Title: Electric field temporal interference stimulation of neurons in vitro.

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Abstract

Electrical stimulation (ES) techniques, such as deep brain and transcranial electrical stimulation, have shown promise in alleviating the symptoms of depression and other neurological disorders *in vivo.* A novel noninvasive ES method, called temporal interference stimulation (TIS), shows great potential, as it can be used to steer the stimulation and selectively activate different brain regions [1]. However, TIS and its effects on neuronal electrophysiological activity have not been demonstrated *in vitro*. To address this, we established an *in vitro* 'TIS on a chip' setup using microelectrode arrays in combination with a current stimulator. The stimulus was applied via four platinum electrodes submerged in the cell medium through a 3D-printed cap. Cultures of rat cortical neurons at 28 days *in vitro* were subjected to two channel stimulation delivering 1) TIS at 653 Hz and 643 Hz, resulting in a 10 Hz frequency envelope, 2) low-frequency stimulation (LFS) at 10 Hz, and 3) high-frequency stimulation (HFS) at 653 Hz. Unstimulated cultures were used as controls/sham. We successfully established a novel stimulation platform for noninvasive ES *in vitro*, showing spatial steerability in a 'TIS on a chip' system [2]. We observed differences in the electric field strengths during TIS, HFS, and LFS. As hypothesized, HFS and LFS did not increase neuronal electrophysiological activity. However, TIS elicited neuronal electrophysiological responses, which were the most prominent 24 hours after stimulation. The approach opens new avenues for studying neuronal responses to TIS to gain insights into its potential clinical applications in treating various brain disorders.

References:

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