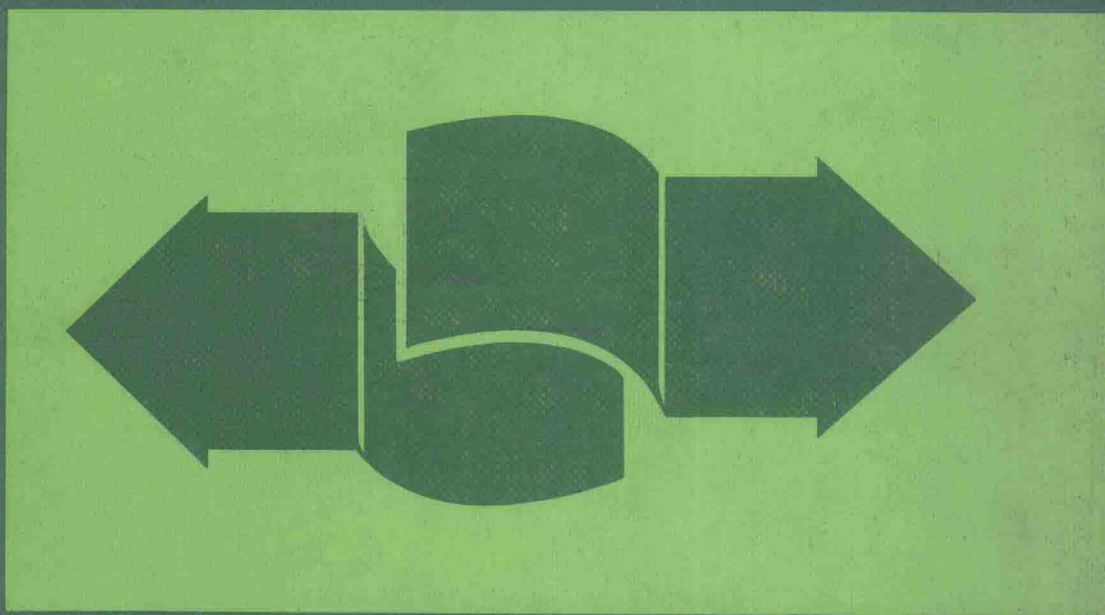
Axoplasmic Transport in Physiology and Pathology

Edited by D. G. Weiss and A. Gorio



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Preface

Cajal and contemporary scientists have laid the basis of the modern concepts of the organization of the nervous system: the circuits of the brain are made up of individual neurons which transfer information via specialized structures called synapses. Soma and dendrites usually receive the inputs, then the signal is carried all along the axon to the target areas. To fulfill this task several types of neurons have developed their unique geometry characterized by a large receptive area (soma and dendrites) and an often very extensive distal branching with the axon terminals. The volume of cytoplasm which constitutes the neuronal periphery is often far larger than the cell body, where the synthetic machinery is located. It is one of the roles of axoplasmic transport to supply the periphery with proper material and to sustain the specialized structures necessary for the physiological activity of the neuron.

Furthermore, it has become more and more clear that target areas also exert effects on the innervating neurons, and these effects are not only mediated via recurrent fibers. Synapses have been shown to be able to pick up material from the synaptic left which is then intraaxonally transported back to the cell body. This retrograde axoplasmic transport has therefore been recognized as another basic mechanism to convey signals from the periphery to the centre. Several articles in this book deal with the role played by anterograde and retrograde transport in the physiology of the normal nervous system, in particular the role played in molecular communication. We now know that in addition to electrical activity, axoplasmic transport is of crucial importance for the function and development of the nervous system.

General effects of several neurotoxins can be explained by their specific effects on axoplasmic transport. Furthermore, the pathogenesis of some viral diseases is incomprehensible without the knowledge of retrograde axoplasmic transport conveying the pathogenic agent from the periphery to the more central parts of the nervous system. Many other alterations of nerve function have been found to be correlated with an impairment of axoplasmic transport. Indeed, it is in most cases not yet clear whether axonal transport causes neuropathies

or whether the disease itself produces changes in axoplasmic transport as a secondary effect. However, several articles collected in this book will show a strong link between the occurrence of neuropathies and alterations of transport.

The publication of this volume was conceived at the occasion of the *Workshop on Axoplasmic Transport* which has held at Schloss Elmau in Bavaria in 1981. Written after that meeting, the contributions to this volume reflect our secured knowledge as well as the questions still remaining to be answered.

The more basic aspects of axoplasmic transport, i.e., its properties and our knowledge and current hypotheses about how its molecular mechanism may work, are the subject of another volume published concomitantly (D.G. Weiss, ed., *Axoplasmic Transport,* contents see p. 193). It is revealing that this volume dealing with the physiology and pathology of axoplasmic transport is the slender one. However, due to the increasing interest of neurologists, neurosurgeons, neurotoxicologists, neuropathologists, ophthalmologists, dentists and others dealing with medical aspects of neurons and the nervous system, this relation is expected to reverse during the next few years.

The preparation of this volume would not have been possible without the help and understanding of many friends and colleagues to whom we would like to express our gratitude. We are especially grateful to FIDIA Laboratories, Abano Terme, Italy, for having supported the preparation of this volume.

München and Abano Terme, August 1982

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Contents

General Properties of Axoplasmic Transport

DIETER G. WEISS [1]

Axoplasmic transport has become a well-established phenomenon whose properties have been described in depth in many publications over the last years. The biological significance of axoplasmic transport is widely recognized. Lacking detailed knowledge of the underlying mechanisms, in this paper all movement of molecules and particles inside nerve cell processes that cannot be related to diffusion or Brownian movement is to be called "axoplasmic transport". This leaves open the question whether one "unitary mechanism" can account for all the phenomena mentioned below, or whether several mechanisms are involved. The term "mechanism" when used in this article is therefore meant to encompass as many different mechanisms as might be needed ultimately to account for all of the phenomena.

This article surveys the general properties of axoplasmic transport which are experimentally verified. It is not intended to cover the literature in detail since an excellent review article was published very recently [41] and since many of these topics are discussed in depth in a recent book [120]. In this article only some, mostly more recent publications are quoted as examples, which do not necessarily represent the first or the only reports on the topics mentioned. If available, preference is given to summarizing and review articles.

The Transported Material

Apparent Lack of Specificity

Axoplasmic transport conveys virtually all axonal and dendritic constituents which have been tested so far. Some of the most common components of nerve cell processes whose transport is well documented are listed in Table 1 without reference to transport velocities and directions. Also, the question whether all or only part of the smaller molecules are bound to macromolecules or organelles during transport is not addressed by Table 1. From this table we can conclude that physicochemical para-

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Table 1. Cellular constituents which are axonally transported

Low molecular weight material:	
Amino acids	[16, 45, 64, 100]
Sugars	[8, 29, 121]
Amines	[57]
Transmitters	[17, 102]
Lipids:	[1, 20]
RNA (tRNA):	[56]
Mucopolysaccharides:	[24]
Neurohormones:	[35, 87]
Proteins:	
Enzymes	[11, 12, 15, 17, 111]
Cytoskeletal elements	[83, 119]
Membrane proteins	[71, 78]
Glycoproteins	[24, 40]
Organelles:	
Mitochondria	[32, 65, 101]
Smooth endoplasmic reticulum	[21, 107, 118]
Multivesicular and multilamellate bodies (prelysosomal particles)	[107, 118, 126]
Synaptic vesicles	[17, 38]
Neurosecretory granules	[27, 35, 87]
Exogenous material:	
Colloidal gold	[19]
Exogenous proteins	[3, 70, 117]
Horseradish peroxidase	[69, 73, 82]
Lectins	[61, 117]
Tetanus toxin	[26, 96, 115]
Viruses	[67]

meters such as size, charge, shape etc. do not determine whether material is transported or not. Thus, when viewed superficially, one might consider axoplasmic transport to be an unspecific phenomenon. However, it will be discussed below that the dynamics of axoplasmic transport of all these substances and organelles differ considerably and display very specific patterns.

Selectivity

Not all proteins or organelles present in the perikaryon are transported. It seems, however, that the process of selection can be separated from the transport proper. Membrane constituents are thought to be selected when certain proteins and lipids are packaged together into membranous organelles derived from the Golgi apparatus [50, 77] which have been proposed to be the exclusive transport form for rapidly transported material [28, 50]. Similarly, the observed selectivity of the transport in identified neurons for certain transmitters can be explained by selective uptake of these transmitters into storage vesicles rather than by selectivity of the transport process

[38, 103]. Ribosomes and dictyosomes are apparently prevented by unknown mechanisms from passing the axon hillock. Again, such mechanisms must be considered not to be part of the transport mechanism since various kinds of exogenous materials, once in the axon, are transported very nicely (Table 1).

The process of loading the material onto the transport machinery may also give rise to the "routing" phenomenon, i.e., transport of various materials in different proportions along the central and peripheral branches of axons of, e.g., dorsal root ganglion cells [83, 92].

Dynamics of Transport

Transport is Directed

Transport may be anterograde (orthograde), that is leaving the perikaryon, or retrograde, that is directed towards the perikaryon. Transport in both directions occurs also in dendrites [79]. The anterogradely and retrogradely transported organelles are different from each other [107, 118]. Reversal of direction of optically detectable organelles is a rare event in undisturbed axons [30, 107].

Behavior at Obstructions

Endogenous or exogenous obstructions of the axon such as nodes of Ranvier, terminals, ligatures, crush or cold blocks cause accumulations of anterogradely and retrogradely transported material at their respective sides [5, 17, 106, 107, 118, 126]. In these cases there is initially no general damming of the axoplasm, but the rapidly transported organelles move towards the obstruction being stopped only in its immidiate vicinity. Part of the material is reversed if the block persists for some time ("turnaround") [6, 107, 110].

Saltatory Movement

Optically detectable organelles (mitochondria, prelysosomal particles) move in a saltatory fashion with periods of movement and periods of rest [31, 97, 109]. The average transport velocity of a population of organelles (see Table 2) is therefore considerably less than the actual saltation velocity of 1–3 μm/s. The dynamics of the saltations are very similar for both directions [108]. The saltation velocities of the visible organelles do not vary with particle size (c.f. [123]). It is unknown whether small organelles below the resolution of the microscope or soluble molecules behave similarly.

Table 2. Characteristic population velocities of materials transported in optic nerve

Transport group	Maximal velocity mm/d	Material transported	Identified components	Destination
I	240–410	Membranous material (tubulo-vesicular organelles), soluble material	Na^+, K^+-ATPase, GAP's	Axon and axon terminal
	20–240	Synaptic vesicles	Protein I (synaptin I)	Axon terminal
II	20–70	Mitochondria and possibly other membrane bounded organelles	F_1 ATPase, fodrin	Axon and axon terminal
III–IV (SCb)	2–20	Axoplasmic matrix ("microtrabeculae"), subaxolemmal specializations, mostly "soluble" proteins	Myosin-like proteins M1 and M2, actin, calmodulin, clathrin, enolase, fodrin, creatine phosphokinase	Axon
V (SCa)	0.5–2	Cytoskeletal elements	α- and β-tubulin, fodrin, 3 neurofilament proteins	axon

Modified from [2] and [72]; for references see [2, 41, 72, 123].
Abbreviations: SCa, SCb: slow components a and b according to Lasek's nomenclature [72, 119]

Constance of Velocity

If the velocity of a whole population of radioactively labeled compounds is determined, one observes that the maximal transport velocities are constant all along the axons [13, 44, 88]. This also holds true for low molecular weight substances [121, 124] whose movement has often been ascribed to diffusion [60, 81]. Constant transport velocities, however, cannot be due to diffusion [100].

Radioactivity Profiles

Characteristic radioactivity profiles occur along the axon if transport is demonstrated in pulse-chase experiments, i.e., if radioactive precursor is available only on one side of the axon and for a limited period of time. The characteristic properties of these profiles are: a leading wave or peak is followed by a plateau or saddle region and the peak is asymmetric, reveals tailing and broadens with time [37, 44, 86, 89, 90]. In rapid transport even the broadening of the peak cannot be explained by diffusion alone [46]. The amount of radioactivity in the moving peaks decreases with time and distance [44, 86, 121]. Similar profiles are obtained for slowly transported materials including cytoskeletal elements in which case the peak broadening is slower [13, 55] and occurs with the velocity expected from diffusion of free protein [46]. Similar

radioactivity profiles have been shown also for low molecular weight substances such as amino acids and sugars, although it is not known whether these are transported as free molecules or sequestered in storage organelles [121, 124].

Transport Velocities

Various types of organelles and molecules move with different, but specific average velocities ranging from 0.5 mm/day up to 370 mm/day. The maximal velocity is in many vertebrate and mollusc nerves 410 mm/day (at 37°C) [23, 44, 88, 104, 121]. Table 2 gives an overview of the transport velocities of a variety of cell constituents. Within the broad spectrum of velocities there are five regions which have been found in mammalian optic nerves to be especially well populated with specific polypeptides [55, 62, 125]. There are, however, substances known to move with intermediate velocities or with a wider range of velocities so that they are reported to be conveyed with more than one transport group (e.g. fodrin, Table 2). Transport groups I–II contain heterogeneous, mostly membrane-bound material and their velocities seem to be very similar for most vertebrates (if temperature is corrected for) [2, 88].

The velocities of the more slowly transported materials vary much more between different species and different nerves (groups IV and V). Apparently the organization of the cytoskeleton is of importance since in nerves with fewer neurofilaments such as the vagus and olfactory nerves the slowest components move with 8–25 mm/day whereas in the optic nerves 0.5–4 mm/day are observed [7, 13, 33, 116]. Table 2 gives the values obtained for optic nerves, however many more data on slow transport in other nerves are required, before we can make more general statements on slow transport velocities.

The question how these components are organized while undergoing transport is presently being debated. The idea was proposed that each one of the five transport groups represents one structural component of the cell [119]. This does not seem to fit the data on the rapid transport groups which contain very heterogenous material [2, 121, 123]. For the slower groups this idea seems to be persuasive in the case of the findings on optic nerves [72], whereas the data on other nerves are less consistent (as is also discussed in [123]).

Retrogradely transported material has been reported to move with rapid velocities of at least 100–290 mm/day [12, 105, 117], but recently also a slow component (3–6 mm/day) has been found [34]. The retrogradely moving material consists mainly of multivesiculate and multilamellar organelles which seem to be destined for degradation in the perikaryon [107, 118]. This material originates from endocytosis at the synaptic level where synaptic membrane components as well as extracellular material are sampled [68, 117] and from the reversal of axonal constituents which are also conveyed anterogradely but in part reverted [3, 4].

Particle Size and Transport Velocity

There seems to exist a weak biphasic correlation of population transport velocity and particle size [47]. The most rapidly transported material (group I) consists of smooth-

walled tubulo-vesicular structures of about 50 nm in diameter and 180 nm in length [107, 118]. Larger material (mitochondria, neurosecretory granules and retrogradely moving prelysosomal particles) seem to move at slower population velocities (group II; [12, 78, 87, 105, 107, 115]). Smaller material seems also to be considerably retarded, although part of it is initially transported rapidly [47, 100]. The subcellular status of such rapidly transported soluble proteins and low molecular weight compounds is an unclarified issue [28, 61, 121, 123]. One could conclude that there is an optimal particle size in the range of maximally 80 nm of diameter, whereas smaller and larger materials move slower. Since the velocity of individual particle saltations is not size dependent [65, 123], the pauses between the saltations must increase for larger organelles.

This correlation seems not to apply to the movement of the group of slowly transported soluble proteins (Group IV) and the cytoskeletal constituents (group V).

Temperature Dependence

The velocities of rapidly and slowly transported materials are within physiological limits linearly [13, 42] or nearly linearly temperature dependent [10, 23]. The maximal transport velocities seem to be only little influenced by length, diameter or functional type of the axon, by the animal species (except arthropods) or by the electrical activity of the axon [41].

Properties of the Transport Mechanism

Independence from the Perikaryon

Removal of the perikaryon or dissection of axonal segments does not impair transport for several hours if the nerve is kept under physiological conditions [44, 51]. This leads to the conclusion that the mechanism, at least for rapid transport, is inherent in any segment of the axon.

ATP, Ca^{2+}, Microtubules, Actin

The mechanism of axoplasmic transport requires ATP [75], microtubules (pharmacological evidence: [52, 66]; morphological evidence: [123]; theoretical evidence: [48]) and most probably also actin (pharmacological evidence: [36, 58]). Ca^{2+} is required for the synthesis and formation of the rapidly transported organelles at the level of the Golgi apparatus [49, 77] and most probably also during transport in the axon ([50, 59, 93], but see also [28, 108]).

Guiding Elements and Directionality

It is generally believed that similarly to other forms of cellular motility axoplasmic transport also depends on the presence of guiding structures (e.g., [84, 104]). This can also be postulated on theoretical grounds since the transport direction can most easily be defined by a polar structure [122, 123]. For both purposes only cytoskeletal elements are suitable. Out of these, the neurofilaments seem to be located in the axon where rapid transport does not take place [32, 39, 94], actin filaments are only randomly oriented in axons [39, 74], whereas microtubules could well fulfill such functions (cf. [122]). Autoradiographic work supports the view that rapid transport takes place in axonal regions where microtubules, mitochondria and smooth endoplasmic reticulum are located [21, 94].

Force-Generating Mechanism

The molecular mechanism which links ATP consumption to the generation of the shear force is unknown. It is generally believed that this is accomplished by force-generating enzymes (ATPases). If such enzymes are involved, they would work effectively only if they were oriented in the proper direction and attached to some structure massive enough to absorb the recoil (for review see [122]). Both dynein-like [28] and actomyosin-like ATPases (e.g., [63]) were proposed, but other, not yet characterized ATPases are also visualizable.

Since a whole variety of cellular constituents are to be transported, these could either be confined in one kind of general transport organelle upon which the shear force then could act specifically, or, if shear force is acting in a non-specific manner upon all the axoplasmic components, no such transport organelle would be needed [43, 122].

Energy Consumption

The energy available for axoplasmic transport can be estimated to be of the order of 5×10^{-9} erg s^{-1} for 1 μm^3 of axoplasm [48]. Since the viscosity of axoplasm is very high for moving organelles [99] organelle movement can for energetical reasons only take place in low viscosity channels between cytoskeletal elements or after local destruction of such elements – provided that the laws of fluid dynamics can be applied also to cytoplasm [48, 98].

Reversible Interactions

There is good evidence that the transported material and the mechanism interact reversibly and intermittently. This is conspicuous in the case of visible material as saltatory movement [108] but it can also be concluded more generally from effects such as axonal retention, tailing, deposition and cooperativity [44, 80, 86, 90, 104]. Such behavior may lead to a spectrum of velocities [85, 123], and it can formally be described analogous to chromatography [113].

Different Transport Velocities

The transport velocity differences can be explained by several means, none of which can be excluded from our present knowledge. (1) Several different mechanisms, each having a characteristic velocity, convey different materials (e.g., [119]). (2) One rapid transport mechanism conveys all materials, the different velocities being due to different duration or probability of these materials to stay on the mechanism (chromatography) (e.g., [14, 47, 90]). (3) One rapid mechanism conveys activily only the rapid material, while other material is moved by drag according to its physical properties (e.g., [76]).

Directionality

The dynamics of anterograde and retrograde transport are very similar (e.g., [108]) so that one would assume the same kind of mechanism to underly both. How shear forces can be generated in the two directions differing by 180° is unknown.

Regulation of Axoplasmic Transport

Even in extreme physiological states of the neuron such as in situations of blocked or increased electrical and exocytotic activity at the synapse there seems to be no significant influence on axoplasmic transport. This is especially true for the transport velocity which virtually cannot be influenced by altering the physiological activity of the neuron (see [41], p. 1188, for review). The amount of transported material can be increased in certain situations of physiological stimulation, especially in the hypothalamo-neurohypophysial system [41, 87]. This may be explained, however, by increased perikaryal synthesis of the transported material, since it is known that transport has a considerable excess capacity and can in some cases accommodate four times the amount of material transported in the resting state [9]. That axoplasmic transport cannot be regulated very effectively is also evident from the fact that by electrical stimulation synapses can be depleted of vesicles and transmission be abolished [54, 127]. Elevated velocities could be observed only if the amount of organelles was increased [37, 103]. Davison [18] had already speculated that due to the lack of regulation the neuron has always to convey a surplus of material, thus necessitating retrograde transport (which itself seems not be regulated either [114]).

There are many proposed models of how axonal transport can be regulated intracellularly [22, 25, 32, 53, 91, 95, 112]. Whether the neuron makes use of any of these mechanisms remains to be elucidated by further experimentation.