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Lubinska Phenomenon: Simultaneous Bidirectional Axoplasmic Flow in Nerve Fibers

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Experiments on live mollusk neurons isolated with a neurite fragmentsat its various levels demonstrated that axoplasm is characterized by mechanical strain realized in the form of retraction up to complete invagination of the axoplasm into the soma. Changes in axon geometry were attributed to neuroplasm movement. It was found that the direction of axoplasm movement depends on the location of adhesion points. It was always simultaneous and oppositely directed, as is the case with contractile myofibrils. The formation of distant paired adhesion sites can promote moving away of the axoplasm mass and organelles carried by it. The velocity and activity of axoplasm movement depend on the quantity and intensity of adhesion points along the axon.

Key Words: *axoplasmic flow; neuronal migration; axon retraction; axon adhesion; bidirectional axonal flow*

A famous paper "Axoplasmic Streaming in Regenerating and in Normal Nerve Fibres" was published more than 50 years ago, in 1964. It was a result of a series of preliminary publications of the author demonstrating persuasive evidence of simultaneous bidirectional axoplasmic (AP) flow, which was acknowledged by the international scientific community.

The mechanism of simultaneous "neuroplasm streaming" of the same fiber in opposite directions has been discussed not once [5]. The morphological method with the use of horseradish peroxidase, based on the cellulopetal transport of the neuroplasm, is traditionally used for studies of the retrograde flow. Simultaneous anterograde and retrograde migration of viruses in axons is in the focus of research in virology, in connection with the development of gene therapy for nervous degenerative diseases [5,9]. Numerous publications describe reversible retraction of dendritic spines in titanium stimulation and other experimental exposures [3,8]. Reversible shortening of the apical dendrites and their harborization in the pyramidal neurons of the cortex, hippocampus, and other compartments of the brain is described under conditions of hibernation [12], under the effect of anesthetics [11], in repeated stress [1,2,4,6,8], and under the effect of some pathological factors [7].

It is only natural that shortening of whole nerve axons simultaneously is regarded as the retrograde molecular transport of the neuroplasm mass. P. Weiss (1972) thought that the movement of a semiliquid axonal "bar" together with the liquid layer of the neurolemma is in fact the AP streaming (APS). The neuroplasm migrates as part of a growing or retracting axon as a whole unit [10]. Despite numerous persuasive data on the bidirectional APS, the hypotheses on the mechanism of this unique phenomenon remain unproven or incompetent. The mechanisms of AP "collar" formation in zones of nerve compression or crossing that serve as the primary evidence of anterograde transport and bidirectional APS remains unclear.

We tried to validate the retractile hypothesis on the mechanism of simultaneous bidirectional APS.

MATERIALS AND METHODS

The study was carried out on 194 living isolated neurons with preserved fragments of axons from 52 *Limnaea stagnalis* mollusks. The cells were isolated from

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the peripharyngeal ganglia by incubating them for 40 min in Ringer's solution with 0.4% pronase (Serva) at 21-23°C. The ganglia were dissociated after repeated sucking. Damaged glia and nerve cells were removed by repeated washing of the resultant suspension in Ringer's solution. The cells were placed into a microchambers (1 cm^3) with a fine slide serving as the bottom. The study was carried out under an inverted phase contrast BIOMED-3I microscope. The neuron behavior was monitored for 2-18 h with a DCM videocamera connected to a PC. The rate of preparation contractions, duration of process, and changes in soma diameter were recorded. Additional groups of non-neuronal cells located at the end of the fiber, neuronal soma, or in the middle part of the contracting axon, served as the natural foci for the preparation adhesion in these experiments. The direction of migration was determined by the direction of migration of ends. Translocation of AP mass (APM) was evaluated by sliding axon volumes and transposition of pigment granules in the soma.

RESULTS

Amputation of a nerve process in a single-axon neuron led to its slow contraction irrespective of the resection method (Fig. 1, *a*, *b*). This obviously indicated retractile activity of its AP. The microscopic picture of the axon contraction, *i.e.* changes in its length or mass looked as AP flow (APF). Contractile movement of AP was observed in the proximal and distal stump of the crossed process. In order to identify the direction of movement of the preparation and neuroplasm mass, we analyzed neurons with adhesion (fixation) sites located in the proximal (distal) ends of the preparation, in the middle, in the two ends simultaneously, or without adhesion sites at all. The first experimental crossing of the living axon showed that the same AP of the same crossed fiber could retract in opposite directions in the stumps, *i.e.* APF could be opposite. The end of the crossed axon after its contraction usually moves towards the stable point of adhesion.

In comparison with the axon, the neuron soma has a larger contractile bulk, is lying on a sublayer, and its adhesion is more significant than that of a light, often floating axon. For this reason, the crossed axon usually moves towards the soma, *i.e.* cellulipetally (Fig. 1, *a*). Importantly that it is not just its "tail" portion is migrating. If we analyze the changes in the contracting fibril geometry, we see that the main AP, moving, enlarges the cell soma volume (let us compare the body volume before and the axon diameter after its retraction). Microscopy clearly shows the time course of AP retracting into the body, transporting the neuronal pigment. The other part of AP is distributed

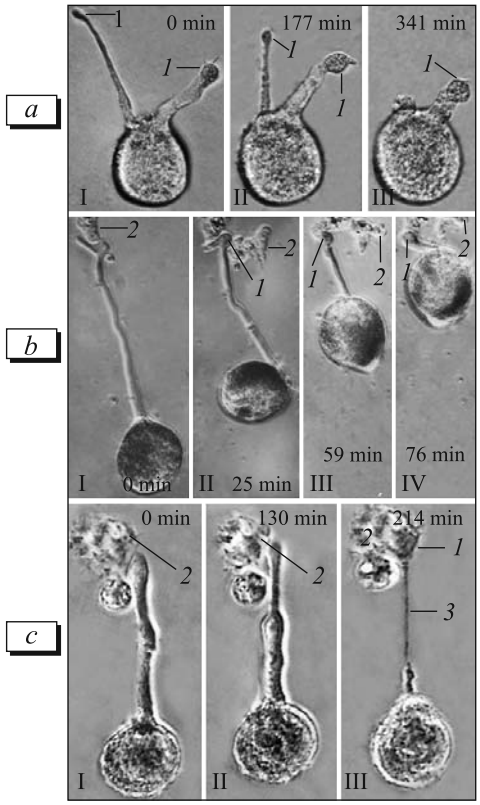

Fig. 1. Changes in retraction vector are determined by changes in the preparation adhesion (fixation) sites. *a-c*) Three variants of neuron retraction: *a*) transposition of nerve processes to neuron body in case of adhesion in the soma; *b*) migration of neuron body towards axon end, with adhesion site by this end; *c*) isometric (volume) retraction of axon in the presence of two adhesion zones; I-IV) process stages; *1*) retraction bulbs; *2*) additional cells providing a new site of preparation adhesion to sublayer; *3*) axon thinning. Here and in Figs. 2, 3: time elapsed since the beginning of observation is shown. Vital microscopy. Phase contrast, ×400.

along the axon sides increasing its diameter (Fig. 1, *a*). However, in order to change completely the AP streaming vector, it is sufficient to change the site of the preparation adhesion. If stray additional cells, left from preparation of the ganglia, lie close to the axon end, thus providing a new site for its adhesion, the direction of the preparation end movement is completely changed to the opposite one. The neuron body is now streaming towards the fiber end (Fig. 1, *b*). This direction is now opposite, while the axon APM is still streaming towards a larger contracting bulk – to the neuron body. These experiments simulate the situation with a strained strip of stretched elastic band, its free ends, if cut, inevitably streaming towards the contralateral fixation points. Numerous studies of isolated neurons with an intact axon demonstrate their similarity to stretched elastic band.

Sometimes both ends of the preparation, the soma and axon end, are immobile and adhere to substrate (Fig. 1, *c*). In these cases. AP movement manifested

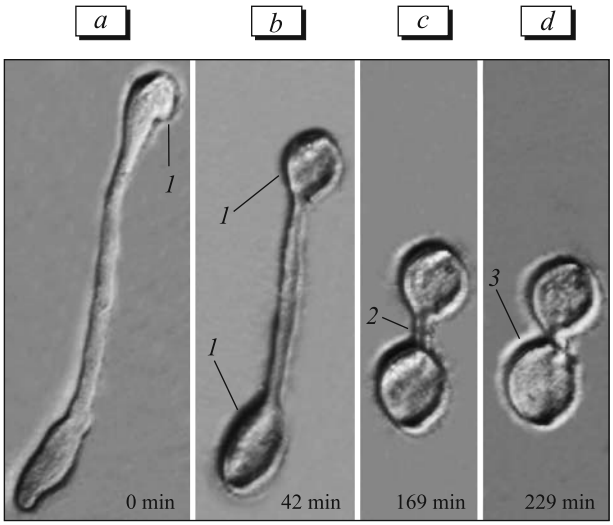

Fig. 2. Bidirectional retraction of lateral APMs of an isolated nerve fibril towards each other; no adhesion sites by the preparation ends. Thinning of the central portion of the axon indicates partial translocation of AP to the sides contralateral from the center. *a-d*) Process stages; *1*) retraction bulb; *2*) thinned part of fragment; *3*) AP fusion into one bulk of spherical shape.

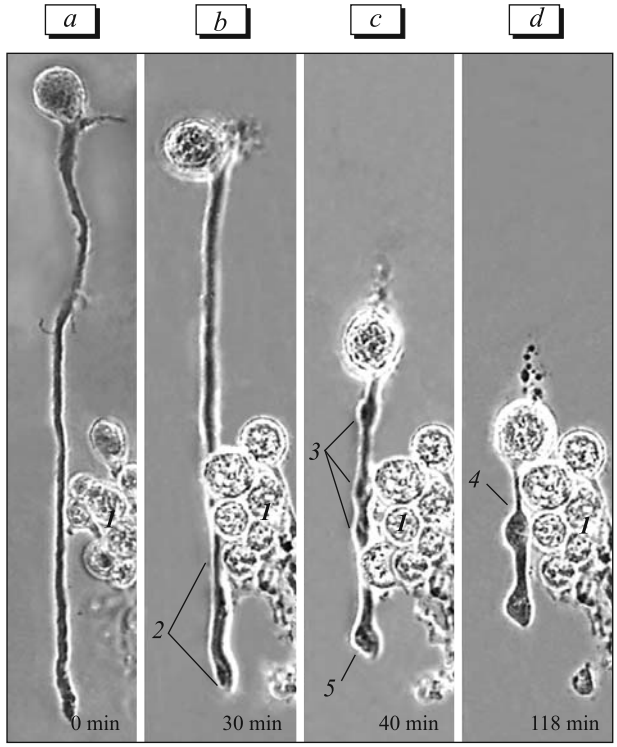

Fig. 3. Transposition of APM in two opposite directions (towards soma and fiber end), with adhesion site in the middle of preparation. Ends of preparations moving in opposite directions. *a-d*: Process stages; *1*) fiber adhesion zone, formed by extra cells; *2*) slow contraction of AP at the peripheral end of preparation; *3*) varicose deformation of axon; *4*) APM thinning in the middle of preparation at the site of adhesion; *5*) rapid transposition of axonal AP.

not by shortening of the axon, but by its thinning – volume shrinkage of APM in the middle parts of the fibril, migrating to opposite sides of the process (bidirectionally), while the preparation ends were increasing in size. This uncommon form of AP transposition could be called isometric contraction. It seemed that this type of nerve fiber retraction was not yet described. However, it could be essential for passive electric properties of the nerve conductor.

The behavior of a completely isolated fragment of the nerve process can be observed by removing the neuron soma, thus making both ends of the preparation free from adhesion. This crossed fiber behaves similarly as an elastic band. Both its ends contract towards the center, towards each other – bidirectionally (Fig. 2). This similarity means that the nerve fibril is characterized by initial strain, tone, and somehow resembles a slowly contracting myofibril. This conclusion is important for neurons, as any kind of muscle contraction is the only example up to the present time, when two sites of a fiber or their organelles during work are moving, inevitably and simultaneously, towards each other, that is, in opposite directions.

If this similarity is really close, the mode of AP streaming in opposite directions is clear and natural and will be observed under a microscope in other cases of nerve fiber retraction.

Videomicroscopic observation of retraction of an isolated nerve fiber retraction shows that its various parts are "testing" intricate tracks of AP transposition. It is clearly seen that a fragment of an isolated nerve process is becoming shorter (Fig. 2, *a-d*). It is clear that the peripheral part of APM is moving from the fragment periphery to its center. In parallel with this, the diameter of the main part of the fiber is thinned (Fig. 2, *c*). This fact indicates that the central part of the fragment APM moves towards the process periphery – in the opposite direction. That is, some parts of AP are moving bidirectionally from the center and from each other simultaneously.

We think that proven retractile activity of the nerve processes is the main explanation of the mysterious phenomenon – bidirectional simultaneous "streaming of AP" and its organelles. We think that AP does not stream, and there are no "streams" of liquid AP; it is the more solid neuroplasm matter, slowly contracting, that is capable of carrying the contents, structures, organelles, and molecules – that is, of carrying itself – simultaneously in the anterograde and retrograde directions. The intensity of this or that opposite direction of movement is regulated by the degree of adhesion of a certain site of axon. The APM is slowly moving towards the site of predominating adhesion under conditions of partial or variable degree of adhesion.

Summing up the results, we should like to say that first, movement of the ends of the preparation often

does not conform to the intricate and contradictory direction of APM translocation. Second, the APM migration vector largely depends on the position, translocation, and intensity of the axon adhesion zones; and, third, the phenomenon of simultaneous and opposite bidirectional translocations of APM is based on the retractile myofibril-like function of the nerve processes.

In cases with some extra cells, left from the glia preparation in the middle part of the axon, forming a new site for the fibril adhesion (Fig. 3), APM is streaming simultaneously towards the neuron soma and to the fiber end, enlarging its volume, that is, in two opposite directions. On the other hand, these enlarged APM will come closer to each other, moving towards each other. In addition, the axon is thinned in the middle – at the site of adhesion under these conditions (Fig. 3, *d*). This indicates partial additional shifting of APM from the middle to the ends of the preparations – to its opposite sides.

These variants of bidirectional APF explain the mechanism of the Lubinska phenomenon. It seems that the known motor proteins, neurotubules, and neurofibers of the cytoskeleton form an entity, organizing in general the overall movement of a fibril as an organ. Our data are in good agreement with the known substrate-cytoskeleton mobility model. All the known molecular cytoskeleton mechanisms quite conform to the hypothetical scheme. A hypothesis is put forward and experimental evidence of the neurofiber contractile tone is offered, realized by the myofibril-like retraction of AP, the "motor" of its movement, with its natural and obligatory characteristic – simultaneous bidirectional movement regulated due to variations in adhesion points.

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