how the general notion of an internal cell skeleton originated and evolved, see Frixione, 2000). The following paragraphs will attempt to summarize the main chapters of this Odyssey.

Precedents before the 19th century

Ever since Antiquity, nerves have been regarded as conduits for some sort of vehicle that rapidly carries information between different parts of the animal body, typically communicating impressions from the sense organs to the brain as well as commands from the latter to the muscles. The carrier itself was originally conceived as a special mixture of air and fire called *pneuma* (see Solmsen, 1961; Temkin, 1977), a term later on Latinized as "spirits" (e.g. Descartes, 1664), which would flow within the nerves just as the blood streams along arteries and veins, only much faster despite the former being in general considerably narrower passages. Several varieties and even doubts started to appear on this model since the Renaissance (reviewed by Clarke, 1968, 1978), yet it was rather surprising that the first microscopical inspections of nerves failed to show any clear ducts in them (Malpighi, 1666; van Leeuwenhoek, 1674). Fortunately, however, a closer look with presumptively improved optics revealed that the nerves are actually composed of "very minute vessels of an incredible thinness ... [i.e. axons] ... running along by the sides of each other ... [and that] the cavity of each of these small vessels is about two thirds its diameter"

(van Leeuwenhoek, 1717; see also Van der Loos, 1967; Brazier, 1984). However, the first known microscopical examination of the contents of such tiny tubes, carried out more than 60 years later, produced results that were deemed quite incompatible with the supposedly swift flow of "spirits" or any other thin fluid along the nerves. As judged from the soft dough that could be extruded from transversely cut nerves, the "primitive cylinders" were filled with "a glutinous, elastic, transparent material, which [...] seemed to be formed of granular filaments, tenacious and elastic, which the water could neither dissolve nor separate" (Fontana, 1782).

The Classic Period

The number of opinions about the fillings of the small cylinders and globules seen to constitute the bulk of all nervous tissues increased along with the expansion of microscopical research in the 19th century. Yet the two main themes that were to dominate the scene can be found exemplified in descriptions provided already in the mid-1820s by two French authors: whereas René-Joachim Dutrochet (1824) could only see a "fluide diaphane" within nerve fibers of the frog, Henri Milne-Edwards (1825) believed those of the rabbit to contain or be composed of "fibres élémentaires", themselves consisting of long chains of diminutive globules. Rapidly evolving technical skills and quality in optics - particularly the introduction of achromatic objectives - paved the way for more reliable observations and further controversy. The longitudinal "cavity" originally described in nerve fibers (van Leeuwenhoek, 1717; see above) was now found clearly visible as a transparent "primitive band" coursing throughout the core of certain (myelinated) nerve fibers of vertebrates (Remak, 1838). But whereas such "cell-cavity" appeared just "to be filled by a firm substance" according to Theodor Schwann (1839), the corresponding axial region of the wider nerve fibers of some invertebrates was described by Robert Remak (1843) as containing an uninterrupted bundle of very delicate filaments or fibrils. Furthermore, when at least some of those nerve fibers were demonstrated to be tubular processes anastomosed to, and therefore continuous with, nearby globules or nerve cells, the central fibrillar bundle within a given nerve fiber would occasionally be seen to reach into the body of an associated cell, and form therein concentric layers of fibrils around the nucleus (Remak, 1844; see Figure 1).

Figure 1: One of the first two known illustrations of the cytoskeleton shows a ganglion cell of the crayfish nerve cord, in which concentric layers of delicate fibrils appear surrounding the nucleus and converging as they enter the axon. The fragile fibrils tend to break down as free grains near the cut end of the axon (Figure 9 in Remak, 1844).

Inconsistencies in the descriptions of internal fine structure in nerve fibers and cells would occur even in the writings of a single author. The highly respected Albrecht von Kölliker, for example, at first interpreted the axial "primitive band" as "obviously quite solid, most generally homogeneous,

but not infrequently also, faintly striated or very finely granular ... and also perhaps with an irregular, even jagged border" (von Kölliker, 1852). It was already evident at the time that at least some of these differences might be attributable to an inherent lability of the fibrillary material, which "on account of its great fragility breaks down easily into a powdery mass" (Remak, 1844, p. 469). There were specific warnings about this susceptibility: "The fibrils rarely appear as straight delicate filaments [...] The lightest pressure, the smallest displacement, breaks and bends them about in various ways, so that with the usual magnifications of 300-600 they always have a slightly granulated appearance" (Waldeyer, 1863). When properly selected and handled, however, some nervous tissues displayed such well-ordered arrangements of protoplasmic fibrils that their actual reality could hardly be doubted. A prime example of this was offered by the large ganglion cells of the torpedo fish, which

"removed from the living animal, and prepared in serum, in which they were capable of being easily isolated, possess, both in their processes and in their proper substance, an exquisitely delicate fibrillar structure [...] giving the impression that the whole mass of fibrils given off by ganglion cells only traverse it [...] and thus the fibrils which are seen traversing the substance of the ganglion cell do not originate in the cell, but only undergo a kind of arrangement in it, and then pass to the axis-cylinder process, or extend into the other branched processes" (Schultze, 1869; see Figure 2).

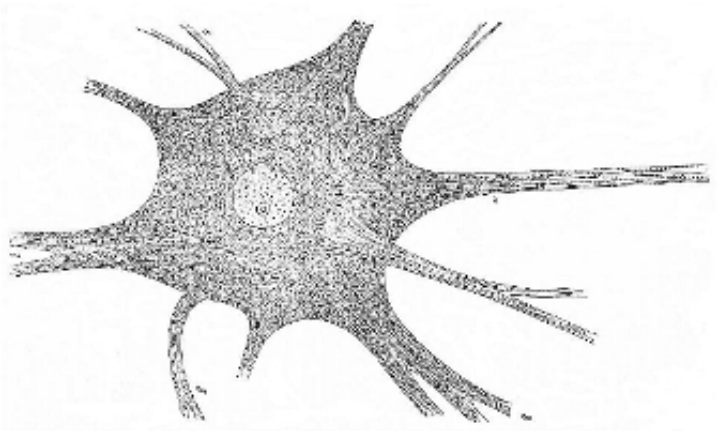

Figure 2: According to Max Schultze, the fibrils merely undergo a rearrangement or change in direction within nerve cells that have multiple branched processes, like this ganglion cell of the torpedo fish (Figure 30 in Schultze, 1869; here rotated 90^{o} clockwise).

Nevertheless, caution was strongly recommended in accepting this picture (Heitzmann, 1883). Moreover, the contents of the large nerve fibers in the ganglionic chain of the crayfish, i.e. the very animal in which the fibrils had been discovered by Remak almost 40 years earlier (see above), were described as " perfectly pellucid, and without the least indication of structure" (Huxley, 1880). In other words, the dispute over the existence of fibrils threatened to persist endlessly. "One author thinks of the nerve cell as granulated, the other as fibrillose; one thinks of the nerve fiber as a bunch of fibrils but another as a liquid column," expressed an understandably frustrated Sigmund Freud (quoted by Jones, 1953), who also investigated the matter himself while he was a research student in the laboratory of Ernst von Brücke at the Institute of Physiology in the University of Vienna. His own unambiguous conclusion after thoroughly inspecting again the crayfish nerve cells and fibers was clearly put in one of his last papers on basic science: "The nerve cells in the brain and in the ventral ganglionic chain consist of two substances, of which one, arranged like a network, is [found also] in the fibrils of the nerve fibres; the other extends homogeneously as the interstitial substance itself" (Freud, 1882; see Figure 3 and Frixione, 2003).

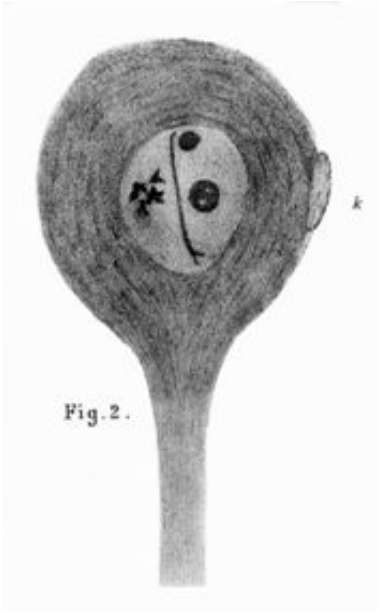

Figure 3: The young Sigmund Freud decided to inspect again the ganglion cells and fibers of the crayfish nerve cord to determine, under the strictest conditions, the truth about the existence of the controversial fibrils described by Remak more than 40 years earlier. His own observations confirmed Remak's findings (Figure 2 in Freud, 1882; cf. Figure 1 above).

Skepticism about the reality of fibrils in the protoplasm of nerve cells and fibers, due to a large extent to their very brief persistence in excised living material before degradation started, diminished following the appearance of a relatively reliable method for staining them in fixed nerve tissue (Kupffer, 1883). Acceptance was also helped by reports of filaments or fibrils in other instances, such as cells undergoing division (Flemming, 1880), egg cells (van Beneden, 1883), and sperm flagella (Jensen, 1887; Ballowitz, 1890). As staining procedures improved, however, a new controversy erupted, this time concerning the function of the fibrils in the nervous system. Not only was their presence reported in an increasing number of animal species, but in addition crisply stained fibrils could be seen coursing their way through long stretches of nerve fibers in favorable preparations taken from invertebrates (von Apáthy, 1897; Bethe, 1898b), as well as from vertebrates (Bethe, 1898a). These findings gained new credibility for a physiological hypothesis that had been put forward originally by Max Schultze (1869), namely that the fibrils, henceforth known as "neurofibrils" (Bethe, 1900), might be the actual conducting pathways for the nerve impulses (see Figure 4).

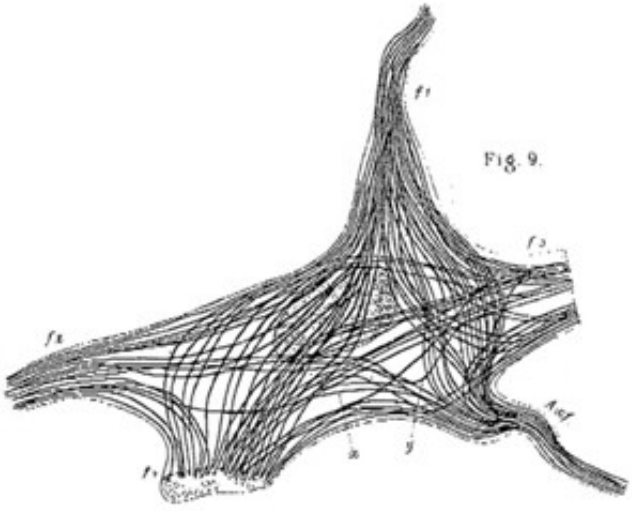

Figure 4: Fibrils coursing across a large cell in the nerve cord of a dog. Some authors, such as Apáthy and Bethe, concluded that neurofibrils could be the actual pathways for the conduction of

nerve impulses, forming a continuous network in which the nerve cells would act just as nodes for the convergence and divergence of neurofibrils (Figure 9 in Bethe, 1898a).

In this view the nerve cells and fibers acted as protective and insulating envelopes for the conducting neurofibrils, which would be wired in a virtually uninterrupted network across the whole nervous system, from sensory terminals to nerve centers to effector muscles. This ingenious model had the added merit of claiming the middle ground between the two currently rival interpretations about the general organization of the nervous system. It retained the basic concept of a continuous reticular array of conducting elements, as inferred from the most advanced staining techniques of the day (von Gerlach, 1873; Golgi, 1886), while accommodating also the novel "neuron doctrine", according to which nerve cells are basically independent units, just like other cells, though extensively interconnected by means of numerous ramifications and long processes (Cajal, 1889; Waldeyer, 1891; see also Shepherd, 1991). For a thorough and conciliatory contemporary review of the various opinions see Pugnat (1901).

The compromise suggested by von Apáthy and Bethe did not sit well with leading hard-core neuronists like Cajal (see Frixione, 2002), who soon objected the idea of assigning a specific conducting function to the neurofibrils because: "The existence of intraprotoplasmic fibrils is a general anatomical law of the cell. More or less modified in their disposition, intracellular threads have been found in skin epithelial cells, in the corpuscles of the lashes, in the egg cell [...] and nobody will think of inferring from this fact that the above mentioned threads constitute the obliged pathway for the light, heat, electric or mechanical waves" (Ramón y Cajal, 1903a).

In fact, Cajal got so excited about the challenge posed by the neurofibril hypothesis to the neuron doctrine, that he promptly developed a superior and quite reproducible method for staining the tiny filaments in nerve tissues of virtually any source (Ramón y Cajal, 1903b), which compared favorably with another excellent procedure (Bielschowsky, 1902, 1903). His own research on this problem produced ample evidence that neurofibrils are strictly intracellular structures, and therefore could not bridge between contiguous nerve cells or their processes as required for conducting impulses (Ramón y Cajal, 1903a; 1908). In addition, he and one of his close collaborators showed that the arrangement of the neurofibrils within nerve cells would change depending upon certain factors affecting the animal, such as hibernation, acclimatization or a pathological condition like infection with rabies virus (Ramón y Cajal, 1904a,b; Tello, 1904; see Figure 5).

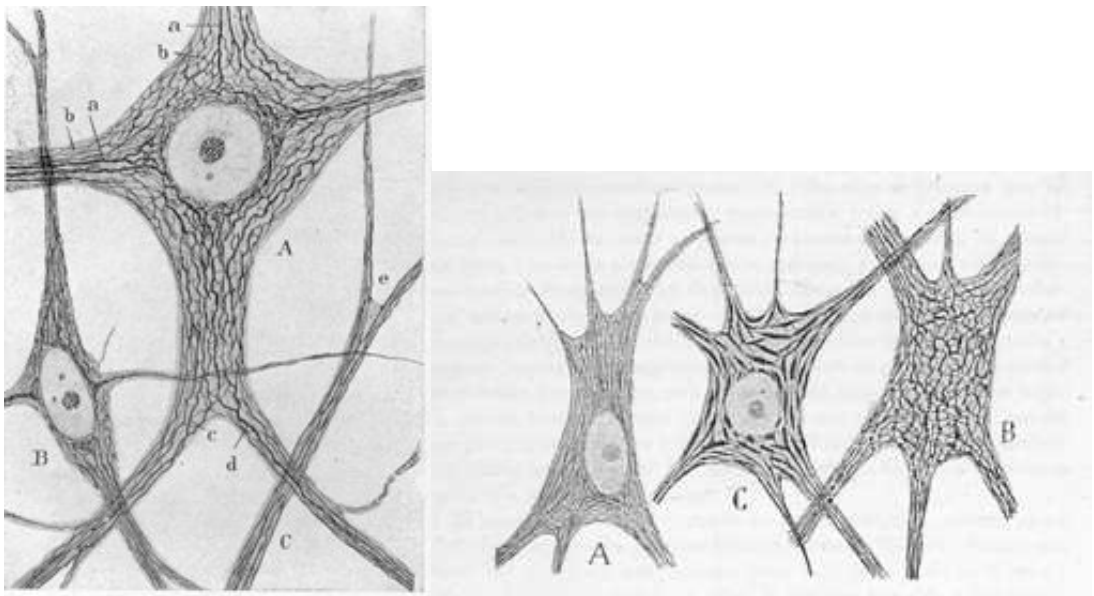

Figure 5: Neurofibrils in neurons from the nerve cord of rabbits kept at different temperatures, prepared by Cajal using his acclaimed staining method with reduced silver nitrate. Normal appearance of the neurofibrils (left), and their altered morphologies after the animals were

exposed to either heat (right, A) or cold (right, B and C) stress (Figures 2 and 5 in Ramón y Cajal, 1903b and 1904b, respectively).

The subject of the neurofibrils became so important indeed for Cajal's concept of the neuron that it occupies a fair portion and five figures of his Nobel Prize lecture (Ramón y Cajal, 1906). A balanced assessment of the issue was also included in the French version of his monumental treatise on the histology of the nervous system (Ramón y Cajal, 1909). Continuing beyond these footsteps, another outstanding member of his school would later on contribute extensive studies on the fibrils characteristic of glial and epithelial cells (Río-Hortega, 1916, 1917).

The first decade of the 20th century was perhaps the zenith of research and vigorous contention about the neurofibrils. Incidentally, it was in the middle of this period when, using Bielschowsky's staining method, Alois Alzheimer first described "very strange changes of neurofibrils" in the brain of a female patient that had been afflicted with the disease now known by his name (Alzheimer, 1906). The hour of neurofibrils soon came to an end, however, in the wake of new electrophysiological findings hinting that the conduction of nerve impulses is a process taking place on the surface of the nerve cells and fibers, rather than through a pathway in their protoplasm (Bernstein, 1902; see also Brazier, 1988). It did not help that neurofibrils, like other intracellular filaments in general, had become suspect of being mere artifactual products of the histological techniques used for the preparation of microscopical specimens (Hardy, 1899; Bayliss, 1915).

A dark interlude

Thus, just after being placed for a short period at the very center of neuroscience theory, the neurofibrils went into relative obscurity. Nevertheless, debate persisted because even if their existence as real cellular components was tentatively granted, the question of their possible functions remained wide open. Any role in the conduction of nerve impulses was fully ruled out as it was realized that these do not necessarily follow the intracellular course of neurofibrils. Thus, for example, crab neurons, in which the axon subdivides into one central and one peripheral branch, would continue to conduct electrical impulses straight from one axonal branch to the other, even after the intermediate cell body and corresponding loop of neurofibrillar tract had been eliminated (see Parker, 1929). An alternative option was that neurofibrils might constitute an internal framework for supporting the shape of the nerve cell (Koltzoff, 1906, 1912; Goldschmidt, 1910; von Lenhossék, 1910; von Szüts, 1914), but this idea was virtually discarded in consideration that the fibrils seemed too flimsy or unstable for that purpose (Marinesco, 1914; Bozler, 1927). Still another possibility referred to the neurofibrils as being involved in the distribution of metabolic influences exported from the cell body into and along its various branches and processes (Parker, 1929; Rényi, 1932).

Persisting skepticism about the neurofibrils was largely the result of understandable reservations about the reliability of observations made on fixed and stained preparations, on one hand, and of the scarcity and contradictions of studies on living material, on the other. The findings were indeed quite inconsistent even in fresh specimens taken from either vertebrate or invertebrate sources (for a brief critical discussion of the state of the matter at the time, see Cowdry, 1928). Distinct neurofibrils were found in living nerve fibers of jellyfishes (Bozler, 1927) and lobsters (Rényi, 1929, 1932), but just a faint longitudinal striation was reported in nerve fibers of frog tadpoles, and then only if these nerves were previously subject to irritation (Speidel, 1935). An analogous delicate longitudinal striation - suspected by the author to be perhaps an artifact or damage caused by manipulation - was observed also in the squid giant axon (Young, 1936), soon to become a key protagonist in basic neuroscience (Hodgkin and Huxley, 1939). And yet birefringence measurements of fresh squid axoplasm suggested that a fraction of its entire protein content was longitudinally oriented, as it would be expected from the presence of neurofibrils (Bear et al., 1937). Similarly, neurofibrils observed in cultured ganglion cells of the chick were interpreted as somehow reflecting the underlying molecular organization of the protoplasm (Weiss and Wang, 1936). It was around this time that a totally new kind of microscopy was introduced in physics and material science laboratories, and it soon began to be tested for use with biological

specimens as well.

Electron microscopy arrives

The inspection of cells with electron microscopy posed significant problems not usually found with inorganic samples. The specimens had to be (1) fixed and fully dehydrated in order to stand in a vacuum, (2) greatly reduced in thickness so that the electron beam could pass through them, and (3) preferably stained with a heavy metal in order to increase image contrast. A straightforward early attempt to inspect the contents of nerve fibers with this powerful instrument consisted of simply smearing droplets of squid nerve axoplasm over thin collodion membranes attached to the small wire-screens used as specimen holders, and then inserting these into the microscope after the preparations had been thoroughly air- or freeze-dried (Richards et al., 1943; see Figure 6).

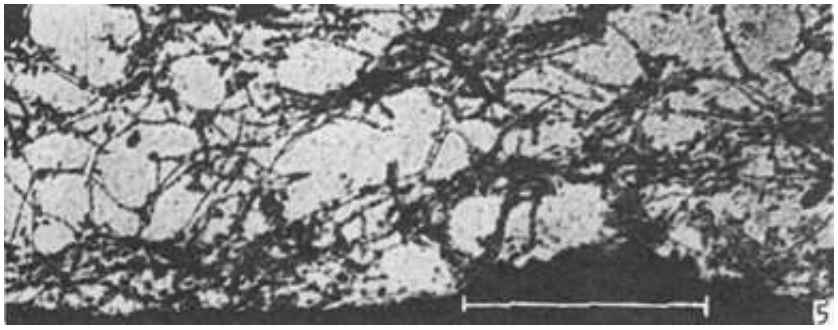

Figure 6: "Axon filaments" (neurofilaments) in a myelinated fiber of rat sciatic nerve, in one of the earliest electron micrographs of nervous tissue (Figure 5 in Schmitt and Geren, 1950).

Numerous fibrils of various sizes and configurations were revealed in these samples, depending on whether they had been left untreated or washed in either distilled water or salt solutions. Another practical approach devised to meet the strict requirements of electron microscopy was to fragment at random fresh or formaldehyde-fixed pieces of tissue, and depositing an aliquot of the resulting suspensions over the collodion film. When different types of nerves from various sources were so prepared, hitherto unknown long fibers measuring 400-800 Å in diameter, presumptively released from within axons and apparently having an empty central cavity, were found and called "neurotubules" (De Robertis and Schmitt, 1948). Within a short time, however, as attempts to produce ultra-thin sections of otherwise whole biological samples began to succeed, and the maintenance of structural integrity consequently improved, it was made clear that axons do not contain such wide "neurotubules" but rather unbranched filaments of indefinite length and just 100-200 Å in diameter (Schmitt and Geren, 1950; see Figure 6). Nevertheless, in certain specimens these individual linear filaments or threads appeared "linked up to form an irregular three dimensional network" (Rozsa et al., 1950; see Figure 7).

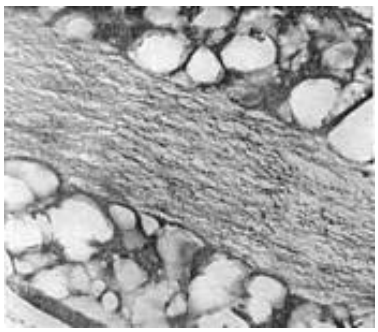

Figure 7: Electron micrograph of a myelinated fiber of rabbit nerve, in which axon filaments "seem to be tied together to form a three-dimensional mesh" (Figure 4 in Rozsa et al., 1950; slightly cropped from the original).

The abundance of these axonal filaments, and the gradual formation of a distinct longitudinal

striation within nerve fibers as they were being fixed while observed under phase-contrast microscopy, led to the suspicion that classic neurofibrils might perhaps consist of those filaments bundled together by the harsh action of osmium-based fixatives (Fernández-Morán, 1952). Indeed, it was soon confirmed that the morphology of neurofibrillar material under the electron microscope depends entirely on the method of fixation employed (discussed by Hughes, 1954). And, being the techniques for the preparation of biological specimens for electron microscopy also an active field of research by itself, in the following years thread-like structures of indefinite length with diameters measuring from just 60-70 Å (Hess and Lansing, 1953; Elfvin, 1958) up to 200-300 Å (Palay and Palade, 1955; Vial, 1958) were described in nervous tissue from a variety of origins.

Two main sets of linear structures with different calibers - approximately 100 Å, and 200-300 Å - were eventually distinguished to co-exist in the axoplasm of both unmyelinated (Elfvin, 1961) and myelinated nerve fibers (Metuzals, 1963). As regards the first set of such structures, until then just occasionally called by their future definitive name of "neurofilaments" (De Robertis and Bennett, 1955), evidence was collected that their intracellular distribution and general arrangement corresponded with those of the neurofibrils described by 19th-century microscopists (Gray and Guillery, 1961). Moreover, alterations of those patterns in response to changes in environmental temperature, essentially as reported from light microscope studies more than half a century earlier (Ramón y Cajal, 1904; Tello, 1904; see above), were confirmed by electron microscopy (Boycott et al., 1961; for a brief discussion of how could 19th-century microscopists have observed filamentous structures of widths well below the theoretical limit of resolution of light microscopy, see Frixione, 2003).

The filaments of the second set were not only thicker but also comparatively straight, and having an apparently clear core as if they had in fact a tubular geometry, to the point of being at first mistaken for "elements of the endoplasmic reticulum" (Palay, 1956; Gray, 1959; Whitear, 1960; see Figures 8 and 9). The presence of these long, cylindrical and seemingly hollow rods became more evident following the introduction of glutaraldehyde, a primary fixative to be added before osmium during the preparation of biological materials for electron microscopy (Sabatini et al., 1963).

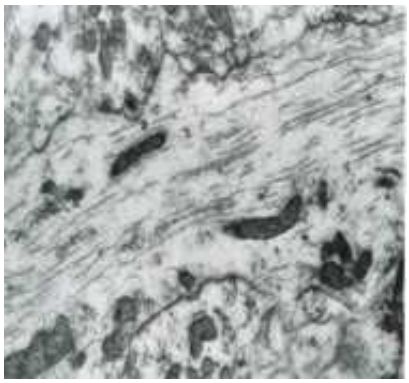

Figure 8: Linear structures in a dendrite in the central nervous system of the rat were originally misinterpreted as "more or less parallel, thin, canalicular elements of endoplasmic reticulum", in one of the first electron micrographs of cytoplasmic microtubules (Figure 1 in Palay, 1956; slightly cropped from the original).

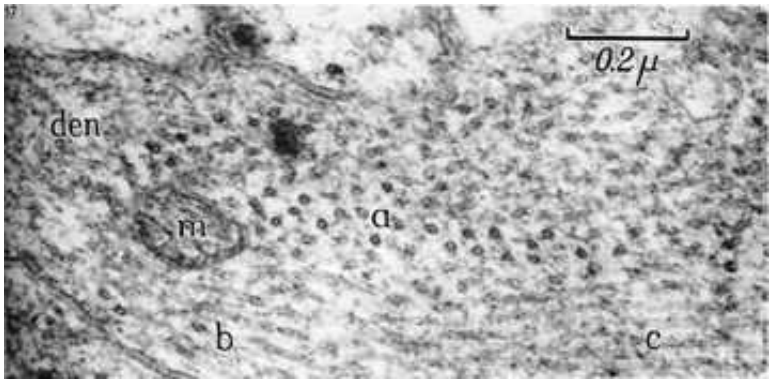

Figure 9: Microtubules oriented in various directions, and therefore cut at various angles in relation to the plane of the section, at a branching point of a large dendrite in the cerebral cortex of the rat (Figure 17 in Gray, 1959).

Similar proteinaceous structures that had previously been either disrupted, otherwise altered or nearly dissolved under the harsh action of osmium-based fixatives, appeared now consistently well preserved in the cytoplasm of a vast majority of eukaryotic cells. These organelles, thus far found consistently in just a few selected specimens and described variously as "ciliary fibrils" (Fawcett and Porter, 1954) or "tiny tubules or canaliculi" (Porter, 1957), among other interpretations, ultimately became known everywhere as "microtubules" (Slautterback, 1963; see also Porter, 1966; Burnside, 1975).

Nevertheless, for some time the microtubules located in nerve cells were occasionally referred to as "neurotubules" (e.g. Gonatas and Robin, 1965), the authors being apparently unaware that such a word had been applied before to a quite different entity (De Robertis and Schmitt, 1948; see above).

Biochemical and functional characterization

Brain tissue was promptly identified as a major source of microtubules, which biochemical work found to consist primarily of two specific proteins later known as "tubulins" (Borisy and Taylor, 1967; Shelanski and Taylor, 1967; Bryan and Wilson, 1971; Feit et al., 1971). Moreover, when incubated under favorable conditions, brain tubulin fractions supported the formation of microtubules in vitro (Weisenberg, 1972; Borisy and Olmsted, 1972). Meanwhile, the characteristic presence of numerous microtubules in nerve fibers, and the high sensitivity of the continuous transport of axoplasmic materials to colchicine, a drug known to inhibit chromosome separation during anaphase in mitosis, suggested that microtubules might play a key role in the mechanism of translocation (Dahlström, 1968; Karlsson and Sjöstrand, 1969; Kreutzberg, 1969; see also Ochs, 2004). Direct evidence was later presented of firm structural associations between migratory organelles and microtubules in neurons (Frixione, 1983b). Not surprisingly, nervous tissue would also become the main source for the purification of force-generating proteins responsible for axoplasmic transport and other microtubule-based examples of intracellular motility (Brady, 1985; Vale, 1985a,b; Paschal and Vallee, 1987; Schnapp and Reese, 1989).

In the meantime "*actin filaments*", i.e. a different sort of linear polymer of protein typically involved in force-generation in muscle fibers, were demonstrated to exist in various non-muscle cells, including nerve cells (Ishikawa et al., 1969). The function of actin filaments, also known as "microfilaments", in nerve cells started to be understood when cytochalasin B, a substance deleterious to microfilaments, was shown to inhibit axonal growth (Yamada et al., 1970; Wessells et al., 1971; see Figure 10). Almost simultaneously, an abundant presence of actin along with myosin, the corresponding molecular motor, was confirmed biochemically in the brain (Puszkin et al., 1968; Fine and Bray, 1971: see Figure 10).

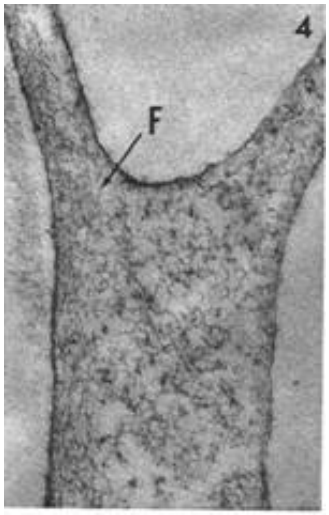

Figure 10: An early electron micrograph of actin filaments (microfilaments) in neurons. The filaments form a loose network within this bifurcating branch (microspike) of the growth cone of an elongating nerve cell, in a culture from explants of chick embryo ganglia. No other cytoskeletal structures, like neurofilaments or microtubules, are present (Figure 4 in Yamada et al., 1970).

In the following years, the sturdy and largely insoluble material constituting the neurofilaments was at last successfully purified from various sources, thus opening the way to its full biochemical characterization. This revealed the neurofilaments as members of the widespread superfamily of the so-called "intermediate filaments" that provide passive structural stabilization to the cytoplasm (see Shelanski et al., 1976; Lazarides, 1980). Comparative studies in well-known and suitable preparations, such as the squid axon, eventually allowed to assess the fractions of total axoplasmic protein corresponding to the three main cytoskeletal components - i.e. tubulin, actin, and neurofilament proteins (Lasek, 1984).

In due recognition

Today, when the cytoskeleton has exploded as a vast and ever expanding field of research in cell biology, it is only fair to point out once again to the seldom remembered work of a those early neuroscientists whose keen observations and well-crafted ideas anticipated some of our current views on the subject. They include of course Robert Remak, the discoverer of neurofibrils (1838, 1844), and Santiago Ramón y Cajal, who contributed the most to the study of their properties in the culmination of the classic period (1903a,b, 1904a,b, and 1908, among many other papers). Correct intuition about the mainly mechanical roles of the cytoskeletal elements was reached by just a handful of microscopists who inspected nerve cells at the beginning of the 20th century (Koltzoff, 1906, 1912; Goldschmidt, 1910; von Lenhossék, 1910; von Szüts, 1914). Still fewer conceived the neurocytoskeleton as a structural system involved in the trafficking of intracellular components along the numerous and lengthy processes of nerve cells (Parker, 1929; Rényi, 1932).

Finally, a word is appropriate also about crustacean nerve cells, upon which many of the classic observations of neurofibrils were made over the years by Remak, Freud, Rényi and others (see above). Such cells proved also excellent specimens for the electron microscopic inspection of the neurocytoskeleton. The crayfish, in particular, once again supplied the large axons of its nerve cord or ganglionic chain to reveal a regular array of uniformly distributed and laterally linked microtubules, in some of the first studies examining the role of microtubules in axoplasmic transport (Fernández et al., 1970, 1971; Burton & Fernández, 1973; Ochs & Burton, 1980). In addition, an elaborate system of overlapping fascicles of microtubules, which seemingly provide a supporting framework for the light-dependent migrations of thousands of screening-pigment granules, was reported in the retinal photoreceptor cells of the crayfish compound eye (Krebs, 1972; Frixione, 1983a,b).

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