Effects of Ethanol and Acetaldehyde on Isolated Nerve Ending Membranes: Study by Atomic-Forced Microscopy

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A new method of fixation of native synaptosomes and synaptosomal membranes from rat striatum was applied for their visualization by atomic-force microscopy. A scheme for examination of the surface of biological material was developed, which helps to distinguish intact synaptosomes from washed synaptic membranes and evaluate damage to synaptic membrane surface caused by ethanol (25 mM) and acetaldehyde (50 μM). The proposed method can be used for evaluation of the damaging effects of ethanol and acetaldehyde on neurons.

Key Words: *synaptosomes; synaptic membranes; atomic-force microscopy; ethanol; acetaldehyde*

Neuronal surface and synaptic contacts, are an important characteristics determining the function of nervous tissue. Isolated nerve endings (synaptosomes) are often used as the model system for investigation of neuronal membranes. As a substrate of signal processes, neuronal membrane is very sensitive to effectors and bioactive compounds [1]. Ethanol (ET) and acetaldehyde (AA) induce specific changes in synaptic structures of the brain, which probably underlie alcohol tolerance [13]. Identification of ET and AA effects on the membrane is a pressing problem [2], because the level of ET and its metabolites in tissues is important for evaluation of the severity of alcoholization and neuron damage.

The aim of this study was to elaborate a method for visualization of synaptosomes and neuronal membranes by atomic-force microscopy (AFM) and detection of possible damaging effects of AA and ET on neuronal membranes.

MATERIALS AND METHODS

Synaptosome-rich fraction isolated from rat striatum as described previously [6] was resuspended in normal Krebs—Ringer solution (pH 7.4) and left for 30 min for recovery of the synaptosome ultrastructure. The suspension (4 μl) containing 300-500 μg protein per 0.1 ml was transferred to slide and fixed by heating.

For obtaining synaptic membranes, dense synaptosome precipitate was frozen and after thawing washed twice with 20 mM sodium phosphate buffer (pH 7.4), the precipitate was resuspended in normal Krebs—Ringer solution (pH 7.4), 4 μl suspension was transferred to slide and fixed.

Synaptic membranes were incubated with ET and AA in final concentrations of 25 mM and 50 μM, respectively, for 20 min. Similar concentrations were detected in rat blood 0.5 h after injection of ET in a dose of 1.5 g/kg [8]. The level of AA is usually 0.1-0.2% of tissue ET concentration.

The surface of synaptosomes and synaptic membranes was examined under a Nanotope-203 computer-assisted atomic-force microscope in a tapping-mode regimen, which represented a combination of contact and contact-free examination. The images obtained after a shock wave reflect topographic and elastic properties of the surface [11,12]. During scanning the phase of the probe oscillations changes yielding the so-called phase images. Phase image is a density map, the maximum alteration of oscillation phase characterizes the maximum changes in elastic properties of the material [3].

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RESULTS

Visualization of native synaptosomes is difficult because of elasticity of biological material. After applying onto slides, the samples were dried at room temperature and under a stream of hot air (90, 150, and 200°C for 1, 3, 5, or 10 min). Optimal fixation was attained at 90°C for 5 min (Fig. 1, *a*).

Washed synaptosomal membranes obtained after synaptosome destruction formed scale-like films (Fig. 1, *b*). Analysis of AFM images showed that the mean thickness of the film is 2225.3 nm (Fig. 1, *d*). It was found that in our samples examined in selected scanning regimens the black zones on the image corresponded to "soft" contact of the point with the surface, while white zones corresponded to "rigid" contact [11,12]. The structure of the film surface was clearly seen on the phase image (Fig. 1, *c*): individual light (rigid) sites were surrounded with dark borders corresponding to soft contact zones presumably at the membrane interfaces.

The total image of membrane film after ET treatment showed continuous films (Fig. 2, *a*) with small fragments at their periphery. The thickness of the membrane film decreased to 1293.3 nm (Fig. 2, *b*). The colors on the phase-contrast images changed to opposite: dark tissue on membrane film was lined with light borders (Fig. 2, *c*). Acute ET exposure exerted a universal fluidizing effect on biomembranes of all types [7], and therefore this color inversion can be due to softening of membrane film in comparison with its thermally fixed borders. Hence, ET modified the surface of synaptic membranes.

Differentiation of ET and AA effects on cell membranes is very difficult [2]. Similar membranotropic effects of ET and AA were noted after chronic alcohol intoxication [9]. The toxicity of AA far surpassed that of ET [4]; this compound is more active: AA changes the properties of cell and subcellular membranes [2] by modifying their phospholipid and protein components [5]. AA can form both stable and labile bonds with amino groups of cell compartment proteins

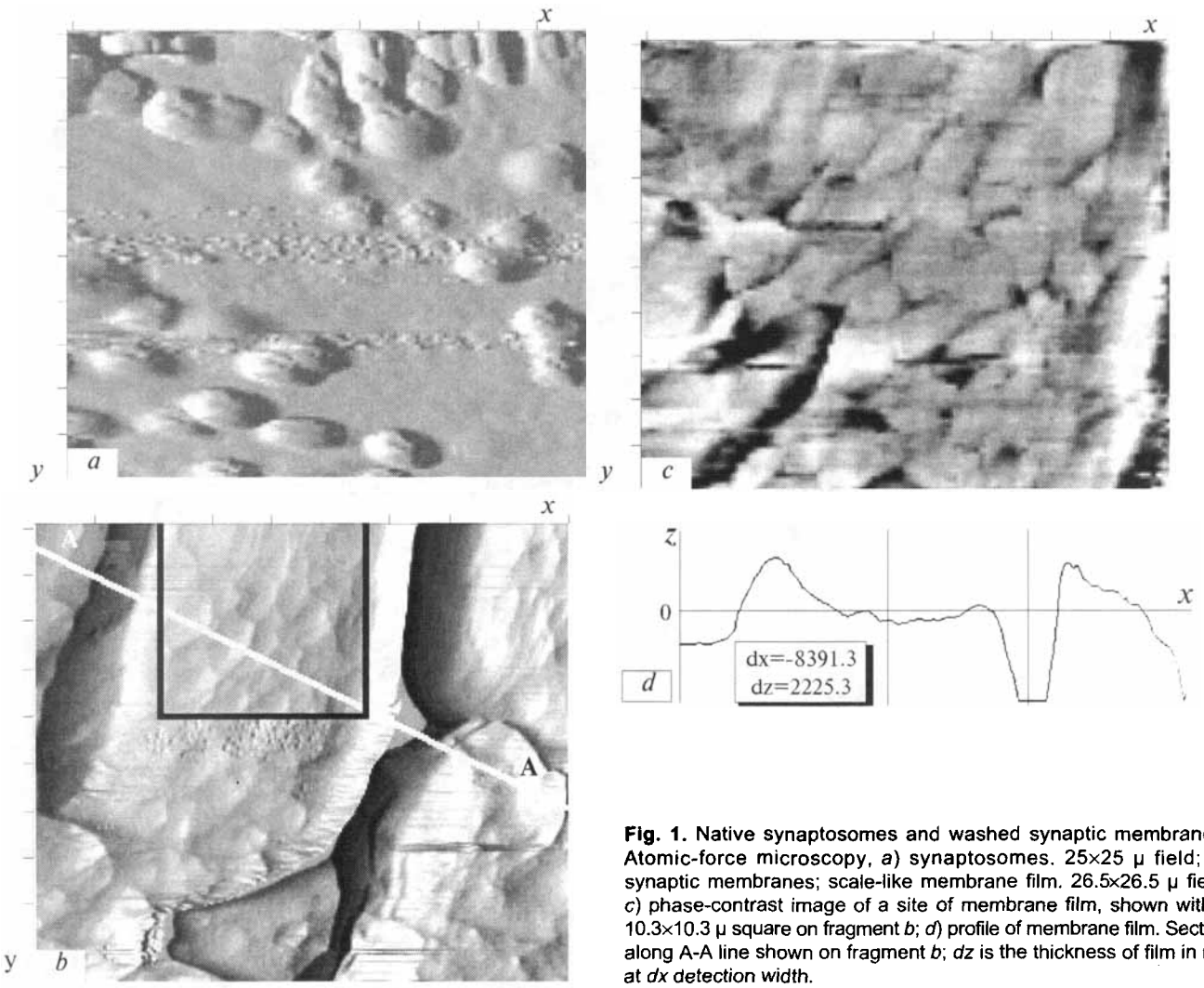

Fig. 1. Native synaptosomes and washed synaptic membranes. Atomic-force microscopy, *a*) synaptosomes. 25×25 μ field; *b*) synaptic membranes; scale-like membrane film. 26.5×26.5 μ field; *c*) phase-contrast image of a site of membrane film, shown with a 10.3×10.3 μ square on fragment *b*; *d*) profile of membrane film. Section along A-A line shown on fragment *b*; *dz* is the thickness of film in nm at *dx* detection width.

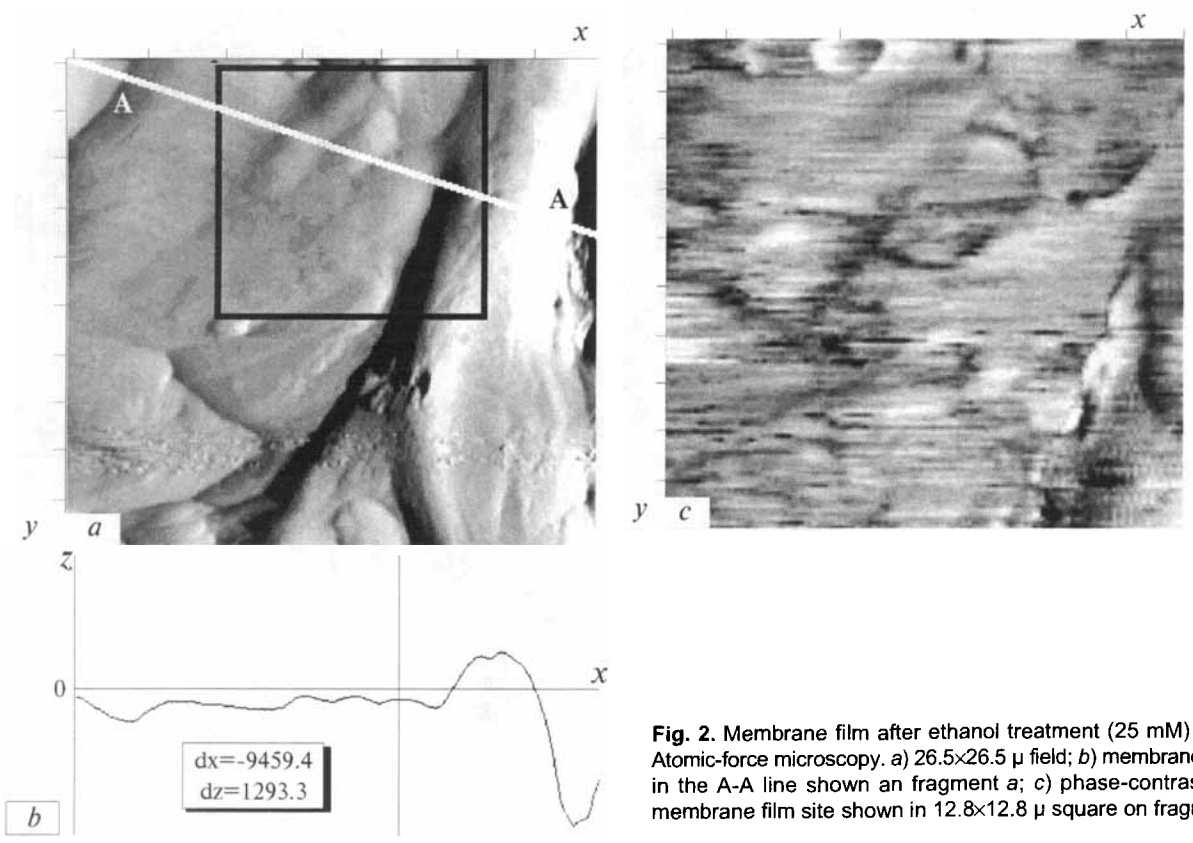

Fig. 2. Membrane film after ethanol treatment (25 mM) for 20 min. Atomic-force microscopy. *a*) 26.5×26.5 μ field; *b*) membrane film profile in the A-A line shown an fragment *a*; *c*) phase-contrast image of membrane film site shown in 12.8×12.8 μ square on fragment *a*.

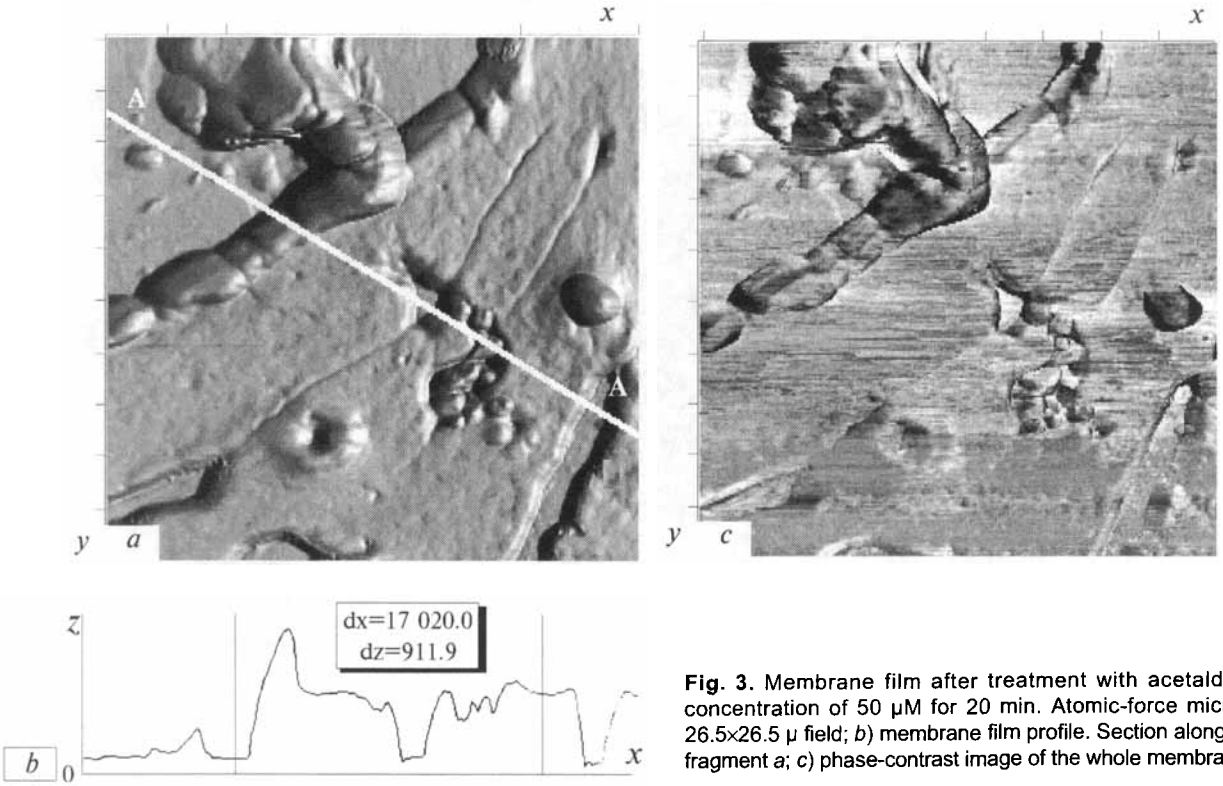

Fig. 3. Membrane film after treatment with acetaldehyde in a concentration of 50 μM for 20 min. Atomic-force microscopy. *a*) 26.5×26.5 μ field; *b*) membrane film profile. Section along A-A line on fragment *a*; *c*) phase-contrast image of the whole membrane film field.

[5]. Unstable compounds are Schiff bases transforming into stable upon reduction of the double bond [10].

In our experiments AA caused even more pronounced flattening of membrane film (mean thickness 911.9 nm, Fig. 3, *b*). Some microsites of the film decreased still more and the characteristic contrasts of its surface structure was virtually leveled. This can be attributed to alteration of the membrane film density

after AA treatment under the effect of intermolecular forces. The formation of a uniform contact for soft tissues under the effect of surface forces is known as the effect of precision contact [12]. Structural changes in the synaptic membrane surface during the first 20 min of ET and AT treatment can be regarded as adaptive shifts. It is not yet clear whether these changes are reversible and compensated, but their presence can indicate the initial atrophic processes in neurons. The results allowed us to recommend the method of synaptosome visualization by AFM for experimental studies. The method of native synaptosome fixation allows investigation of the synaptic membrane surface and evaluation of the differences between intact synaptosomes and washed synaptic membranes.

AFM revealed that washed synaptic membranes differently react to ET and AA in physiologically compatible concentrations .

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