Use of optogenetic technology in cell culture models

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Abstract. Optogenetic is a powerful method that allows to modulate cellular physiological properties. In our article, we demonstrate changes of electrical properties of cellular membranes on HEK-293T and hippocampal neurons transfected with channelrhodopsins and halorhodopsins induced by blue and orange light stimulation.

1. Introduction

Brain is one of the most complicated and poorly understood parts of the human body. There is a large group of disorders related to abnormalities in brain activity called neurodegenerative diseases. At the moment etiology and pathological basis of these diseases are unknown. That's why the fundamental assays in neurobiological field become more and more important. New technology that allows scientists to solve these biomolecular problems is called optogenetics. Optogenetics is a modern approach to modulate physiological status of excitable cells, including neurons. This modulation is achieved by combining the techniques of genetic engineering and photonics [1, 2, 3].

2. Materials and methods

2.1. Human embryonic kidney cell line 293T

HEK-293T cells were cultured in DMEM with addition of 10% FBS, 1% L-glutamine and 1% PEST, after 3 days of culturing, medium was refreshed. Transfection with FCK-ChR_2-HALO plasmid using turbofect transfection reagent was performed in order to perform optogenetic experiments.

2.2. Whole-cell patch recordings in HEK-293T cells

Whole-cell recordings in external solution (140mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 10mM HEPES) were performed in voltage-clamp mode (MultiClamp 700B amplifier) unsing 5–10 MΩ pipettes filled with internal solution (35mM NaCl, 2mM $MgCl_2$, 10mM EGTA, 10mM HEPES). Following establishment of whole-cell configuration, the depolarizing current steps 1 sec in duration from 20 pA to 50 pA in amplitude were injected and the corresponding potential changes were recorded.

2.3. Primary hippocampal neuron cultures

The hippocampal cultures of mice were established from postnatal day 0–1 pups and maintained in culture as we described previously [4, 5]. Briefly, after dissection and dissociation, neurons were plated on coverslips (pre-treated with poly-lysine) and cultured in neurobasal A medium with addition of 1% FBS and 2% B27. At third day in vitro (DIV3), Ara-C (4 _M) was added to prevent glial cell growth. At DIV7 and DIV14, 50% of medium was exchanged with fresh neurobasal A medium containing 2% B27 without FBS. In these culture conditions, the astrocytes constitute about 10–20% in total cells in our cultures at DIV15 as determined by GFAP staining (data not shown). For assessment of synapse morphology, hippocampal cultures were transfected with TD-tomato for easily detection of transfected cells and for optogenetics experiments -FCK-CHR_2 plasmids at DIV7 using the calcium phosphate method.

2.4. Whole cell patch recordings and loose patch recordings in hippocampal cultures

Whole cell recordings in ACSF external solution (124mM NaCl, 26mM $NaHCO_3$, 10mM glucose, 5mM KCl, 2.5mM $CaCl_2$, 1.3mM $MgCl_2$, 1mM NaH_2PO_4) were performed in a current-clamp mode (Axopatch-200B amplifier) using 5–10 MΩ pipettes filled with internal solution (K-Gluconate 140mM, MgCl2 2mM, NaCl 2mM, ATP-Na2 2mM, GTP Mg 0.3mM,HEPES 10mM). Following establishment of whole-cell configuration, the depolarizing current steps 1 sec in duration from 10 pA to 100 pA in amplitude were injected and the corresponding potential changes were recorded.

Loose patch recordings in Hibernate A solution with B27 and glutamine (Life Technologies) were performed in a voltage-clamp mode (Axopatch-200B amplifier) held at 0mV using 5–10 MΩ pipettes filled with ACSF external solution. A loose patch (>100MΩ) was generated at the neuron soma close to the axon hillock. Spontaneous action potential currents were recorded 10min from each cell.

3. Optogenetic approach in HEK cells

Cells of human embrionic kidney (line HEK-293T) are easily transfected so they are often used as an object of study [6]. In our preliminary experiments HEK-293T cells were transfected with Channelrhodopsin and Halorhodopsin constructs. The responses were recorded using the patch-clamp technique in voltage clamp whole-cell mode using blue (depolarisation, Fig. 1,A) and orange (hyperpolarisation, Fig. 1, B) light stimulation.

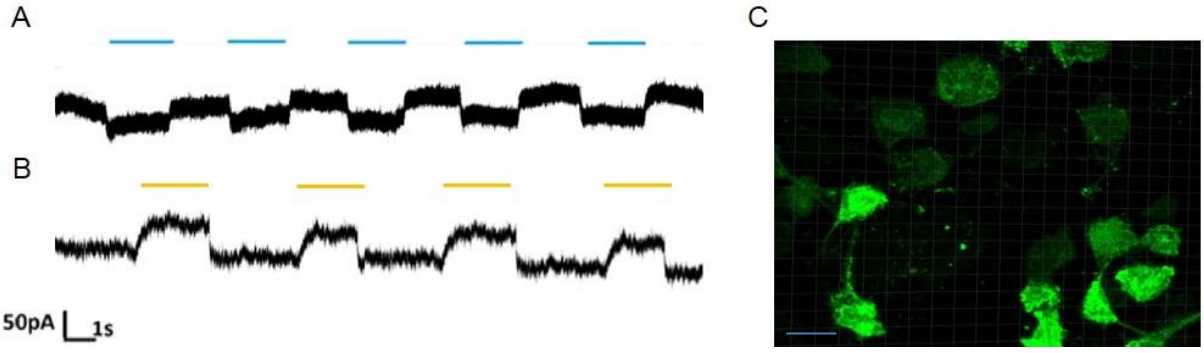

Figure 1. (A, B, C). (A) Depolarization of the cell membrane during the optogenetic stimulation of transfected by ChR_2-GFP plasmids cells lines HEK-293 (λ_{ex} = 470 nm, whole-cell, voltage-clamp mode); (B) Hyperpolarization of the cell membrane during the optogenetic stimulation of transfected by HR-GFP plasmids cells lines HEK-293 (λ_{ex} = 590 nm, whole-cell, voltage-clamp mode); (C) confocal image of transfected cell lines HEK-293T.

4. Optogenetic approach in hippocampal neurons

After successful approbation on HEK cells, optogenetic experiments were conducted in mouse primary hippocampal neuron cultures. Neurons were transfected with ChR_2-GFP plasmid (Fig. 2) and stimulated

by blue (470 nm) light. Traces of neurons activity were recorded in whole-cell and loose-patch, voltage and current clamp modes (Fig. 3, 4).

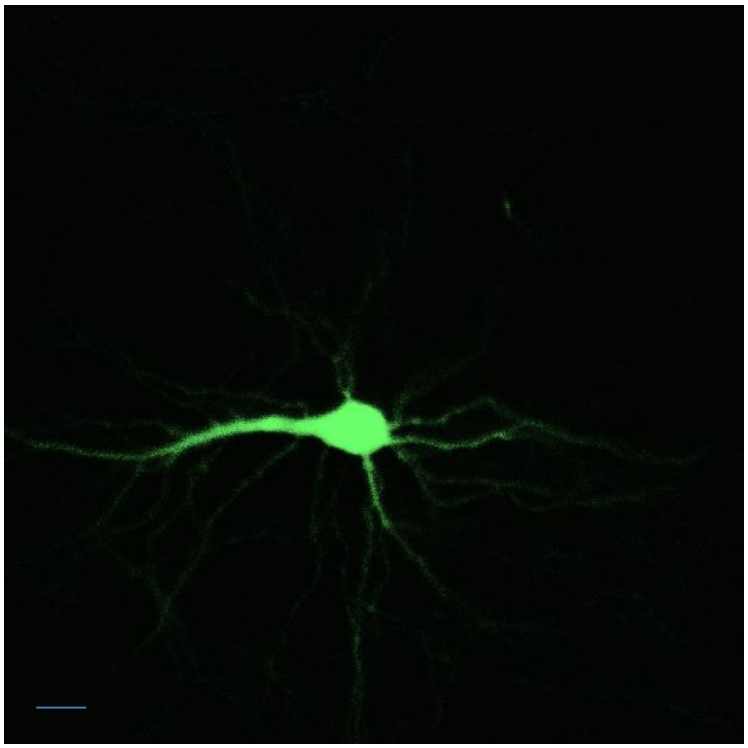

Figure 2. Confocal image of transfected hippocampal neuron expressing ChR_2-GFP (scale bar 20 μm, λ_{ex} = 470 nm)

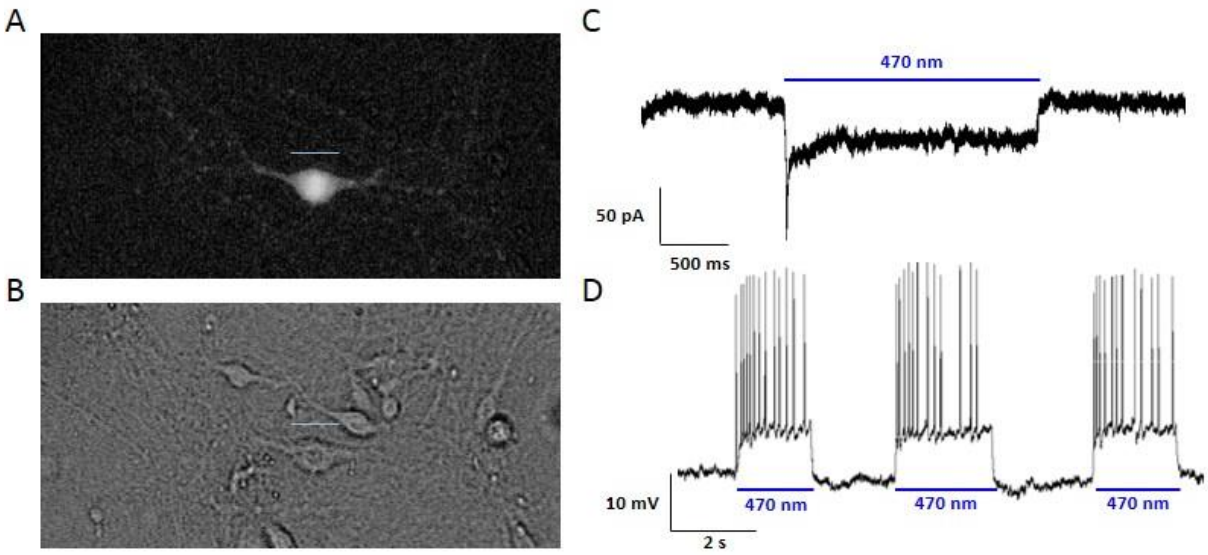

Figure 3. (A, B, C, D). (A) Hippocampal neurons expressing dTomato and ChR_2-GFP (scale bar 20 μm); (B) Hippocampal neuron culture (DIV15); (C) Inward current in voltage-clamped neuron evoked by 470 nm light (indicated by blue bar, whole-cell, voltage-clamp mode); (D) Voltage traces showing response to light stimulation (indicated by blue bar, whole-cell, current-clamp mode).

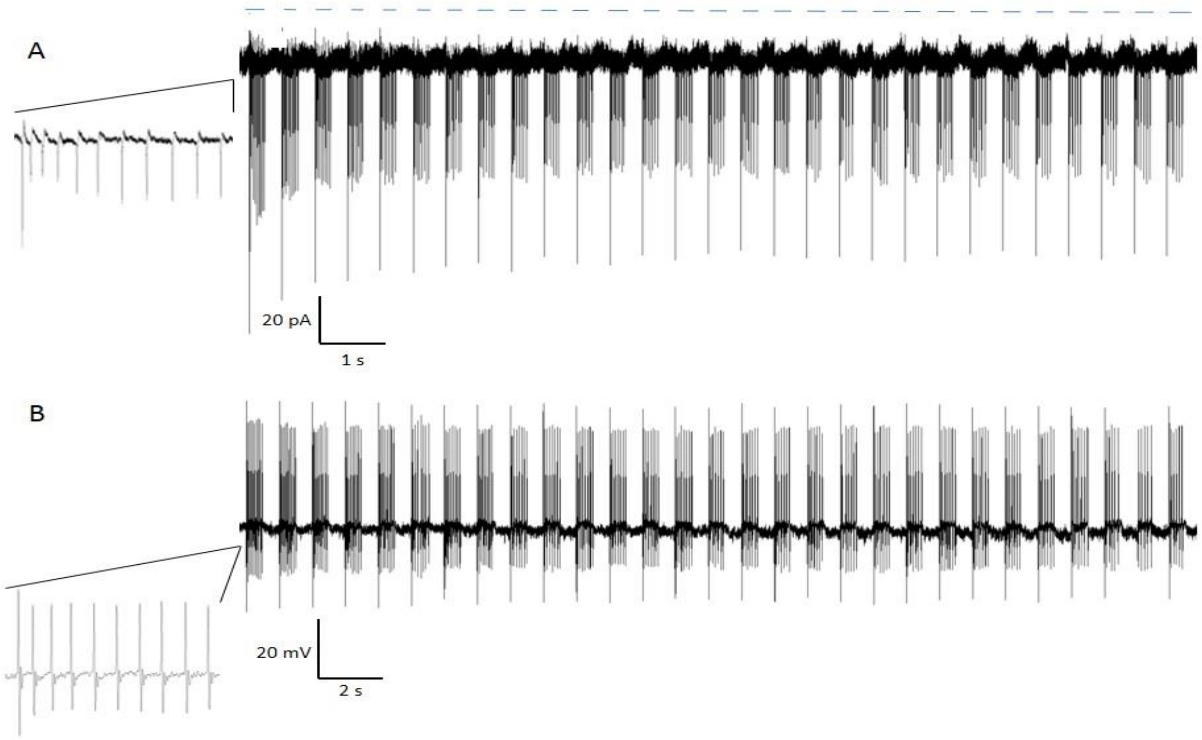

Figure 4. (A, B). Neuron activity evoked by blue light 470 nm (indicated by blue bar); (A) Inward current in voltage-clamped neuron (loose-patch, voltage-clamp mode) (B) Voltage traces showing response to light stimulation (loose-patch, current-clamp mode).

5. Future plans and conclusions

Alzheimer's disease (AD) and aging are resulting in impaired ability to store memories, but the mechanisms responsible for these defects are poorly understood. It is known that electrophysiological response of mutant mouse neuron cultures in case of electrical stimulation results in significant decrease in frequency of action potentials [7]. Our future plans include use of optogenetics in slices and live animals from AD models. For these studies we expect to use optoelectronic implant.

Acknowledgments

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