Electric Field Stimulation (EFS) of human iPSC-derived neuron using Hamamatsu FDSS/μCELL

Natsumi Kato, Sunao Hisada, and Fumio Iwase, Systems Division, Hamamatsu Photonics K.K., Hamamatsu 431-3196, Japan
Ko Zushida and Hideo Saotome, iPS PORTAL Inc., Kyoto 602-0841, Japan

Introduction

Hamamatsu developed a 96-channel electrode array system that is mounted on the FDSS/μCELL. It adds electric field stimulations (EFS) to all 96 wells in a microplate simultaneously while fluorescence/luminescence signals are monitored. Using this instrumental setup, we electrically stimulated human iPSC-derived neurons (iCell® Neurons, Cellular Dynamics International) to evoke a Ca^{2+} response, transient increase of intracellular Ca^{2+} concentration, which was measured with calcium-sensitive fluorescent dyes.

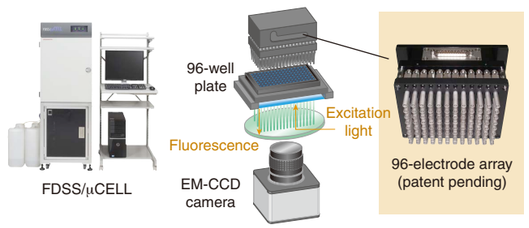
Materials & Methods

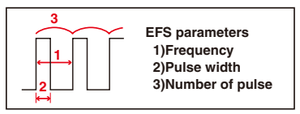
Intracellular Ca^{2+} measurements in neurons using FDSS/μCELL

The human iPSC-derived neurons (iCell® Neurons, Cellular Dynamics International) were cultured in 96-well microplates (Coster). The cells were incubated with 5 μM of a calcium-sensitive fluorescent dye, Cal-520/AM (AAT Bioquest), with 1.25 mM probenecid (Sigma-Aldrich) and quencher for 1 h at 37 °C in 5% CO_2. The fluorescence images of all wells in a microplate were taken every 0.08 s to capture changes in intracellular Ca^{2+} concentration using FDSS/μCELL (Hamamatsu Photonics K.K.), a kinetic plate reader for cell-based fluorescent assays that acquires fluorescent/luminescent signals of all wells in a microplate simultaneously.

Electric stimulation of neurons using the electrode array mounted on the FDSS/μCELL : the EFS system

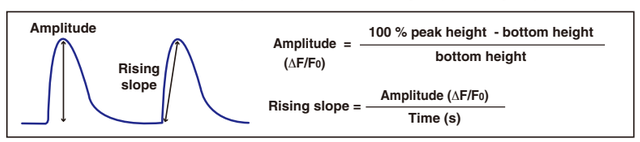
Our developed 96-channel electrode array is used with the FDSS/μCELL. The electric field pulses are given to all 96 wells in a microplate simultaneously by the electrode array that is positioned at the upper side over the microplate. The excitation light is introduced from the bottom side, and fluorescence signals are monitored with an EM-CCD camera.





Analysis of calcium waveform

The intracellular Ca^{2+} concentration changes (calcium waveforms) were analyzed using the FDSS Waveform Analysis Software for Cardiomyocytes (Hamamatsu), which estimates peak rate, peak width, peak-to-peak time, rising slope, falling slope, and more.

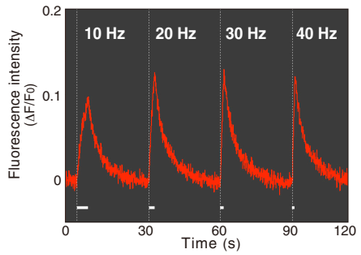


The FDSS/μCELL EFS system should not be used for optically detecting/monitoring change in transmembrane potential of the cells.

The FDSS/μCELL EFS system should not be used on any cell or cells in which the user or anyone else has expressed target ion channels.

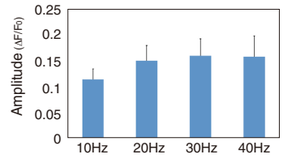
Results ; Intracellular Ca^{2+} concentration changes by EFS in human iPSC-derived neurons

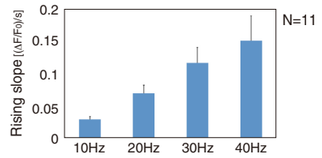
(1) Ca^{2+} response evoked by Electric Field Stimulations

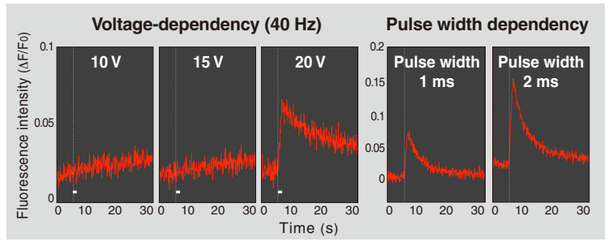


Electric field stimulation (EFS) parameters

Voltage	20 V
Pulse Width	2.0 ms
Number of pulse	50 times at each stimulation
Frequency	10, 20, 30, 40 Hz

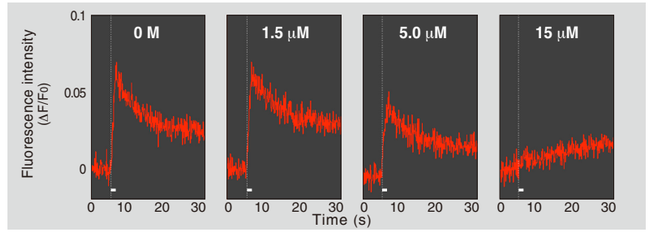


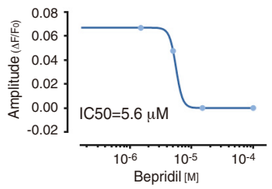




Human iPSC-derived neurons were cultured in a 96-well plate. To evoke the Ca^{2+} response, 50 times of electric field pulses (2 ms of pulse width) were given to the neurons at various frequencies. The left figure shows the intracellular Ca^{2+} concentration changes by adding EFS at 10, 20, 30 and 40 Hz sequentially. The white bar indicates the time during which the EFS pulses were repeatedly added (5, 2.5, 1.67, and 1.25 s for 10, 20, 30, and 40 Hz, respectively). In an experiment, we observed results of 11 wells in a microplate. In every 11 wells, the Ca^{2+} responses were observed similarly. The amplitudes and the rising slopes of these Ca^{2+} responses were shown (left graphs).

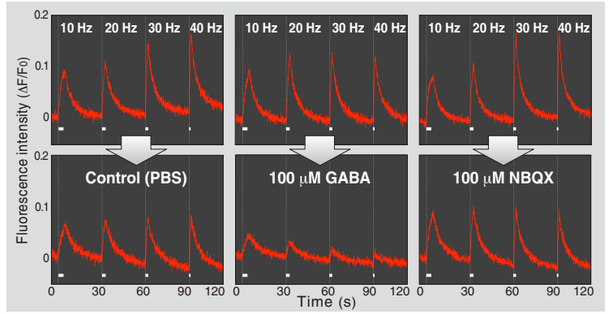
(2) The Ca^{2+} response is inhibited in the presence of a calcium channel blocker, Bepridil





Bepridil, a calcium channel blocker, was added to the wells at each concentration described, incubated for 20 min, and then EFS pulses (5 ms of pulse width, 40 Hz) were given. The upper figures show intracellular Ca^{2+} concentration changes. At more than 15 μM, the Ca^{2+} response was completely inhibited. The IC_{50} value was estimated to be 5.6 μM (lower graph).

(3) Effects of GABA and an antagonist for non-NMDA receptor, NBQX



We examined effects of an inhibitory neurotransmitter, GABA, and an antagonist for non-NMDA receptor, NBQX. From left to right of each figure, the EFS pulses were added at 10, 20, 30, and 40 Hz sequentially. First, we measured the Ca^{2+} response evoked by EFS in the absence of any compounds, and after the compound was added, the Ca^{2+} response were measured again. GABA largely reduced the amplitude of Ca^{2+} response. In contrast, NBQX had little effect.

Summary

Human iPSC-derived neurons (iCell® Neurons, Cellular Dynamics International) were electrically stimulated by Hamamatsu EFS (Electric Filed Stimulation) system mounted on the FDSS/μCELL, while intracellular Ca^{2+} concentration changes were monitored with calcium-sensitive fluorescent dyes. Transient increase of intracellular Ca^{2+} concentration was observed upon EFS.

We examined effects of a calcium channel blocker, an inhibitory neurotransmitter, and an antagonist for non-NMDA receptor on such Ca^{2+} response upon EFS.

The results show that the EFS system on FDSS/μCELL is able to evoke the Ca^{2+} response in human iPSC-derived neurons, which would be useful phenomena for characterization of these cells and also in disease-model studies and drug discovery with human iPSC-derived neuronal cells.

For further information, questions, and comments, please contact **natsumi.kato@sys.hpk.co.jp** or **SDu@hamamatsu.com**