Mechanisms for the light-cell interface in optical cell stimulation

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Advances in optical neurostimulation have shown advantages over electrical approaches by translating precise optical stimuli into electrophysiological signals on a single cell level. Using a neuroblastoma cell line, we studied the effects of different optical stimulation methods. The different strategies were evaluated to make a first attempt at assessing the feasibility of an optical hearing aid.

1 Introduction

Electric neurostimulation has become an important means of therapy for several neurological pathologies. However, an electrode cannot deliver a stimulus with sufficient precision to stimulate single cells in a dense neural network. Concerning sensory devices like a cochlear implant, an imprecise stimulus due to electronic crosstalk results in a blurred sensory impression.

Optical approaches might overcome these limitations by generating the stimulus with high precision on a single cell level. We focused on a gold nanoparticle mediated approach for optical stimulation and investigated the related biochemical effects in a murine neuroblastoma cell line. As a perspective, this data is put into the context of alternative stimulation mechanisms, transmitter uncaging and optogenetics, to evaluate the applicability of the approach for long term neural stimulation.

2 Methods

Cell culture: Cells of the murine neuroblastoma cell line N2A were cultured in Eagle's MEM containing EBSS, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, and incubated at 37°C, 5% CO_2. Before experiments, cells were cultured without serum for 48 to 72 h to promote differentiation.

Laser stimulation: Approx. 3 h before stimulation, 0.5 µg/cm^2 of 200 nm gold nanoparticles were seeded upon the cells. Then cells were stained with the calcium dye Fluo 4-AM according to the manufacturer's protocol (Thermo Fisher Scientific). Laser stimulation was performed with a picosecond pulsed Nd:YAG laser at 532 nm for 40 ms. Either only one or five consecutive pulses were applied. The fluorescent signal of the dye was recorded before, during, and 60-90 seconds after the laser pulse. Viability and cell perforation were assessed with propidium iodide.

Inhibitors: In some stimulation experiments, inhibitors were added 10-15 minutes before stimulation at the following concentrations: CGP-37157 20 µM, 2-APB 75 µM, lidocaine 100 µM.

Glutamate uncaging and optogenetics: 30 µM Ru-Bi-glutamate was added to an N2A culture for uncageing experiments. For optogenetics experiments, N2A cells were transfected with Chronos. In both cases the evoked currents in response to blue light pulses were recorded by patch clamping.

3 Results and Discussion

It has been shown that action potentials are accompanied by fast calcium transients [1]. By applying a laser pulse to gold nanoparticles attached to the cell, its membrane quickly heats up, producing transmembrane currents which may evoke action potentials [2], [3], [4]. The N2A cells reliably produced a calcium transient which led to a 2-5-fold increase in fluorescence (Fig. 1).

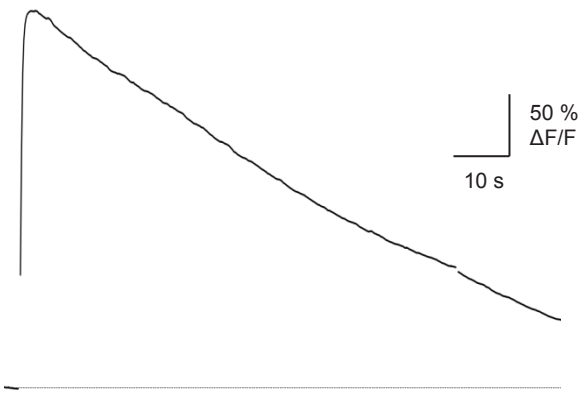

Fig. 1 *Representative calcium trace after laser pulse.*

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However, the transient that would be expected from an action potential is both faster and of lower amplitude [1], [4].

Different pulse energies between 17 and 51 mJ/cm^2 were used. The change in fluorescence did not depend on the laser power. However, with increasing power the time between laser pulse and peak fluorescence decreased.

An elevated calcium level in response to an unspecific stimulus is often an indicator of stress [5]. To further investigate whether the observed transients are part of an electrophysiological or a stress response, different inhibitors were employed (Fig. 2). When either Ca^{2+} release from intracellular compartments was blocked or no extracellular calcium was present, peak fluorescence values decreased compared to medium control.

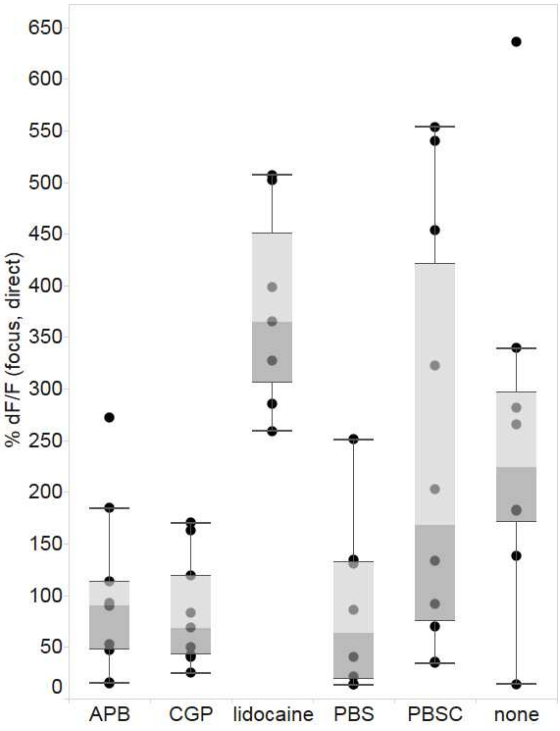

***Fig. 2** Peak change in fluorescence for each inhibitor. APB and CGP: block Ca^{2+} release from ER and mitochondria, respectively. Lidocaine: blocks voltage-gated sodium channels. PBS: phosphate buffered saline without Ca^{2+}. PBSC: PBS + Ca^{2+} and Mg^{2+}. None: Eagle's MEM only.*

This behavior hints at calcium induced calcium release (CICR), where Ca^{2+} release from intracellular stores is promoted by inflow of extracellular Ca^{2+}, or store-operated calcium channels, which open up in response to large amounts of Ca^{2+} released from internal stores. The prolonged elevation of cytoplasmatic calcium, independent of its origin, poses considerable stress on the cells [5]. It is therefore likely that this stimulation method would cause longterm damage to cells when applied at high frequencies.

Preliminary experiments show that both transmitter uncaging and optogenetic manipulation might be suited for stimulation of N2A cells. Inward currents were evoked using both methods. In cells transfected with the light sensitive opsin Chronos, currents of 100 pA were reliably evoked at frequencies as high as 100 Hz, which might be enough to induce action potentials. Further experiments will determine the limitations of spatio-temporal resolution of these approaches. Diverse caged compounds and opsins are available, so that experiments can be designed flexible to meet the requirements of certain cell lines or experimental outcomes.

4 Conclusion

The studied cell line and laser source does not seem to be suitable to reproduce the promising results from nanoparticle mediated stimulation experiments that were obtained from primary cells [3], [4]. However, our results implicate that this method poses a considerable amount of stress on the cells and is not a favorable approach at least for high frequency stimulation.

Transmitter uncaging and optogenetics allow for more flexible experimental designs. Both have drawbacks for in vivo applications – a steady supply of caged transmitters is required for uncaging, and gene therapy in humans is still not approved. But with a sophisticated design, these approaches might be used to mimic actual neuronal activity.

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