Optogenetic light pulses generator

A I Erofeev¹, M V Matveev¹, O A Zakharova¹, S G Terekhin¹, V A Kilimnik¹, I B Bezprozvanny¹² and O L Vlasova¹

¹Molecular Neurodegeneration Lab, Peter the Great Polytechnic University, Saint-Petersburg, 195251, Russia
²Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, USA

Abstract. To date, optogenetics is one of the most popular methods in the world in neuroscience. There are new equipment and devices created to keep the progress of this method. This article describes a light pulse generator developed at the Laboratory of Molecular Neurodegeneration, designed for optogenetic experiments.

1. Introduction
On the world market there are a large number of equipment able to manage specified wavelength LEDs, but most of them do not allow to set the necessary parameters of the light stimulation, such as duration, frequency, number of light pulses, which would be required for optogenetic experiments [1, 2, 3]. Therefore was developed light pulse generator, which allows to set required parameters of the light and which was tested on neuron cultures.

2. Materials and methods

2.1. 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-CHR₂ plasmids at DIV7 using the calcium phosphate method.

2.2. Whole cell patch recordings in hippocampal cultures
Whole cell recordings in ACSF external solution (124mM NaCl, 26mM NaHCO₃, 10mM glucose, 5mM KCl, 2.5mM CaCl₂, 1.3mM MgCl₂, 1mM Na₂HPO₄) 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-Na 2mM, GTP Mg 0.3mM, HEPES 10mM). Following establishment
of whole-cell configuration, the light with period 1 s in duration from 10 ms to 500 ms were illuminated and the corresponding potential changes were recorded.

3. Light pulses generator
The developed device (4) is a part of the optogenetic research system, which illustrated in Figure 1. It allows to set the specific light pulses settings. The duration, frequency and number of optogenetic impulses are controlled by the integrated microcontroller.

![Figure 1. Optogenetic research complex 1-Digidata 1440A - Data Acquisition System; 2-Axopatch 200B - Patch-clamp amplifier; 3-Inverted microscope; 4-Optogenetic impulse generator; 5-DC4100 - 4-channel LED driver; 6-Personal Computer.](image)

The developed device is a driver for a LED source controlling. The pulse generator is used in the complex of optogenetic studies. This unit is used both as an independent regulator of light pulses, and as a programmable unit. In the second case, the device is built into the chain of adaptive feedback and performs the function of signal tuning. The control signal is generated based on the data from the biopotential amplification block. Figure 2-3 shows a schematic diagram and 3D scheme of the developed device.
Figure 2. Optogenetic pulse generator (General scheme).

Figure 3. Optogenetic pulse generator (3D scheme).

4. Test light pulses generator on the hippocampal neurons culture
Checking the device was carried out in conjunction with thorlabs DC4100. Experiments were conducted in transfected with ChR2-GFP plasmid mouse primary hippocampal neurons. Traces of neurons activity were recorded in current-clamp mode (whole-cell configuration) in response of 470-nm light. The results are shown in Figure 4.
Figure 4. Action potentials evoked by 470 nm light (indicated by blue bar, T=1s) with duration A – 500 ms, B – 10 ms recorded in current-clamped neuron in whole-cell configuration.

5. Future plans and conclusions
The created devices could affect neurons by light stimulation with the specified parameters, particularly close to physiological conditions. Our future plans include the development of optogenetic brain implantable system for in vivo experiments.

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