Magnetoelectric Nanoparticles-Based Wireless in vitro Neuron Stimulation With sub 100-µm Spatial Differentiation

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**Theory and Hypothesis**

- **Principle of Operation:** Magnetoelectric Nanoparticles (MENPs) convert an applied changing magnetic field into a local changing electrical field, which can act on and stimulate individual neurons.
  - Our previous in vivo work proved the principle of operation (Guduru et al, “Magnetoelectric ‘spin’ on stimulating the brain”, NANOMEDICINE, May 2015 https://doi.org/10.2217/nnm.15.52)
  - Ground Truth Verification: Ca++ imaging of GCAMP6 expressing neurons at individual neuron resolution.

- **Hypothesis:** Through control of the distribution of MENPs and applied magnetic field, neurons that are less than 100 um apart can be differentially stimulated.
Magnetoelectric Nanoparticles (MENPs)

Unlike other nanoparticles, our MENPs display a strong magnetoelectric (ME) effect, coupling an applied magnetic field to a localized electric field that can act on neurons nearby.

**Coreshell Magnetoelectric Nanoparticles (MENPs)**

Transmission Electron Microscopy (TEM) (proving a coreshell configuration)

**M-H Loop of 30-nm MENPs**
(no superparamagnetic effect due to a relatively high magnetic anisotropy energy)

**Nanofabrication Approach:** To maximize the Magnetoelectric (ME) effect of the MENPs, we developed CoFe$_2$O$_4$ – BaTiO$_3$ coreshell nanostructures. In these nanostructures, with a size of 20-40 nm, the ME effect is due to the lattice-matched surface interface between the magnetostrictive core and the piezoelectric shell. **These structures showed a record high ME coefficient of above 1 V/cm/Oe.**

*“Colossal magnetoelectric effect in coreshell magnetoelectric nanoparticles,” Nano Letters 20 (8): 5765-5772 (2020)*
Spatial Resolution by Shifting Magnetic Field

Fig. 1. Experiments to investigate the effect of shifting a non-uniform 10 pulse/s (pps) pulsed magnetic field by a distance of ~50μm relative to a cell culture dish on the stimulations of the same set of neurons in a GCAMP6 expressing cortex culture. (a) Location 1 of the non-uniform pulsed magnetic field relative to the culture. (b) Location 2, which is shifted ~31μm from field location 1, and (c) Location 3, which is shifted ~50μm from field location 2, ~80μm from field location 1. Most of the same cells are in all three FOVs, which are 330μm x 330μm and the resolution is 0.33μm/pixel. (d) Illustration of the shift of the pulsed magnetic field. Plots of Ca++ intensity of neurons 4 (red) and 5 (green) in the two FOVs are shown in (e) and (f) on next page.
Shifting Magnetic Field → Stimulates Different Neurons

Fig. 1 (continued). (e) Plots of Ca++ intensity of neurons 4 (red) and 5 (green) in field location 1 as shown in (a), in response to field application turned “On” (green line) and “Off” (red line). (f) Plots of Ca++ intensity of neurons 4 (red) and 5 (green) in field location 3 as shown in (c). The field generating (f) is shifted by ~80μm from field generating (e), thus neurons 4 and 5 in (f) are under different magnetic field gradient than neurons 4 and 5 in (e). (e) shows that neuron 5 had no detectable response in location 1 but had significant response when shifted to location 3 as shown in the plots in (f). Conversely, neuron 4’s response decreased when the field is shifted to location 3. Red and green vertical lines indicate the approximate on and off times of magnetic stimulation. The 4 large spikes in (e) are manual adjustment to counter defocus drift in the microscope.
Comparison of MENP-Induced Response to Spontaneous Activity

Fig. 2 Traces of Neurons 4 and 5 in comparison with the traces of two spontaneous firing neurons (neurons 7 and 28). Note the waveform and magnitude of the red trace (neuron 4) is comparable to that of the trace of neuron 28. (b) Zoomed in waveform of neuron 4 response under magnetic stimulation highlighted in the white circle. It shows a 10 Hz response, the same frequency of the applied magnetic field.
Fig. 3. Top: Bars in the chart show the average Stimulation Response Ratios (SRR) of neurons 4 and 5 at locations 1 to 3. The SRR is computed as the ratio of the average peak-peak variation of Ca++ intensity over that prior and after the ith magnetic stimulation. Thus, a SRR of 1 indicates no response. The error bars are the standard deviation.

Bottom: Data from experiments 1085 to 1090, shown in a table of SRR of the responses of neurons 4 and 5 to the ith magnetic stimulation.

The data shows with a p-value of 1.43E-08 that a ~50μm shift of the stimulating magnetic field from location 2 to location 3 causes neuron 5 to respond which did not respond prior to the second shift. Neuron 4's response becomes weaker when shifted to location 3.
Our experiments showed that

- Applying a pulsed magnetic field to MENPs can stimulate nearby individual neurons.

- The combined effect of shifting an applied magnetic field profile and the corresponding change in MENP distribution can differentially stimulate individual neurons that are less than 100 μm apart.