METHODS

Study of Regular Intracellular and Membrane Processes in Neurons by Laser Interference Microscopy

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Regular fluctuations in the height of phase profile of isolated neuron were studied by laser interference microscopy. The resultant spectra of frequencies of changes in the phase profile height indicate regular local changes in the optical density and/or geometry of neurons. The frequency spectra for the central and primembrane regions of the cells are different.

Key Words: *interference microscopy; neuron; phase profile*

Dynamic processes in cells (rhythmic changes in membrane potential, cytoskeleton restructuring, oligomerization and lateral diffusion of plasma membrane proteins and lipids, migration of cytoplasm components, *etc.*) are now intensively studied [3]. Dynamic processes in neurons are amply studied by probe fluorometry, registration of changes in double refraction, light scattering, and resonance Raman spectroscopy [6-8]. Laser interference microscopy is also used for the studies of dynamic processes in nerve cells. Regular local changes in optical density and/or geometry of the neuron and axon correlate with its rhythmic activity or effects of neurotransmitters [1,4].

We studied differences in the regular dynamic changes in optical density and/or geometry of the neuronal membrane and cytoplasmic subcompartments.

MATERIALS AND METHODS

In contrast to light microscopy, during which the distribution of light intensity is recorded, laser interference microscopy shows phase distribution in the interference image of the object. The work of laser phase microscope is based on measurements of local phases of light wave reflected by the object [2]. The interference picture of the object is obtained by superimposing the object wave and reference mirror wave. Modulation of the supporting arm length creates the alternating periodical signal, whose phase determines path-length difference. The measured value is standardized for the wavelength so that the phase height of the object (phase profile height, PPH) in a certain point is determined. The phase portrait is the sum of phase shifts measured for all points of the object image; it carries information about geometrical size of the object and distribution of the refraction coefficient in its volume.

The studies were carried out on an experimental model of modulation interference microscope MIM 2.1 (Amfora Laboratories) [1]. The device is a laser interferometer with separated optical tracts of the subject and object arms (modified Linnik interferometer). The interference image is recorded by a coordination sensitive receiver during modulation of the length of supporting arm. The laser source wavelength is 532 nm, power 40 mV (radiation power for the object 2 mW), time of information reading from one pixel 2 msec.

Neurons of *Hirudo medicinalis* segment ganglia were studied. Analysis of transmission spectra of iso-

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lated neurons carried out using a Leitz Weitzlar MPV-2 microscope fitted with diffraction monochromator showed no appreciable absorption at λ=532 nm (wavelength of laser used in MIM-2.1 device). Hence, this light source produced no appreciable changes in the function of neurons.

Isolated neurons were put into hermetically closed chamber with a reflecting base (aluminum mirror with Al_2O_3 coating) and rigid fixation of the slide filled with normal saline (110 mM NaCl, 4 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4, at 18-22°C).

Phase image of the object was obtained using specially designed software (Fig. 1). The image relief is determined by optical heterogeneity of the cell due to the presence of organelles and cytoskeleton elements. Scanning line was selected using phase image of the cell. Scanning was carried out at 4-6 sites in the center of the cell and near its interface in each series of measurements.

Changes in PPH were recorded using an objective with magnification 27 with 0.15 aperture; visual field 1024×1024 pixels (27×27 μ), scan line length 16 pixels (0.42 μ), width 1 pixel, size of 1 pixel 26×26 nm, scanning rate 500 pixels/sec. The resultant data massive is a two-dimensional matrix with the number of dots (usually 16) along the X axis and time along the Y axis. Hence, a set of values, presenting PPH values in time, was obtained for each point. During data processing, fast Fourier transform with subsequent standardization of the amplitude was carried out for each point of the scan line.

RESULTS

Preliminary calibration of the device for the frequency and amplitude was carried out using a mirror with aluminum coating fixed on a piesoceramic modulator and low frequency generator (0-20 Hz). The frequency and amplitude of oscillations applied to the mirror varied. Oscillation frequency was also evaluated by the frequency meter. The mirror oscillation frequency, determined by the Fourier analysis program, coincided with the value determined by the generator and recorded by the frequency meter. The minimum recordable amplitude of the mirror oscillations during signal delivery from the generator, for which the frequency could be estimated, was 0.06 nm. In case of absence of the mirror modulations, oscillation frequency in the spectrum was below 0.02 nm and was "white noise".

The chamber needed for operations with isolated neurons increased the background noise, which was evaluated in a cycle of control measurements. The level of low frequency (up to 0.1 Hz) noise was elevated during evaluation of the spectrum of natural oscillations on the mirror in the chamber (Fig. 2, *a*). Therefore, 0.3 Hz was selected as the lower border of the frequency band. The amplitude of oscillations in the rest part of the spectrum was 0.06-0.15 nm.

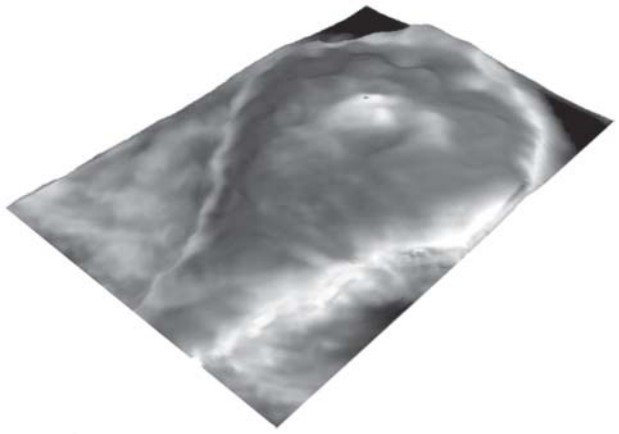

Fig. 1. 3D-phase image of a cell obtained using MIM-2.1 device.

It is known that the processes coursing in the cell can be tentatively divided into membrane and cytoplasmic. Therefore, scanning lines in the experiment were drawn in the center of the cell and near the plasmatic membrane. PPH changes in the base served as the control (Fig. 2, *a*). PPH changes in the central part of the cell presented as a spectrum with individual frequencies 2.2 and 15.5 Hz and frequency groups 0.3-2.0, 4-6, and 10-12 Hz (Fig. 2, *b*). Such scanning recorded changes in PPH in the cytoplasm and plasma membrane. It is obvious that these regular changes in PPH are a result of many cell processes (movements of the cytoplasm and organelles, exo- and endocytosis, changes in the cell volume, plasma membrane viscosity, *etc.*).

Registration of PPH fluctuations in the primembrane (membrane with the adjacent cytoplasm sites) subcompartment gave a spectrum represented by only one frequency group of 0.3-2.0 Hz (Fig. 2, *c*). This group of frequencies is also characteristic of the central part of the cell (Fig. 2, *b*), but their amplitude in the primembrane region is 1.5-2.0 times higher. This difference can be explained by peculiarities of scanning (the scanned membrane volume at the cell interface is 2-3-fold more than during scanning in the center). It seems that the 0.3-2.0 Hz frequency group in PPH fluctuations is associated with processes in the primembrane subcompartment. In addition to this frequency group, frequencies 2.2, 2.5, 2.8, and 3.4 Hz were observed, but their emergence in the spectra of PPH fluctuations was not regular.

Hence, regular changes in PPH fluctuations in different compartments of the neuron were detected. The frequencies of these fluctuations are different in the membrane and cytoplasmic regions of the cells. The findings indicate a complex of cellular processes, manifesting by regular local changes in optical density

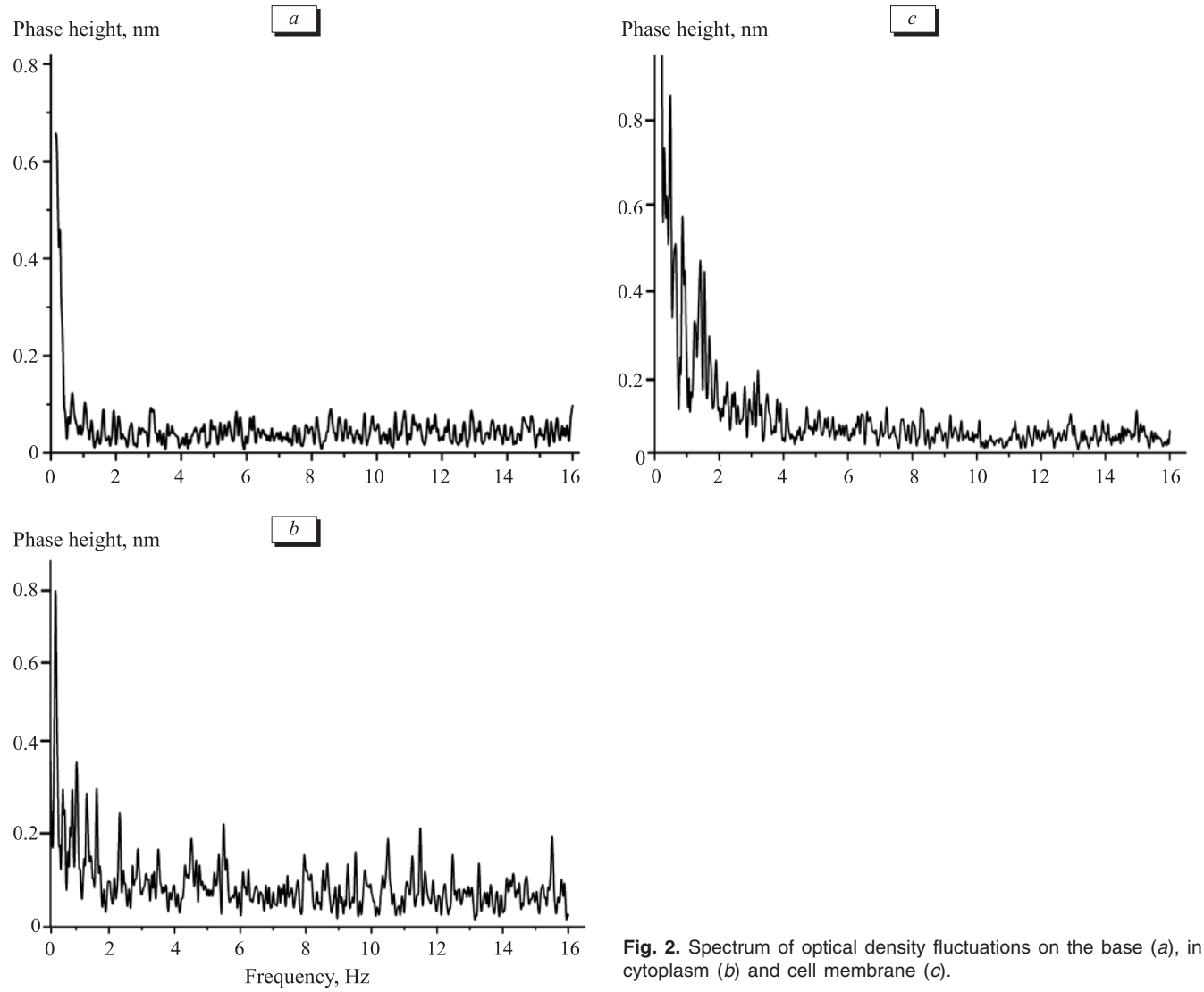

Fig. 2. Spectrum of optical density fluctuations on the base (*a*), in cytoplasm (*b*) and cell membrane (*c*).

of the membrane, cytoplasm, and/or geometry of the neuron. We consider that use of interference microscopy for the studies of regular changes in neurons will extend the range of the studied phenomena and will help to analyze more fine structure of the processes regulating the membrane and cytoplasm status during generation and conduction of impulses in health and disease.

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