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Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation *in vitro*

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Background
The neocortex is the most common target of subdural electrotherapy and noninvasive brain stimulation modalities, including transcranial magnetic stimulation (TMS) and transcranial current simulation (TCS). Specific neuronal elements targeted by cortical stimulation are considered to underlie therapeutic effects, but the exact cell type(s) affected by these methods remains poorly understood.

Objective
We determined whether neuronal morphology or cell type predicted responses to subthreshold and suprathreshold uniform electric fields.

Methods
We characterized the effects of subthreshold and suprathreshold electrical stimulation on identified cortical neurons *in vitro*. Uniform electric fields were applied to rat motor cortex brain slices, while recording from interneurons and pyramidal cells across cortical layers, using a whole cell patch clamp. Neuron morphology was reconstructed after intracellular dialysis of biocytin. Based solely on volume weighted morphology, we developed a parsimonious model of neuronal soma polarization by subthreshold electric fields.

Results
We found that neuronal morphology correlated with somatic subthreshold polarization. Based on neuronal morphology, we predict layer V pyramidal neuronal soma to be individually the most sensitive to polarization by optimally oriented subthreshold fields. Suprathreshold electric field action potential threshold was shown to reflect both direct cell polarization and synaptic (network) activation. Layer V/VI neuron absolute electric field action potential thresholds were lower than layer II/III pyramidal neurons and interneurons. Compared with somatic current injection, electric fields promoted burst firing and modulated action potential firing times.

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Conclusions
We present experimental data indicating that cortical neuron morphology relative to electric fields and cortical cell type are factors in determining sensitivity to sub and supra threshold brain stimulation.

Keywords transcranial magnetic stimulation; direct current stimulation; cortex; layer; rTMS; motor threshold; functional electrical stimulation; TDCs; electroconvulsive therapy

Clinical application of transcranial magnetic stimulation (TMS) and transcranial current stimulation (TCS, encompassing transcranial direct current stimulation [tDCS], cranial electrotherapy stimulation, transcranial electric stimulation [TES], and electroconvulsive therapy) are promising noninvasive approaches for the treatment of a number of psychiatric, neurologic, and pain disorders[1-6] as well as the study of human cognitive function and neural plasticity.[7-10] Because the electric field (voltage gradient) in the extracellular space induced in the brain by TMS/TCS decays with distance from the stimulating coil or electrode, the neocortex is the most common target of noninvasive electrotherapy.[11-15] Invasive cortical stimulation that uses subdural strips/arrays is indicated for a range of therapeutic and diagnostic applications, including pain and preoperative brain mapping.[16,17]

Fundamental questions remain regarding the cellular targets of each cortical stimulation paradigm, including the relative activation of morphologically and functionally diverse groups of inhibitory interneurons and excitatory pyramidal cells.[18] Stimulation waveform, direction, and frequency is thought to preferentially affect specific cortical cell types[18-20] and/or specific segments of a neuron such as axonal bends and terminations.[11,21,22] Neuronal segments oriented toward the stimulating anode (virtual anode for electric fields induced by TMS[22]) have been shown to hyperpolarize, and concomitantly the segments oriented toward the (virtual) cathode depolarize (Supplementary Figure 1).[23,24]

The effects of electric field-induced polarization has traditionally been categorized as "subthreshold" changes in ongoing neuronal processing/timing,[25-27] or "suprathreshold" stimulation that directly triggers action potentials.[26,28,29] Clinical brain stimulation modalities, and associated therapeutic outcomes, may depend specifically on subthreshold (eg, tDCS) and/or suprathreshold (eg, TMS) neuronal effects (reviewed in Wagner et al.[30]). Cortical cell types,[31] distinguished by their laminar position, network connectivity, and neuronal morphology/biophysics, play defined roles in network processing and thus merit investigation in the context of both subthreshold and suprathreshold stimulation paradigms.[18]

In response to the unique electric fields induced by each brain stimulation modality,[22,32-34] neuronal membranes are considered to polarize in a "compartment" specific manner; the polarized compartments interact according to the electrotonic decay along the neuron (Supplementary Figure 1). Neuronal modeling[35-38] and *in vitro*[25,39] studies of electric field stimulation have identified morphologic features that govern the polarization of (interacting) neuronal compartments, including branching patterns and membrane space constants. Changes of compartment angle relative to an applied electric field (eg, activating function, the second derivative of the extracellular voltage along the neuronal membrane), branch terminations, or changes in intercompartment impedance can determine the locations of entry and exit of induced transmembrane currents that lead to polarization.[21,23,35,38,40] The neuronal space constants (λ), and related diameter of axons and dendrites, govern the axial distribution of these induced transmembrane polarizations, and therefore regulate the degree to which neuronal compartments interact.[38,39,41,42] Concurrent polarization of individual segments of a neuronal tree can lead to complex changes in overall neuronal function by modulating cellular biophysics,[43,44] including nonlinear voltage-gated conductances, synaptic efficacy, and action potential (AP) threshold or timing.[23,28,29,45,46]

The goal of this study was to determine whether the distinct morphologic features of cortical cell types affect their response to stimulation by electric field. We performed whole-cell recordings, of pyramidal cells and interneurons in rat motor cortex brain slices, during uniform electric field stimulation *in vitro*. Morphologic reconstructions of biocytin-filled neurons were correlated with electrophysiologic responses to electric fields. We considered differences between cortical cell types in their response to both subthreshold and suprathreshold stimulation. These data were used to consider the cellular targets of clinical cranial stimulation therapies.

Methods

Brain slice preparation

Coronal slices (300 μm) of primary motor cortex (M1) were prepared from male P21-25 Sprague-Dawley rats on a vibratome (Integraslice 7550 PSDS, Campden Instruments, Lafeyette, Indiana) as previously described.[25,47-49] In brief, rats were anesthetized with intraperitoneal ketamine (7.4 mg/kg) and xylazine (0.7 mg/kg) and euthanized by decapitation. After decapitation, the brain was quickly removed, blocked, and placed into ice-cold (4°C) oxygenated artificial cerebral spinal fluid (ACSF). ACSF contained (in millimolars) 125 NaCl, 26 $NaHCO_3$, 3 KCl,