

Results Summary: Controlling Chaotic
Bursting in Distributed, *In-Vitro* Neuronal
Networks¹

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1 Goals

During the course of this project, we set out to accomplish several goals hierarchically contingent and increasing in terms of their relevancy to clinical application. They are enumerated as follows,

1. Show the existence, to some statistical certainty, of deterministic laws governing spontaneous bursting in biological, *in-vitro* neural networks (Fig. 1).
2. Show that the dynamics of bursting are unstable and chaotic
3. Show empirically the existence of hidden modes in these bursting dynamics
4. Control chaotic bursting

Our motivation for this set of goals stems from the prevalence of probabilistic descriptions of computational dynamics at the network level and the need to ameliorate the general lack of elegance (and resultant detriments to human health) of current neural stimulation interfaces used in the clinic. Our philosophy hinged on a desire to understand the intrinsic dynamics of neural activity, from some formal perspective, before the imposing stimulation methodologies to alter neural function.

2 Results

Each subsection below comments on the group's progress in relation to each project goal.

2.1 Spontaneous Bursting in Neural Cultures is Deterministic and Nonlinear with Additive Noise

Using phase-randomized surrogate data, we tested each IBI series, $\{s_1, s_2, \dots, s_N\}$, against a class of 'uninteresting' processes:

- H_0 : The series was produced by a linear stochastic process

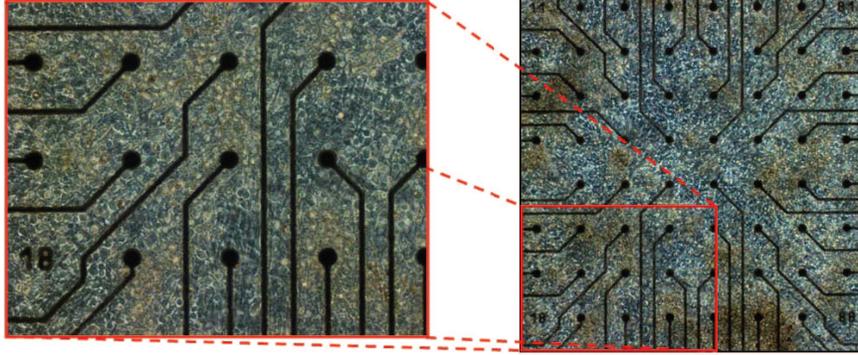


Figure 1: 1000X and 400X (left and right, respectively) phase-contrast magnification of a cultured neuronal network. Each network is grown on a microelectrode array of 59 recording electrodes (Titanium nitride electrodes, $200\ \mu\text{m}$ spacing) and a single ground electrode. These cultures contain about 20,000 living neurons derived from pre-natal rat cortex. They can survive for years with proper maintenance [1].

Phase-randomization preserves the power spectrum and first order statistical properties of the data while destroying higher order correlations [2, 3]. Using a time-asymmetry metric for nonlinearity,

$$\phi_{rev} = \frac{1}{N-1} \sum_{n=2}^N (s_n - s_{n-1})^3 \quad (1)$$

and a simple rank-order test we consistently rejected the null hypothesis (Table 1). Thus we can say with known degree of statistical certainty that bursting in these neuronal networks is a noisy nonlinear process.

2.2 Spontaneous Bursting in Neural Cultures is Probably Chaotic

Using the So transform [5, 6], we were able to consistently extract period-1 periodic orbits from IBI event series data (Fig. 2). The existence of an infinity of unstable periodic orbits (UPOs) embedded in the solution set of an attractor is a hallmark of chaotic dynamics. Because we looked at period-1 orbits only, we could not say conclusively whether the system was in fact chaotic, but a local linear analysis of dynamics about a Poincare surface

culture	d.i.v.	num. surrogates	rank	α
3-2	31	39	39	0.1
	32	39	40	0.05
	33	39	40	0.05
2-5	31	39	40	0.05
3-1	31	39	37	–
4-2	29	39	1	0.05
6-1	24	39	40	0.05
6-3	21	39	20	–
	24	39	40	0.05
7-2	35	39	3	–
8-1	34	39	38	–
8-3	31	39	39	0.1

Table 1: Nonlinearity tests on bursting event series in mature cultures. α corresponds to a level of significance $(1 - \alpha) \times 100\%$. Data from [4]

section of the the orbit seemed to indicate it was generally an unstable saddle. The existence of an unstable fixed point in a recurrent system informally provides the necessary ingredients for chaotic dynamics: local instability and global mixing.

2.3 Periodic Pacing Reveals Hidden Modes in Bursting

The period of bursting corresponding to a significant periodic orbit, T^* , found by the So transform corresponds to a natural mode of the system, possibly hidden in unforced bursting behavior by instability. Excitation of the system at this frequency should produce a resonance phenomenon. To test this hypothesis, we carried out periodic pacing by stimulating the network at three different frequencies:

$$f^* = 1/T^* \tag{2}$$

$$f_s = 1/(T^* + d_1) \tag{3}$$

$$f_f = 1/(T^* - d_2) \tag{4}$$

where d_1 and d_2 are positive constants. The amplitude of stimulation (2 ms duration, biphasic, 50% duty square wave applied at a single electrode) was

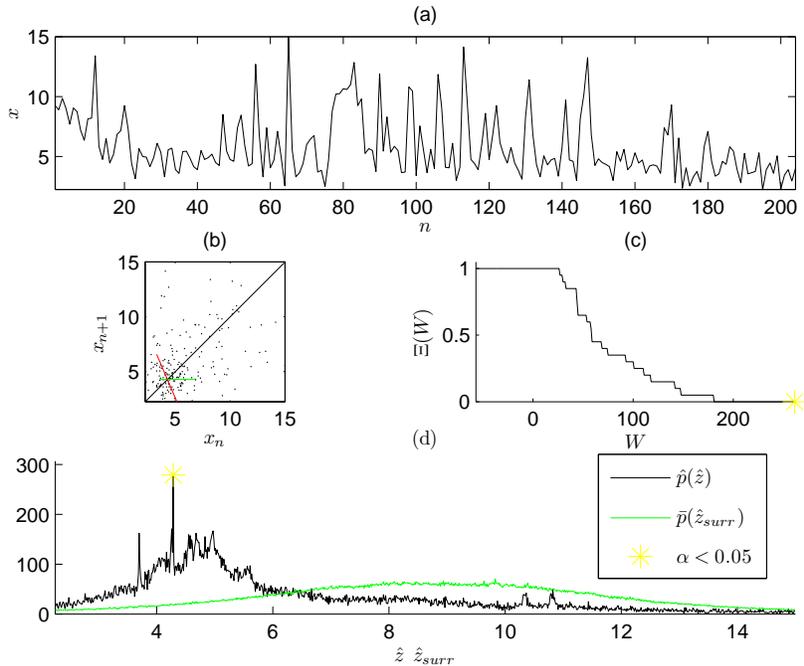


Figure 2: Unforced analysis summary for data collected from culture 12035 at age 35 d.i.v. (a) IBI event series from a 30 minute recording. (b) Delay embedding ($d=2$) of IBI series. The stable and unstable manifolds of the reported fixed point are shown in green and red, respectively. (c) Non-parametric extreme statistics test [5, 7] of peaks in $\hat{p}(\hat{z})$ versus that of peaks in the transformed surrogate data. The star appears at the power, W , of the significant peak and shows that probability of this peak occurring in surrogate transforms ($\Xi(W)$) is unobservably small for a 95% significance level. (d) Data under the fixed point magnification action of the So transform (\hat{z}). The location of the significant peak is the period of a UPO in the evolution of array wide burst timing.

decreased if a burst was elicited within some small error time around the delivery of stimulation, and increased otherwise. The goal was to find the amplitude that could reliably drive bursting at each frequency (3). Some typical data for this experiment is shown in Fig. 3.

Although there was variability in facilitation of burst generation when stimulating at f^* , we only witnessed one experiment (of 5) in which pacing

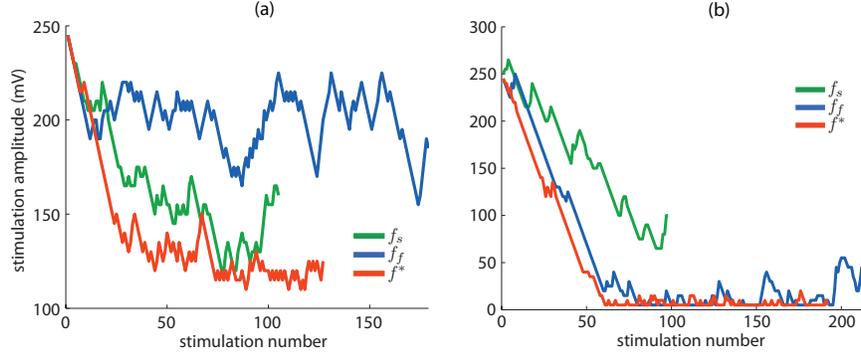


Figure 3: Typical periodic pacing results show that a lower amplitude stimulation was required to elicit bursts at the frequency corresponding to that of the period-1 orbit rather than faster or slower frequencies. (a) Culture MKC03112009 at 33 d.i.v. T^* for this culture was 6.88 seconds. d_1 and d_2 were 6.88 and 3.44 seconds, respectively. (b) Culture MKA01082009 at 61 d.i.v. T^* for this culture was 4.45 seconds. d_1 and d_2 were 4.45 and 1 seconds, respectively.

at f_f actually outperformed pacing at f^* . Pacing at f_s never outperformed pacing at f^* . These preliminary data are strong indications of the usefulness of understanding the natural dynamics of these neural system in terms of periodic orbit theory. When cast into this formalism, effective stimulation protocols become functions of the periodic orbits dictating intrinsic bursting dynamics.

2.4 Chaotic Bursting Can Potentially Be Controlled

Constrained linear regression about a fixed point (Fig. 2), using a temporal sequence of points before and after a close encounter, allows for the estimation of its invariant manifolds. If we assume that the dynamics governing burst timing are smooth, this provides a linear approximate model of state dynamics close to the fixed point. With this in mind, a simple linear controller will be sufficient to stabilize the fixed point and to control burst timing[8, 9] (Fig. 4).

We implemented a controller based on this philosophy with limited success. Time limitations and inadequacies of the real-time system prevented a full exploration of the cause of this failure. However, the theoretical potential

of this control scheme is clear and can be seen in Fig. 5.

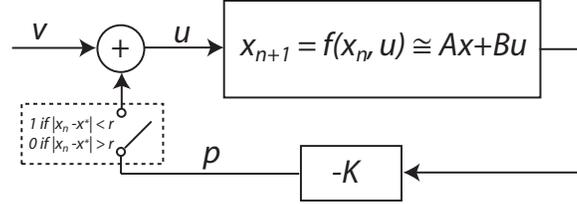


Figure 4: Block diagram of the pole-placement control scheme used for chaos control. Because dynamics are assumed to be chaotic and ergodic, the controller waits for the unforced plant to perform a close approach to the fixed point where dynamics are easily dominated by a simple linear controller. Then the fixed point is stabilized using small perturbations (pulse frequency modulation).

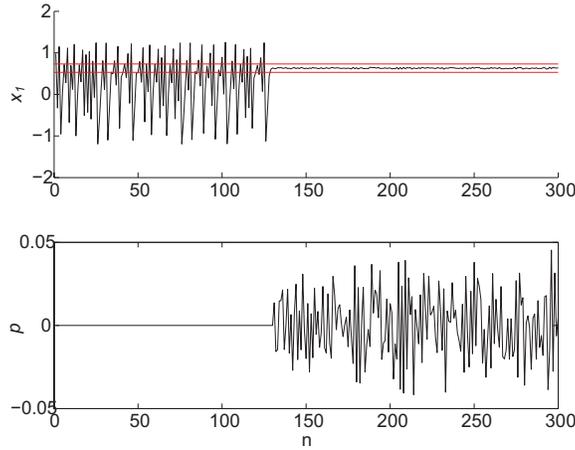


Figure 5: The local pole-placement control scheme (Fig. 4) used to stabilize the chaotic Henon map with additive white noise: $x_1 = 1 - ax_1^2 + b * x_2 + p + \text{unif}[-D, D]$, $x_2 = x_1 + \text{unif}[-D, D]$. The system waits for a close approach to the fixed point $x_1 = x_2 = x^*$, represented by the red lines, and then the controller is activated to stabilize the unstable point. The control perturbation was strictly bounded at $|p| < p_{max} = 0.05$ to demonstrate that only small perturbations are necessary to exert control.

3 Concluding Remarks

Although not all the goals of this project were met, the results outlined above do provide a unique insight into the nature of spontaneous bursting in neuronal cultures grown on MEAs. We feel that during our second attempt at these goals, armed with these results, we will succeed in the control of chaotic bursting. Because our real-time system is now fully operational, we will spend less time dealing with the bugs inherent in the practical application of control during the 15-30 d.i.v. period where offline analysis shows that our methods are the most applicable. Some improvements we plan to make are as follows:

1. Improvement of real-time system interface to prevent mistakes in experiment as it progresses.
2. Possible use of a ‘gold standard’ real-time system.
 - (a) Meabench
 - (b) Neurorighter
3. Design a more accurate methodology for estimating linear approximate model close to the fixed point.
4. Solidification of periodic pacing protocol by standardizing normalized experiment runtime and perturbation (d_1, d_2) amplitude.
5. Functional implementation of pole-placement controller.
 - (a) Implement controller in younger networks (10–30 d.i.v.).
 - (b) Creation of a controller using variable dimension delay space models.
6. Standardization of data collection and representation methods.
7. Culture rejuvenation. Current theories state that bursting is the result of a homeostatic increase in culture excitability in response to a lack of input during development [10, 11]. Can this process be reversed by addition of baseline stimulatory input?
8. Search for higher period orbits to show conclusively the existence of chaos in bursting.

9. Deal with non-stationarity/plasticity during experiments.
 - (a) Rigorous protocol to show that plasticity is non-significant during control. i.e. before/after snapshot of the dynamical landscape by using the So transform at the beginning and end of the experiment.
 - (b) Track the smooth progression of orbits over long-time records by windowing data into overlapping sets.
 - (c) Does stimulation at the frequencies required for periodic pacing and pole-placement induce ‘elastic LTD’ as shown in [12]?
10. Design a less arbitrary method for choice of stimulation site. This could be chosen as the spatial center of activity during a burst or the burst initiation site specific to a particular cell culture.

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