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Multi-site Stimulation Quiets Network-wide Spontaneous Bursts and Enhances Functional Plasticity in Cultured Cortical Networks

Radhika Madhavan, Zenas C. Chao, Daniel A. Wagenaar, Douglas J. Bakkum, Steve M. Potter

Abstract-**We culture high-density cortical cultures on multi-electrode arrays (MEAs), which allow us to stimulate and record from thousands of neurons. One of the modes of activity in these high-density cultures is dish-wide synchronized bursting. Unlike *in vivo*, these synchronized patterns persist for the lifetime of the culture. Such aberrant patterns of activity might be due to the fact that cortical cultures are sensory-deprived and arrested in development. We have devised methods to control this spontaneous activity by multi-electrode electrical stimulation and to study long-term functional neural plasticity, on a background of such burst-quieting stimulation. Here, we investigate whether burst quieting reveals long-term plasticity induced by tetanic stimulation. Spatio-temporal activity patterns (STAPs) that result from probe pulses were clustered and quantified in quieted and non-quieted cultures. Burst-quieted cultures show more tetanus-induced functional change than cultures which are allowed to express spontaneous bursts. The methods developed for this study will help in the understanding of network dynamics and appreciation of their role in long-term plasticity and information processing in the brain.**

I. INTRODUCTION

Dissociated neural cultures on multi-electrode arrays (MEAs) allow for observation and manipulation at an individual cell level as well as the network level [1], [2], [3]. MEAs consist of 60 electrodes made of titanium-nitride embedded on a glass substrate which are connected to amplifiers and a computer that allows continuous stimulation of and recording from neurons lying on or near electrodes.

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R. Madhavan is with the Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA (phone: 404-385-4083;email: mradhika@neuro.gatech.edu)

Z.C. Chao is with the Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA (email: zenas@neuro.gatech.edu).

D.A. Wagenaar is with the Division of Biological Sciences, University of California, San Diego, La Jolla, CA, USA (email: dwagenaar@ucsd.edu).

D.J. Bakkum is with the Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA(email: bakkum@neuro.gatech.edu).

S.M. Potter is with the Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA(email:steve.potter@bme.gatech.edu).

Intracellular electrode recordings reveal properties of single neurons, but fail to provide information about the dynamics at the network level. We are interested in studying network-level dynamics in our dense cortical cultures and the emergent properties that result from them. We are especially interested in the study of network level long-term neural plasticity and learning mechanisms in this model system.

One of the dominant patterns of activity in these cultures is network-wide bursting [4], [5], [14] which might interfere with plasticity-inducing stimuli. During bursts, a large fraction of cells in the culture rapidly increase their firing rates by a factor of ≥10. Here, we investigate whether the control of spontaneous synchronized bursting *in vitro* makes cortical cultures more amenable to induction of learning-related plasticity. We have developed a multi-site distributed electrical stimulation protocol to quiet spontaneous dish-wide bursting [10]. We hypothesize that controlling bursts could help in maintenance and induction of plasticity, or make such plasticity easier to detect. To test this hypothesis, we compared the level of burst quieting to the amount of plastic change induced in the network by tetanic stimuli delivered via substrate electrodes.

This *in vitro* study of functional plasticity provides new methods that could also be used *in vivo*, with two-way electrode arrays, to investigate brain function as well as neurological disorders like epilepsy.

II. MATERIALS AND METHODS

A. Cell culture

Neocortex was dissected from rat embryos (E18) under sterile conditions. Cells were dissociated and plated in a dense monolayer (~50,000 cells consisting of both neurons and glia) on MEAs (Multichannel systems, Reutlingen, Germany) as described previously [6]. Cultures were maintained in dishes sealed with gas permeable membrane [6] to prevent infection and evaporation. Cultures were maintained in an incubator at 35°C, 65% R.H., 5% CO2, and 9% O2. All experiments were performed inside this incubator, guaranteeing stability of environmental conditions. All recordings were done on 2-3 week old cultures.

B. Electrodes and Recording System

We use glass MEAs with 30μm titanium nitride electrodes insulated with silicon nitride (Multichannel systems, Reutlingen, Germany). There are 59 such electrodes in an 8X8 grid with 200μm distance between the electrodes.

Signals were amplified using an MEA60 preamplifier, and digitized using an MC Card PCI board (both MultiChannel Systems). Data acquisition, artifact suppression [7] and visualization were controlled using our open-source MeaBench[1] software. Stimulus pulses were delivered using our custom-built 64-channel stimulator [8]. All electrical stimuli (tetani, quieting pulses and probes) were biphasic voltage-controlled pulses (500-800 mV) with durations of 400μs per phase [9].

C. Experiment protocol

Quieted experiments

25 electrodes that evoked responses in pre-experimental probing [1] (A probe is a stimulus at a single electrode) were arbitrarily chosen as quieting electrodes, and were stimulated in cyclic order with inter-stimulus interval of 20 ms. This resulted in reversible cessation of spontaneous bursting (Figure 3). Plasticity-inducing stimulation in these experiments was a 15 minute tetanus. Tetanization consisted of trains of stimulus pulse pairs delivered to two electrodes, with inter-electrode interval of 5-10 ms. Each train consisted of 20 pulse pairs, with 50-100 ms between each pair. A complete tetanization sequence consisted of 20-150 trains at 2-6 s intervals. Quieting stimulation was suspended during the tetanus. Probe pulses (600mV) were delivered to the two tetanus electrodes and 4 other electrodes in cyclic order with 1s between stimuli. The voltage for probe stimulation was chosen to be the voltage which evoked a reliable response at the probe electrode [Wagenaar et al. (manuscript submitted)]. Quieting was suspended 50 ms before and 200 ms after a probe pulse, so that the responses to probe stimuli could be measured without interference. In all experiments, probe-pulse-evoked activity was recorded for two hours before and two hours after the tetanus sequence. The tetanus sequence was repeated twice but we only used periods before the second tetanus for our analysis. An hour of spontaneous activity was recorded before and after each experiment. There were N=3 *Quieted experiments* from N=3 cultures (20-23 days *in vitro*) in this study.

Non-Quieted experiments

These experiments were similar to the *Quieted experiments* except that there was no burst-quieting stimulation. The probe pulses were 800mV. The voltage for probe stimulation was chosen to be the voltage which evoked a reliable response at the probe electrode [Wagenaar et al. (manuscript submitted)]. There were N=3 *Non-quieted experiments* from N=3 cultures (13-16 days *in vitro*) in this study.

The results in this paper were obtained by further analysis of experiments previously described (Wagenaar et al., submitted).

D. Analysis

Spatio-temporal Activity Pattern (STAP) of probe responses

For each probe electrode, the firing rate histogram was generated by counting number of spikes in 5ms moving time window (Time step = 500μs) for all spikes within 100ms of each probe stimulus. The Spatio-temporal Activity Pattern (*STAP)* for each stimulus is a 60xN dimensional vector where N= number of bins. For ever probe electrode, the *Mean STAP* was obtained by averaging *STAP*s from every five consecutive probes (step = 5 probes).

Clustering of Mean STAP

To investigate whether there was structure in probe responses, *Mean STAP*s for probe responses were compared against each other for correlation [11]. A dendrogram (paired clustering algorithm) was used to sort the correlation matrix. A contrast function was calculated to determine optimal number of clusters. The peak of the contrast function indicated the level at which the most distinct grouping of clusters occurred [11]. ***Occurrence*** represents when probe responses in different *Mean STAP* clusters happen (Figure 1). ***Integrated Occurrence*** is the total number of *Occurrences* of probe responses in 30 probe-wide moving window (step =5 probes).

Comparison between drift and tetanus-induced change

The Pre period is the probe period before any period of tetanization. The periods after the Nth tetanization are denoted as PostN (e.g. the period after first tetanus is Post1, after the second tetanus is Post2 and so on). *Integrated Occurrences* were measured from two parts of the Pre period separated by the same time as the duration of the tetanus and the first part of the Post1 period. Centroids of *Integrated Occurrence* during these periods were calculated. We then calculated the following quantity:

$$\Delta D_{ij} = \frac{mean(d_{ij})}{mean(d_{ii})}$$

where mean(d_{ii}) is the average Euclidian distance of each *Integrated Occurrence* in period i to the centroid of period i, and mean(d_{ij}) is the average Euclidian distance of each *Integrated Occurrence* in period i to the centroid of period j.

Therefore, ΔD_{ij} indicates the ratio of the change from period i to period j and the drift within period i. If $\Delta D_{ij} \sim 1$ then there's no significant change from period i to period j.

For all experiments, we measured two quantities: ΔD_{12} and ΔD_{23}. ΔD_{12} represents the ΔD_{ij} of between two parts of the Pre period (Pre1 and Pre2) separated by a duration equal to the duration of the tetanus, and ΔD_{23} represents the ΔD_{ij} of the 2nd part of the Pre period (Pre2) compared to the 1st part of the Post1 period. This allows testing whether the change across the tetanus was more than the drift within the periods Pre and Post 1.

The significant difference between the drift within the Pre period and the change across the first tetanus was tested by Wilcoxon's Rank sum test for equal medians between ΔD_{12} and ΔD_{23}.

Identifying Spontaneous bursts

Bursts were identified by a burst detector algorithm described elsewhere [10]. Briefly, any 100 ms window with more than 4 spikes on one electrode was considered to be a

[1] http://www.its.caltech.edu/~pinelab/wagenaar/meabench.html

part of a burst. Bursts that occurred within 10 ms of a stimulus were considered to be evoked and were excluded from this analysis. Bursts come in different sizes [14] and this analysis deals with dish-wide spontaneous bursts. We identified dish-wide spontaneous bursts as spontaneous bursts in which at least 50% of the active electrodes in the dish participated. These *Spontaneous dish-wide bursts* were used in further analysis. Burst rates (per minute) of the spontaneous dish-wide bursts (not evoked by stimulation) were determined in the Pre, Post1 and Post2 probe periods and mean burst rate during these probe periods was calculated as BR_{quiet}. The spontaneous burst rate was also determined in the periods where there was no stimulation as BR_{spont}.

Quieting Index

To determine how quiet the dish was during the probe periods, we calculated the following Quieting index:

$$Quieting\ Index\ (QI) = 1 - (BR_{quiet} / BR_{spont})$$

For dishes in which there are fewer spontaneous bursts (more effective quieting of bursts) during the probe periods, QI is closer to 1. In dishes where there are more spontaneous bursts (less effective quieting of bursts), QI is closer to 0. If the quieting were to induce additional dish-wide bursts, QI would be negative.

III. RESULTS AND DISCUSSION

It has been successfully demonstrated that activity-dependent modification, measured by probe responses, can be induced by a local tetanic stimulation [12], [13]. But, on trying to replicate the same experimental protocol, we found that the probe responses changed continuously and unpredictably (drift). This drift made it difficult to determine whether the changes were due to the tetanus or the probes themselves. We hypothesized that drift is due to the presence of spontaneous dish-wide bursting and devised methods to quiet spontaneous bursting using multi-site stimulation [10]. We then performed similar experiments on the background of such burst-quieting stimulation (Methods).

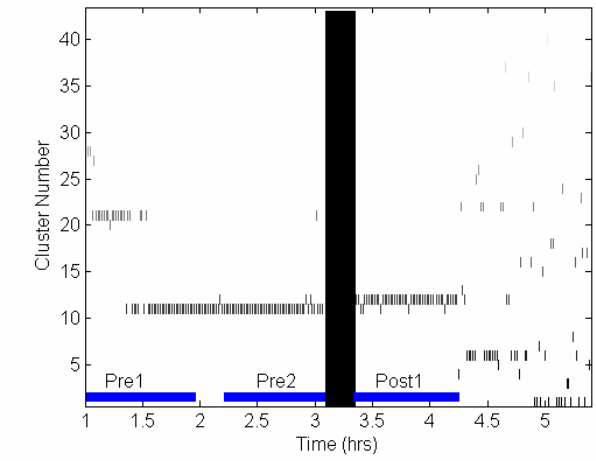

Figure 1: The *Occurrence* of clusters of *Mean STAP* of probe responses was stable before a tetanus and changed after the tetanus. This figure shows the *Occurrence* of *Mean STAP* for responses to a single probe stimulus for one Quieting experiment. Each stroke is the *Occurrence* of *Mean STAP*. The color indicates different clusters. The black vertical bars indicate tetanus periods. This experiment had a total of 6 probe electrodes.

Figure 1 shows the *Occurrence* of *Mean STAP* for probe responses to a single probe from one experiment. The black bars indicate tetanus periods. It is seen from figure 1, that the *Occurrence* of *Mean STAP* of probes responses remains stable before tetanus, and changes after the tetanus. This is a *Quieting experiment*.

The statistics comparing change across tetanus to the ongoing drift for N=6 cultures is shown in Figure 2. The quieting was suspended for 50 ms before and 200 ms after probe pulses, so that responses to probe stimuli can be measured without interference. The red dashed line in figure 2 indicates $\Delta D_{ij} = 1$; if the bars are close to the red line it indicates that there was hardly any change across the periods i and j. The drift indicates change within the two parts of the Pre period separated by same duration as the duration of a tetanus. Change indicates the change from second part of Pre period to first part of Post1 (across the tetanus). Figure 2 shows that in two experiments the change is significantly higher than the drift (*-$p<0.001$). These experiments were Quieted experiments (Methods).

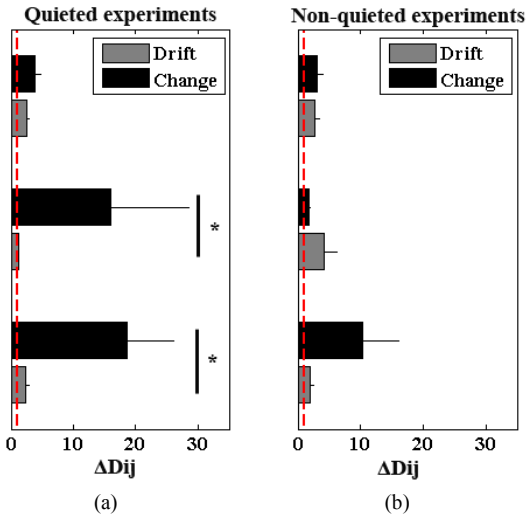

Figure 2: The change *Integrated Occurrence* of *Mean STAP* of probe responses across tetanus (ΔD_{23}) is significantly higher compared to the drift (ΔD_{12}) for at least two of N=3 *Quieted experiments* (* - Change is significantly higher than drift, $p<0.001$). Figure 2(a) shows drift and change across tetanus for Quieted experiments. Figure 2(b) shows drift and change across tetanus for Non-quieted experiments. The red dashed line indicates $\Delta D_{ij}=1$, which means that there was hardly any change of ΔD_{ij}. The error bars indicate standard error. Each bar is mean ΔD_{ij} for all the probes in the experiment. Drift is the ΔD_{12} within the Pre period and Change is the ΔD_{23} across the tetanus.

In figure 2, two out of three Quieted experiments show significant change compared to drift ($p<0.001$), while two of the three Non-quieted experiments show change comparable to the drift. To investigate whether the change after tetanus was more in dishes that had fewer bursts (more burst quieting) during the probe periods, we compared the Quieting index (QI) to the amount of change (ΔD_{ij} across tetanus). The mean QI for Quieted experiments was

0.98±0.01 and the mean QI for Non-quieted experiments was -0.87±0.5. A higher QI (QI~1, fewer spontaneous dish-wide bursts) corresponds to a more significant ($p<0.001$) change across a tetanus (Figure 2).

Figure 3 shows the dish-wide spontaneous burst rates for one of the experiments. It is clearly seen that in this experiment the burst quieting stimulation was successful in quieting bursts (QI for this experiment is 0.94). Also, the spontaneous bursts came back after the quieting stimulation was turned off. The spontaneous burst rate during the quieted period (black line) was much lower than the burst rate during spontaneous (non-stimulated) periods (grey line).

A dominant point of view in the burst literature is that bursts are needed to increase the reliability of communication between neurons [15]. It is important to note the difference between our network-wide bursts which include a majority of the active electrodes and typically last several 100 milliseconds and single-neuron bursts that last tens of milliseconds. These network-wide bursts resemble synchronous activity present during epileptic seizures [19], during certain sleep stages [18] or waves of activity during development [16]. *In vivo*, bursting occurs during development as a means to establish suitable connections in the brain [16], [17]. But, soon these bursts are replaced by more spatially heterogeneous spiking activity. Hence, the persistence of bursting in our cultures can be viewed as a sign that they are arrested in development [3].

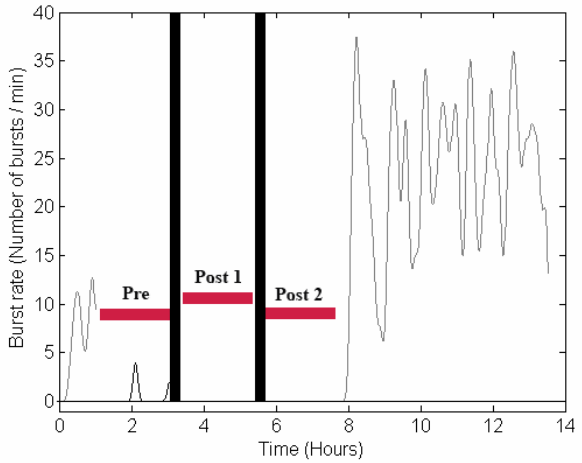

Figure 3: Burst rate of quieted cultures. The gray line indicates burst rate (number of spontaneous bursts/minute) in the spontaneous periods before probing. The black line indicates spontaneous burst rate in the period where there is quieting (and probes in this case) stimulation. The black vertical bars indicate the duration of the tetanus. It is clearly seen that the spontaneous burst rate is very low in the quieted period compared to the spontaneous period. This is data from a single experiment (same experiment as Figure 1).

IV. CONCLUSIONS

We have successfully demonstrated that cultures quieted by multi-site stimulation show more change in spatio-temporal activity patterns (*Occurrence* of *Mean STAP*) after tetanic stimuli. In our study, we use a measure of spatio-temporal patterns, *Occurrence* of *Mean STAP,* that remains stable in quieted cultures before a tetanus and changes significantly after the tetanus ($p<0.001$). In contrast, in cultures that are allowed to burst spontaneously, change in *Occurrence* of *Mean STAP* before the tetanus is comparable to change across tetanus (Figure 2). Thus, the drift, which might be caused by ongoing spontaneous activity can obscure or negate plasticity induced by external stimuli. Bursts are known to have an effect on tetanus-induced plasticity [5] and modeling studies in our lab have shown that spontaneous bursts change the synaptic weights in a simulated network [20]. Therefore, we suggest that by controlling spontaneous dish-wide bursts, we can make cultured cortical networks more suitable for study of distributed information processing and learning.

REFERENCES

[1] Y. Jimbo, H.P. Robinson and A. Kawana, "Strengthening of synchronized activity by tetanic stimulation in cortical cultures: application of planar electrode arrays," *IEEE Trans Biomed Eng.* vol. 45(11), pp.1297-304, 1998.

[2] S. Marom and G. Shahaf,, "Development, learning and memory in large random networks of cortical neurons: lessons beyond anatomy," *Q Rev Biophys.*, vol. 35(1), pp.63-87, 2002.

[3] M.A. Corner, J. van Pelt, P.S. Wolters, R.E. Baker and R.H. Nuytinck, "Physiological effects of sustained blockade of excitatory synaptic transmission on spontaneously active developing neuronal networks--an inquiry into the reciprocal linkage between intrinsic biorhythms and neuroplasticity in early ontogeny," *Neurosci Biobehav Rev.*, vol. 26(2), pp.127-185, 2002.

[4] H. Kamioka, E. Maeda, Y. Jimbo, H.P. Robinson and A. Kawana, "Spontaneous periodic synchronized bursting during formation of mature patterns of connections in cortical cultures", *Neurosci Lett*, vol. 206(2-3), pp.109-12, 1996.

[5] E. Maeda, H.P. Robinson and A. Kawana, "The mechanisms of generation and propagation of synchronized bursting in developing networks of cortical neurons", *J Neurosci*., vol. 15(10), pp.6834-6845, 1995.

[6] S.M. Potter and T.B. DeMarse, " A new approach to neural cell culture for long-term studies," *J.Neurosci. Methods*, vol. 110, pp.17-24, 2001.

[7] D. A. Wagenaar, and S. M. Potter," Real-time multi-channel stimulus artifact suppression by local curve fitting," *J. Neurosci. Methods*, vol. 120, pp.113-120, 2002.

[8] D. A. Wagenaar, and S. M. Potter, "A versatile all-channel stimulator for electrode arrays, with real-time control," *Journal of Neural Engineering*, vol.1, pp.39-45, 2004.

[9] D. A. Wagenaar, J. Pine, and S. M. Potter, "Effective parameters for stimulation of dissociated cultures using multi-electrode arrays," *J. Neurosci. Methods*, vol.138:1-2,pp.27-37, 2004.

[10] D.A. Wagenaar, R. Madhavan, J. Pine and S.M. Potter, "Controlling bursting in cortical cultures with closed-loop multi-electrode stimulation," Accepted in J Neurosci. Nov. 2004.

[11] Beggs J.M. and Plenz D., "Neuronal avalanches are diverse and precise activity patterns that are stable for many hours in cortical slice cultures", *J Neurosci*., vol.24(22), pp.5216-29, June 2004.

[12] Y. Jimbo, T. Tateno, and H. P. Robinson, "Simultaneous induction of pathway-specific potentiation and depression in networks of cortical neurons", *Biophys J*, vol 76(2), pp-670-678, February 1999.

[13] T. Tateno and Y. Jimbo, "Activity-dependent enhancement in the reliability of correlated spike timings in cultured cortical neurons", *Biol. Cybern,* vol. 80(1), pp.45-55, January 1999.

[14] D. A. Wagenaar, J. Pine, and S. M. Potter, "An extremely rich repertoire of bursting patterns during the development of cortical cultures", *BMC Neuroscience,*7(1), 2006.

[15] Lisman JE, "Bursts as a unit of neural information: making unreliable synapses reliable", *,Trends Neurosci* 20,pp.38-43, 1997.

[16] M. Meister, R.O. Wong, D.A. Baylor and C.J. Shatz, "Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina", *Science* 252, pp.939-943, 1991.

[17] X. Leinekugel, R. Khazipov, R. Cannon, H. Hirase, Y. Ben-Ari, G. Buzsaki, "Correlated bursts of activity in the neonatal hippocampus in vivo", *Science* 296, pp.2049-2052, 2002.

[18] R. Krahe and F. Gabbiani,, "Burst firing in sensory systems", *Nat Rev Neurosci*, 5, pp.13-23, 2004.

[19] E.J. Furshpan and D.D. Potter, "Seizure-like activity and cellular damage in rat hippocampal neurons in cell culture", *Neuron*, 3, pp.199-207, 1989.

[20] Chao, Z. C., Bakkum, D. J., Wagenaar, D. A. and Potter, S. M, "Effects of random external background stimulation on network synaptic stability after tetanization: a modeling study" . *Neuroinformatics* 3(3): pp 263-280, 2005.