Magnetic Stimulation of Dissociated Cortical Neurons on a Planar Mulitelectrode Array*

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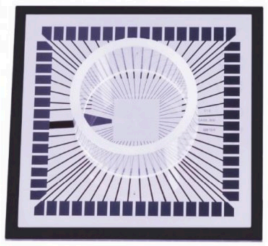
Abstract—We perform experiments to study the magnetic stimulus-induced changes in neural activity in dissociated cortical neurons with different stimulation parameters. The goal of performing these studies is to build on the results from our previous work that suggested magnetic stimulation may lead to improved performance of cochlear implants. A magnetic stimulator is assembled using a micro-scale coil. To detect small changes in activity, we use glass substrate MEAs to measure culture-wide synaptically-mediated response to stimulation, rather than the direct activation of individual neurons. Our initial findings show magnetic stimulation is associated with changes in network-wide firing rates, beyond those expected by spontaneous drift in activity. This suggests that the magnetic stimulation parameters we used were able to evoke neural activity. However, we observe substantial differences in the type of change induced in neural activity in different cultures and with different stimulation parameters, some showing increases in activity and others showing decreases in activity. This may be due to differences in the number and type of neurons (inhibitory or excitatory) activated by stimulation in different experiments, which in turn may be affected by differences in stimulator location and alignment, differences in stimulus pulse waveform and amplitudes, or differences in culture density or cell morphology. We also compare the power consumption and heating of this stimulation technique with that of electrical stimulation. Finally, a need to optimize the experimental setup to allow longer experiments is identified, to reach definite conclusions.

I. Introduction

Cochlear implants are one of the most successful neural prosthetic devices in the world with an estimated user base of 320,000 patients across the globe [1]. The state-of-the-art cochlear implants use electrical stimulation to convey sound information to the auditory neurons. However, this electrical stimulation leads to tissue inflammation, undesirable electrochemical reactions, and nonspecific spatial stimulation due to current spread; resulting in sub-optimal performance of the device [2][3]. Such sub-optimal performance has consequences for a patient's quality of life, through abnormal pitch perception and their inability to enjoy music [4]. In our earlier work we used finite-element analysis to show that magnetic stimulation of neurons promises better spatial resolution than electrical stimulation, which may lead to better frequency resolution in these implants [5]. To experimentally validate this finding, we performed a set of *in vitro* experiments on dissociated cortical neurons observing the neural activation by magnetic stimulation, limiting ourselves to establishing a platform for future work in this study. The small changes in neuronal activity are detected using glass substrate multielectrode arrays (MEA: Fig. 1). By performing these experiments without synaptic blockers, the inherently unstable population dynamics of the neural culture acts as a sort of 'amplifier' of stimulation-induced activity, enhancing our ability to detect activation of a small number of neurons. We applied external electrical stimulation, in addition to magnetic stimulation, to these neurons using a commercially fabricated thin-film array and compared some of the effects due to both these stimulation techniques.

Figure 1. An image of the MEA



II. Methods

We used MEAs manufactured by Multichannel Systems (Multichannel Systems MCS, GmbH) to record the neuronal data. These MEAs have a glass ring that forms a well, so that the culture can be kept submerged in a culturing medium at all times (Fig. 1). To maintain sterility, this ring is covered using Teflon lids. These lids also prevent rapid evaporation of culture media, thereby allowing experiments lasting a few days. However, this lid needs to be taken off during media changes and was kept off during the experiments reported in this paper. As a result, we were limited to a two-hour window for performing our experiments.

A. Preparation of the magnetic stimulator

15-mils solder wire (Kester, Itasca, IL, USA) is used to solder commercially available inductors (100 nH, MLZ2012N100LT000, TDK Corporation, Tokyo, Japan) that are 1 mm long and 0.5 mm wide, with 34-AWG copper wire (Belden, Richmond, IN, USA). This assembly is glued to a plastic pipette for precise placement of the inductor and insulated with a conformal, precise 10 μm layer of Parylene-C.

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When this assembled magnetic stimulator makes contact with media containing cells for experiments, the inductor and media behave like two plates of a capacitor and the Parylene-C coating acts as the dielectric between them. So, while the Parylene-C coating is known to be pin-hole free at a thickness of 5 μm, an additional 5 μm is coated, to reduce this capacitive coupling between the inductor and the media.

B. *Magnetic stimulation drive*

Fig. 2 shows a block diagram representing the driving circuitry. A waveform from the waveform generator is applied as input to the Pyramid amplifier (PB717x, Pyramid, Brooklyn, NY), which has a gain of 4.3 V/V. During preliminary experiments the amplifier was observed to introduce distortions or non-linear behavior at frequencies higher than 12 kHz and for voltages higher than 5 V. As a consequence, all experiments are performed within these limits, to avoid any non-linearity in the setup. The range of stimuli tested are presented in Table I. All stimuli are presented at a repetition rate of 2 Hz and the average experiment time for recording neuronal response is five minutes.

Figure 2. A block diagram of the driving circuit for magnetic stimulation. Here, (a) Agilent 33522A 30 MHz function/ arbitrary waveform generator, (b) Pyramid PB717x 1000W audio amplifier, (c) Agilent E3634 A DC power supply for the amplifier, (d) inductor connected in series with a 1-Ω resistor, and (e) Neurorighter for analog channel recordings.

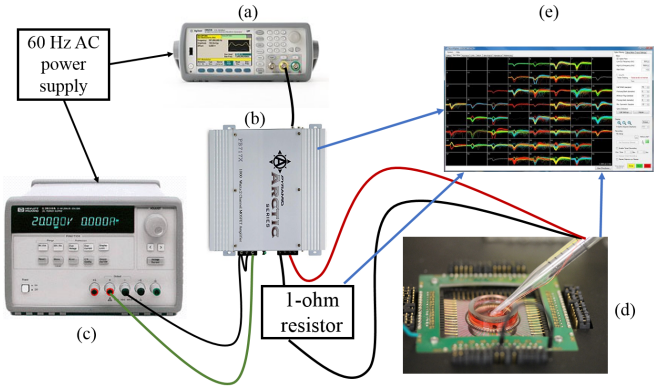


TABLE I. STIMULATION PARAMETERS TESTED DURING MAGNETIC STIMULATION

Voltages (V)	0.175 - 4
Frequencies (kHz)	2.5 - 10
Number of cycles per pulse	1 - 4

C. *Preparation of cultures for in vitro analysis*

Dissociated cortical neurons of embryonic day 18 rats received from BrainBits LLC (Springfield, IL) are plated on sterilized MEAs. The plating density is 1000 - 3000 cells per μL, and ~20 μL of dilute cell suspension is applied to each MEA[1]. For sterilizing the MEAs, they are soaked in ethanol for 30 minutes while under UV light in a laminar flow hood, then they are left to dry overnight under UV light. The medium based on the recipe described by Jimbo et al [7] is used to maintain the cell cultures as it has been observed to maintain high levels of activity in neurons. For the experiments reported in this paper, three sets of plating were performed on 7 seven different MEAs.

D. *Recording apparatus and data acquisition*

The MEAs used for experiments in this paper have 60 electrodes, including 59 recording electrodes arranged to span an area of 4.68 mm^2, and one distal reference electrode 0.4 mm away from the rest. An MCS array with four different quadrants is used for the ease of differentiating responses based on position of the electrodes (60-4QMEA1000iR-Ti-gr, MCS, GmbH).

E. *Experimental protocol for in vitro experiments*

Physical movement of a plated MEA can cause significant change in activity of the neurons. To avoid any effects of this movement on our recording, all the cultures were allowed to rest for at least 20 minutes before inserting the magnetic or electrical stimulator into them. Once the stimulator was inserted, another 15 minutes were allowed before recording the first set of spontaneous data.

F. *Data analysis*

Neurorighter, an open source electrophysiology tool, provides raw data in the form of a 64 x n matrix, where 64 corresponds to 60 MEA electrodes and 4 auxiliary channels, and n is the number of samples [8]. First, the size of this raw data is reduced by eliminating noisy and grounded channels. Next, stimulation artifacts are removed from the raw recording, using custom MATLAB files. Then the neuronal spikes are detected using a spike detector with an unconventional threshold of 7 times the average channel noise *vrms*. This higher threshold ensures that only neuronal spikes are detected, thereby making further analysis easier.

In general, synaptic blockers[2] are used to eliminate culture wide bursts, making the interpretation of data easy. In our case, we observed that the spontaneous spiking reduced significantly upon addition of synaptic blockers. So, we performed the experiments without adding these blockers to the culture. This allowed the amplification of neuronal response, which may otherwise go unnoticed. At the same time, it increased the level of complexity in data analysis several times. Hence, we lean towards a more statistical approach to determine the effect of stimulation and calculate the firing rate of these neurons.

III. RESULTS

For the results reported in this section, experiments are performed on plated MEAs, where micro-scale magnetic stimulator is inserted into the MEA well and stimulation is delivered using parameters listed in Table II. It is ensured that the stimulus is provided for a duration of 400 μs, which corresponds to 1 cycle of sinusoidal signal at 2.5 kHz, or 4 cycles at 10 kHz. This pulse width has been shown to be most effective for exciting neurons on MEAs [9]. The recorded raw files are analyzed using MATLAB, and the important findings are presented.

[1] The plating procedure closely follows the one outlined by Hales et al. [6].

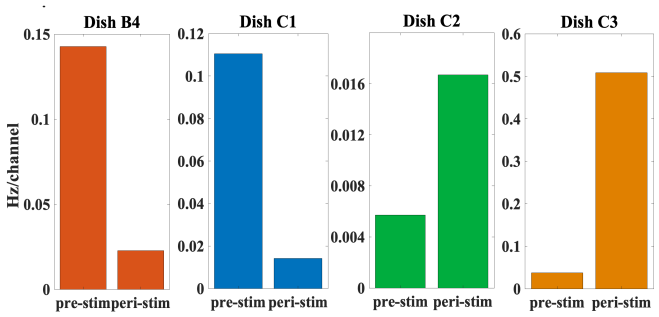
[2] 100 μm APV (amino-5-phosphonopentanoic acid), 50 μM Bicuculline (Bicuculline Methiodide), and 10 μM CNQX (6-cyano-7-nitroquinoxaline-2, 3-dione).

A. *Magnetic stimulation results in a change in firing rate*

Firing rate per channel of neurons during spontaneous activity before stimulation is compared with the firing rate of neurons during stimulation, and a test for statistical significance is performed. For each experiment reported here, 600 stimulus pulses are delivered over a period of 5 minutes and the response to stimulus is recorded. Table III reports the parameters tested and the results for statistical significance.

The null hypothesis tested here is: *There is no significant difference between the firing rate per channel between the two data sets tested* with a 5 % significance level. An h-value of zero means the hypothesis cannot be rejected, whereas an h-value of one indicates rejection of the null hypothesis. The Wilcoxon signed rank test is used to obtain the h-values, where the data sets compared contain the firing rate per channel before and during magnetic stimulation. This test is used because the firing rate does not follow a normal distribution and the two data sets: spontaneous and stimulation induced are not correlated.

Figure 3. Firing rate before and during stimulation for dishes B4, C1, C2, and C3 are plotted.



Four out of the six cultures that were tested show a statistically significant change in firing rate in response to stimulus (Fig. 3). To confirm that this change is because of stimulation and not a spontaneous change in behavior of neurons, ten minutes of spontaneous activity is recorded from three different cultures and the acquired data is divided into two five-minute long data sets. The same statistical significance test is performed on these data sets. The rejection of null hypothesis in all these recordings suggests that, there is no significant change in spontaneous activity during a short span of ten minutes in absence of stimulus. We performed these tests on firing rates for electrical stimulation of neurons as well. This electrical stimulation was introduced using commercially available thin film arrays and driven using a Plexon stimulator. The threshold for neuronal activation was found to be ±500 μA. We use this value for all later analyses.

B. *Analysis of factors that may cause a change in firing rate*

To ensure that the change in firing rate was indeed due to magnetic stimulation, we ensured that the inductors were properly insulated before and after each experiment by measuring the resistance between the two ends of the inductor. Any leakage in the insulation layer presents itself in the form of an increased resistance between these two ends. Thereby ensuring that the induced activity was not due to electrical effects.

TABLE II. THE SIGNED RANK TEST ON 'BEFORE' AND 'DURING' STIMULATION FIRING RATE OF CULTURES

Dish	Voltage (V)	Frequency (kHz)	# of cycles	h-value
B4	3	10	4	1
B2A	3.5	2.5	1	0
B1	4	5	2	0
C1	3.5	2.5	1	1
C2	0.5	2.5	1	1
C3	0.25	2.5	1	1

Additionally, we calculated the temperature changes on a plated MEA, in response to magnetic stimulation at one of the higher voltages tested, 3.5 V, over a period of five minutes, and recorded a net change of 0.23°C in temperature. This rise in temperature is not sufficient to significantly alter the firing rate as suggested by literature [10]. Additionally, physical movement can cause significant change in activity, but our experimental protocol ensures that this effect has subsided before the experiments are performed. Hence, based on the above analysis we conclude that the observed change in firing rate during experiments were in fact due to the applied magnetic stimulus.

C. *Temperature changes at 2 Hz repetition rate*

A Traceable® Excursion-Trac™ USB Datalogging Refrigerator/ Freezer thermometer (Cole Parmer, Vernon Hills, IL) is used to record temperature changes during five-minute-long experiments, where the stimulus is applied at the maximum values tested, that is, ±500 μA for electrical stimulation and 3.5 V for magnetic stimulation. A general increase in temperature is observed as the media, and the thermometer along with it, is adjusting to the incubator temperature. The recorded temperature changes were less than 1 ºC suggesting that temperature changes are not significant for both electrical and magnetic stimulation at low repetition rates.

D. *Power comparison: magnetic vs. electrical*

Power consumption is of prime importance for an implantable device, as it has a direct impact on the frequency of battery recharge/change. Hence, we calculate the power consumption for both magnetic and electrical stimulation in the cases tested during our experiments. For magnetic stimulation pulses delivered at 2 Hz in the form of a 2.5 kHz single cycle of sine wave, the average power consumption is:

- 60.4 mW at an amplitude of 3.5 V to the amplifier, and
- 370 μW at an amplitude of 250 mV to the amplifier.

The effect of amplifier's amplification is included in these calculations. For electrical stimulation pulses delivered at 2 Hz in the form of a biphasic current waveform with peak amplitude of ±500 μA, the average power consumption is 40 μW. Here the electrode impedance is considered to be 0.2 MΩ which is at the higher end of the spectrum [11].

IV. DISCUSSION

While the results presented above establish a promising baseline, the range of both the effects produced by stimulation, and the possible factors that may have modulated those effects

make it difficult to draw precise conclusions from them. Future work may isolate the impact of stimulation waveforms and stimulator location by using a range of such parameters for each culture. This will extend experiment duration, which will require methods to maintain culture osmolarity and temperature. Experiment duration can be decreased by increasing the reliability of the measured response to stimulation, for example by measuring the responses of individual cells.

Magnetic stimulation has been applied in clinical studies. However, the scale of stimulation analyzed in this study is much smaller than standard studies. This scale of stimulation promises a more localized impact area and may be used to study intact neural networks in greater detail. As a first step, we try to understand these networks *in-vitro*, which allows us to study a simpler cause-effect scenario, as the system is isolated. Ideally, the next step would be an *in-vivo* animal study before moving on to human trials.

Finally, there is a need to understand and characterize micro-scale magnetic stimulation better. Studies so far, including this one, show the effects of magnetic stimulation on a set of neurons. However, similar to electrical stimulation studies, there is a need to study the mechanisms of stimulation in greater detail, and the first step toward that goal is to have some basic standardized protocols to follow. For micro-scale magnetic stimulation, most studies use the same 100 nH inductors, and audio amplifier as used in this study. Some studies have reported innovative coil designs, while others study different types of neurons in vitro, and in vivo [12] [13]. Most of the stimulation pulses used are either sinusoidal or rectangular pulses, but a well-established threshold has not yet been defined. Multielectrode arrays do provide a standard testing platform that is capable of functioning with different types of neurons and may be the platform that this field of research needs.

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