Dynamic Steering of In Vitro Cortical Neurons Using Field Stimulation

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Abstract— **Neurological disorders are often characterized by abnormal neuronal activity. In the case of epilepsy, this can manifest itself in the form of uncontrolled synchronous activity often in the form of bursting. Pattern steering is the ability to apply stimulation to a network that effectively changes its dynamical firing pattern. In an epileptic network, the stimulation would be used to move the seizing network from its abnormal state to a normal state. This idea is explored here in cultured networks of cortical neurons plated on microelectrode arrays. Stimulation was applied to the bath resulting in an electric field generated throughout the network. This field was verified as sub-threshold in strength using a finite element model simulation. Stimulated networks showed a significant suppression in the number of bursts and increase in the interburst interval as compared to control networks. This observed burst suppression suggests that the sub-threshold stimulating field moved networks from a state of high frequency bursting to a state of low frequency bursting.**

I. INTRODUCTION

Neurological disorders can be attributed to an abnormal state of the brain, characterized by an unusual pattern of neuronal activity. In the case of epilepsy, this abnormality takes the form of high frequency uncontrolled synchronous activity often in the form of bursting [1], [2]. Upon detection of an epileptic seizure the ability to apply a sub-threshold stimulation that moves the brain out of this abnormal epileptic state to a non-seizing state would be a huge development towards the control of seizures. This shift from seizing to non-seizing can be thought of as pattern steering. At a high level, the idea of steering is the ability to move a dynamical system from one behavior to another using an applied stimulus. This change is permanent in the sense that the system will not move back to its original state or another state unless a subsequent stimulation is applied. Stimulation of neuronal networks has proven to be effective in changing neuronal behavior, such as bursting [3], [4], [5]. However, conducting neuronal stimulation through electric fields would provide an environment more similar to naturally occurring endogenous fields within the brain. These endogenous fields are low frequency and sub-threshold electric fields hypothesized to play a significant role in guiding cortical activity [6]. It has been demonstrated that the application of relatively low frequency (1-50 Hz) electric fields could modulate neuronal activity while the stimulation was active, entraining neuronal firing to the oscillations of the stimulating field. When the field was turned off, activity returned to its non-entrained state [6], [7], [8], [9].

These results did not demonstrate pattern steering since the observed change in activity occurred only during stimulation. To study the potential capabilities and mechanics involved in network steering, primary neuronal cultures plated on microelectrode arrays (MEAs) were used. These networks are spontaneously active, displaying a variety of network behavior. The capability of MEAs to simultaneously record extracellular neuronal activity at each electrode across the network makes them an invaluable tool for the study of network dynamics [10], such as network connectivity [11], [12] and the effects of electrical stimulation [13]. The use of MEAs allows for the continuous monitoring of the spontaneous firing patterns before and after stimulation.

To further explore the influence of electric fields on neuronal activity, in vitro networks of cortical neurons plated on MEAs were stimulated with electric fields through the arrays bath. This stimulation setup was chosen due to its robustness to various experimental designs. Results from a finite element modeling simulation determined that the stimulating field was sub-threshold and distributed homogenously throughout the network. The electric fields generated were characteristically similar to endogenous fields in the brain, oscillating at a low frequency and sub-threshold in strength. In particular, the effects on network bursting were examined. Network burst parameters were calculated and used to determine any influence of stimulation on the networks dynamics. Network activity was examined up to half an hour after stimulation in order to ascertain whether or not a persistent change occurred. The percent change of burst parameters as compared to baseline values were calculated for all MEA networks. Results showed a statistically significant difference between stimulated and control networks for the percent change in the number of bursts as well as interval between bursts. This supports the notion that we were able to change the dynamical behavior of the network, essentially steering the network from a high frequency bursting state to a low frequency bursting state.

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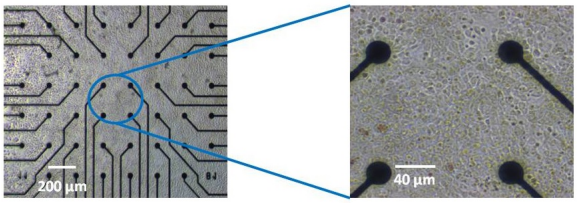


Fig. 1. Representative in vitro network of cortical neurons plated on a microelectrode array (MEA). The MEA is plated with E18 mouse cortical cells at a density of approximately 150,000 cells per array. Cultures were incubated at 37° with 10% CO_2 and maintained by a 50% media exchange twice a week until recording and experimentation at 28 DIV.

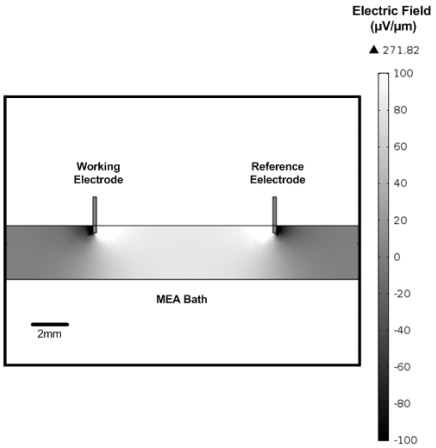
II. MATERIALS AND METHODS

Animal procedures were approved by George Mason Universitys Institutional Animal Care and Use Committee (IACUC) under protocol 0221. Cortical neurons from embryonic day 17, CD-1 mice were cultured on MEAs as previously described in detail [14]. Cultures were incubated under controlled temperature (37°) and humidity (10% CO2) until recording at 28 days in vitro (DIV), as suggested by prior work [15], [16]. A typical MEA dish at 28 DIV shows a carpet of cells covering the electrodes (Fig. 1).

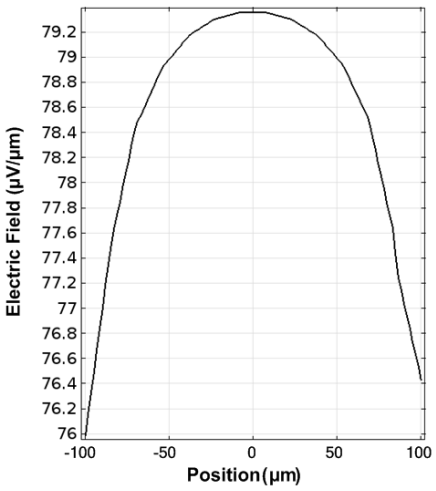
Our experimental protocol consisted of three periods: baseline recording, treatment period and post-treatment recording. A 30 minute baseline recording of spontaneous network activity was established. Networks (n = 5) then underwent ten minutes of field stimulation. A continuous low frequency sinusoidal wave, 1 Hz and 1 V peak-to-peak (Vpp), was applied to the networks with an Agilent 33220A function generator (Agilent, Santa Clara, CA) through two silver/silver chloride (Ag/AgCl) electrodes placed into the media of the MEA well. Immediately after the stimulation period, a 30 minute post-treatment recording of the networks spontaneous activity was obtained to determine if a persistent change in the network behavior occurred. Additional control networks (n = 4) went through the same protocol except that they did not receive any electrical stimulations.

Extracellular recordings were obtained with a Multichannel Systems (MCS) set-up (Reutlingen, Germany) and temperature was maintained at 37°C with a TC02 Temperature Controller (Multichannel Systems, Reutlingen, Germany). A threshold for spike detection was individually set for each electrode to 4.5 standard deviations of the base level noise, as determined by the recording software (MC Rack). Individual spikes were not sorted to distinguish separate units since the interest was in the effect of stimulation on network dynamics as a whole. Recorded extracellular activity was homogenous across the network during the course of the experiment.

Burst analysis was conducted for each active electrode in a network. A burst can be described as a sequence of action potentials whose interspike interval (ISI) is less than some determined threshold. Burst definition was done as detailed in [17]. After examining the distribution of the network logISI, a burst was characterized as at least five spikes with an ISI of at most 100 ms. Once initiated, a burst terminated when an ISI greater than 100 ms occurred.



(a)



(b)

Fig. 2. (a) Results of the COMSOL simulation of the electric field distribution throughout the MEA when the sinusoidal stimulation (1 Hz and 1 Vpp) was applied to the MEA bath through the Ag/AgCl electrodes. (b) Zoomed in results of the electric field over a 200μm length at the bottom center of the dish (denoted as 0) where the neuronal cells are plated.The resulting field generated in this region is approximately 79.3 μV/m. This implies that each 10 m cell is exposed to around 0.793 mV, validating the sub-threshold nature of the stimulating field.

To quantify a networks bursting behavior interburst interval (IBI), burst duration and total number of bursts were calculated for each channel. Outliers were removed if they were at least two standard deviations from the mean. Each channel was normalized to its network average during baseline. These normalized values were then averaged within a network. Percent change from baseline was compared between stimulated networks and control networks using a Wilcoxon rank-sum statistical test. This analysis was done for each of the three burst measures.

To determine the strength of the stimulation that reached the neurons a 2-D finite element model (COMSOL, Burling-

ton, MA) was developed. The MEA well was modeled as a rectangle (3 mm $\times$ 35 mm) which contained cell culture media with permittivity of 80 and conductivity of 1.38 S/m [18], [19]. Stimulating and reference electrodes were modeled as two bars, 1 cm apart, each having 200 μm width and 2 mm height. Only 400 μm of the electrodes were immersed in the cell culture media. The distance between electrode tips and the center bottom of the well was 2590 μm. The conductivity and permittivity of the electrodes were 6e7 S/m and 1, respectively. For sake of simplicity, the electric field was calculated at the center bottom of the well where the neurons were presumably located.

III. RESULTS

In the bath stimulation setup, the electric field can be calculated by the equation below:

$$E = -\nabla V$$

where E is the generated electric field and is the gradient of the potential [20]. This differential equation was simulated as the governing physics in COMSOL given the described model setup in the Methods section. The resulting electric field generated throughout the MEA chamber when the stimulation was applied to the bath can be seen in Figure 2a. While the field strength varies throughout, within a 100 m radius of the center of the array the electric field was homogeneous. This region of the MEA is where the neuronal cells were plated. Restricting our attention to this 200 μm diameter, the generated field was approximately 79.2 μV/m (Fig. 2b), which implied that a typical 10 μm cell was exposed to 0.792 mV. Such an external field was considered sub-threshold from the perspective of a neuronal cell in the cortex, validating the stimulation setup.

The percent change in number of bursts, IBI and burst duration, as compared to baseline, were calculated for stimulated and control networks. Stimulated cortical networks exhibited a decrease in the number of spontaneous bursting events after stimulation as compared to baseline. In contrast, control networks did not show any noticeable difference. Raster plots showing this effect in a representative control and stimulated network can be seen in Fig. 3a and Fig. 3b respectively. The data showed that networks which received stimulation significantly decreased the number of bursts compared to control networks Ws = -2.45, $p < 0.05$. There was also a significant increase in IBI for stimulated networks compared to controls Ws = -2.45, $p < 0.05$. However, there was no significant difference between stimulated networks and controls for burst duration, Ws = -1.60. A detailed description of these results is summarized in Table 1.

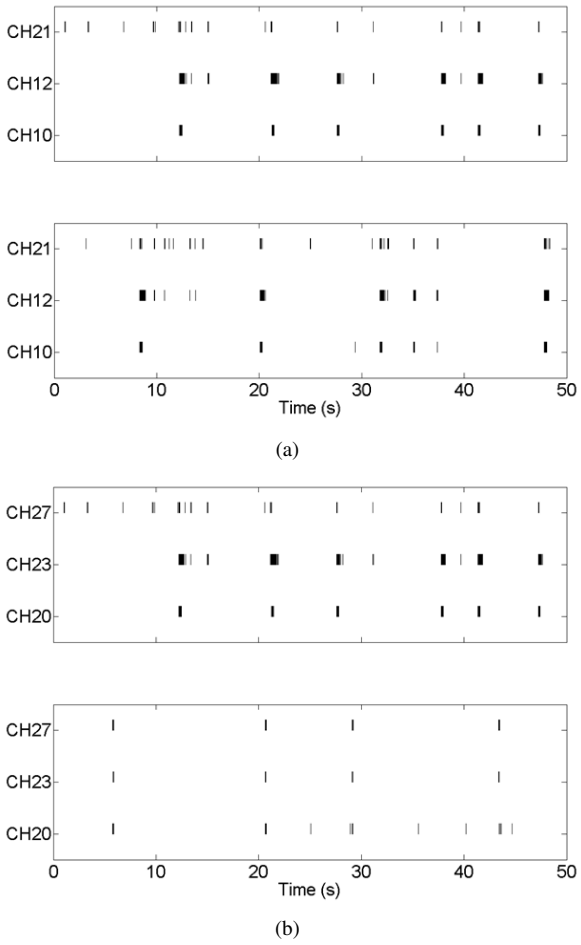


Fig. 3. (a) Raster plots displaying 50 seconds of spontaneous network activity from three active channels of a representative control network. Baseline activity (top) and post-treatment activity (bottom) after ten minutes of no stimulation. There were no visibly noticeable differences between these two periods. (b) Raster plots displaying 50 seconds of spontaneous network activity from three active channels of a representative stimulated network. Baseline activity (top) and post-treatment activity (bottom) after ten minutes of electric field stimulation. There was a noticeable suppression of network bursting after exposure to the sub-threshold field.

IV. DISCUSSION

The results presented here show that pattern steering can be achieved in neuronal networks. By applying a sub-threshold electric field to several cortical networks in vitro we were able to change the fundamental dynamics of the system. Specifically, the networks in a state of high frequency bursting were shifted to a state of low frequency bursting. Such a change manifested itself in a decrease in number of bursts and an increase in the IBI. These changes were observed after stimulation had ended, showing a persistent effect of the stimulation on the network dynamics.

Using a stimulating electric field similar to biologically occurring endogenous fields would be a natural way to conduct the steering of network activity. Besides the benefit of having similar features to a biological phenomenon, the sub-threshold nature of the field would ensure that there are no adverse effects from the stimulation process. The COMSOL simulation confirmed that the area over the recording grid where neurons can be found was exposed to a sub-threshold electric field. This suggests that the observed decrease in spontaneous network bursting was the result of an actual change in the network dynamics caused by exposure to the electric field rather than the result of a stimulation-induced excitotoxicity.

TABLE I

Network	No. Networks	No. Bursts (%)	IBI (%)	Burst Duration (%)
Control	4	$-17 \pm 4\%$	$+15 \pm 0.07\%$	$-3 \pm 6\%$
Stimulated	5	$-70 \pm 10\%$	$+660 \pm 244\%$	$-43 \pm 15\%$

Summary of stimulation results. Reported values indicate average percentage change in dynamical measure (number of bursts, IBI, burst duration) as compared to baseline. (-) indicates a decreasing percentage and (+) indicates an increasing percentage. (*) indicates a statistically significant difference ($p < 0.05$) between the percent change as compared to baseline between stimulated networks and control networks. Statistical analysis showed significance for the difference in percentage change of number of bursts and IBI. This suggests that the applied field stimulation suppressed the spontaneous network bursting.

If a network can be steered to a state of decreased bursting, it should be able to be moved back to its original bursting state. This is the subject of future experimental work. The idea of steering in general suggests that by applying a stimulus to a network you should be able to move the system to its different dynamical regions. This capability would give greater insight into the different behaviors of the brain and in general how the brain functions. Exploring the different stimulation characteristics, in terms of waveform, frequency, duration and voltage, necessary to enact these different steering patterns is the subject of future research. However, the results presented here provide the groundwork for these experiments.

V. CONCLUSION

We have demonstrated that sub-threshold electric field stimulation can be used to change the dynamical behavior of neuronal networks. Networks of cortical neurons cultured on MEAs in vitro were administered a 1 Hz 1 Vpp stimulation through the bath. The resulting electric field generated throughout the network was modeled by COMSOL, and this simulation verified that neurons in the network were exposed to a sub-threshold field. This stimulating field was shown to have a large impact on the dynamics of the neurons. Our results suggest a significant difference in percent change from baseline between stimulated networks and control networks for number of bursts and IBI. Specifically, the stimulating field showed the ability to suppress the spontaneous bursting of networks. Generalizing the application of this study to the *in vivo* brain presents clear challenges, as the collective dynamics *in vivo* differ from those observed in *in vitro* networks. While further investigation is needed to determine how these effects seen here *in vitro* would translate to *in vivo*, the results do indicate that sub-threshold field stimulation can have a strong influence on network firing patterns.

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