Int. J. Devl Neuroscience 38 (2014) 184–194

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Synaptic signal streams generated by *ex vivo* neuronal networks contain non-random, complex patterns

Sangmook Lee[a,b], Jill M. Zemianek[a,b], Abraham Shultz[a,c], Anh Vo[a], Ben Y. Maron[a], Mikaela Therrien[b], Christina Courtright[b], Mary Guaraldi[a,b], Holly A. Yanco[a,c], Thomas B. Shea[a,b,*]

[a] *Center for Neurobiology and Neurodegeneration Research, UMass Lowell, Lowell, MA 01854, United States*
[b] *Department of Biological Sciences, UMass Lowell, Lowell, MA 01854, United States*
[c] *Department of Computer Science, UMass Lowell, Lowell, MA 01854, United States*

ARTICLE INFO

Article history:
Received 25 February 2014
Received in revised form 19 August 2014
Accepted 19 August 2014
Available online 27 August 2014

Keywords:
Neuronal network
Synaptic activity
Cultured neurons
Multi-electrode array

ABSTRACT

Cultured embryonic neurons develop functional networks that transmit synaptic signals over multiple sequentially connected neurons as revealed by multi-electrode arrays (MEAs) embedded within the culture dish. Signal streams of *ex vivo* networks contain spikes and bursts of varying amplitude and duration. Despite the random interactions inherent in dissociated cultures, neurons are capable of establishing functional *ex vivo* networks that transmit signals among synaptically connected neurons, undergo developmental maturation, and respond to exogenous stimulation by alterations in signal patterns. These characteristics indicate that a considerable degree of organization is an inherent property of neurons. We demonstrate herein that (1) certain signal types occur more frequently than others, (2) the predominant signal types change during and following maturation, (3) signal predominance is dependent upon inhibitory activity, and (4) certain signals preferentially follow others in a non-reciprocal manner. These findings indicate that the elaboration of complex signal streams comprised of a non-random distribution of signal patterns is an emergent property of *ex vivo* neuronal networks.

1. Introduction

Cultured embryonic neurons develop networks that transmit synaptic signals over multiple sequentially connected neurons as revealed by multi-electrode arrays (MEAs) embedded within the culture dish (Wagenaar et al., 2004; Morin et al., 2005; Zemianek et al., 2012a,b, 2013a,b). Signals generated by such *ex vivo* networks convert over the course of approximately one month in culture from a pattern predominated by high-amplitude individual spikes to a "mature" pattern that is rich in complex bursts (van Pelt et al., 2004a,b; Madhavan et al., 2005; Wagenaar et al., 2006; Ikegaya et al., 2008; Chiappalone et al., 2009; Zemianek et al., 2012a,b, 2013a,b). Establishment of this mature pattern is dependent upon a critical neuronal density and a balance of inhibitory and excitatory activity (Serra et al., 2010; Zemianek et al., 2012a). External stimulation hastens the establishment of a signaling pattern characteristic of mature cultures (Jimbo et al., 1998; van Pelt et al., 2004a; Wagenaar et al., 2004; Wagenaar and Potter, 2004; Chao et al., 2005; Madhavan et al., 2005; Zemianek et al., 2012a,b).

These *ex vivo* findings indicate that establishment of networks that transmit signals over distances that require multiple synaptically connected neurons is an inherent property of neurons. We noted that signal streams of *ex vivo* networks contain spikes and bursts of varying amplitude and duration, which prompted us to question whether or not various signal patterns were elaborated randomly or instead displayed a non-random occurrence. Coupled with our prior demonstration that *ex vivo* neuronal networks can undergo changes in signaling in response to stimulation (Zemianek et al., 2012a), non-random occurrence of signals would suggest that, despite the inherent random connectivity that accompanies dissociated neuronal culture, *ex vivo* neuronal networks may contain some degree of self-regulation that underlies the basic aspects of synaptic activity *in situ*.

Visual and computer-based quantification of the frequency of occurrence of signal patterns allow us to demonstrate herein that (1) certain signal types occur more frequently than others, (2) the

* Corresponding author at: Center for Neurobiology and Neurodegeneration Research, UMass Lowell, Lowell, MA 01854, United States. Tel.: +1 978 934 2881; fax: +1 978 934 3044.
E-mail address: Thomas_shea@uml.edu (T.B. Shea).

http://dx.doi.org/10.1016/j.ijdevneu.2014.08.008
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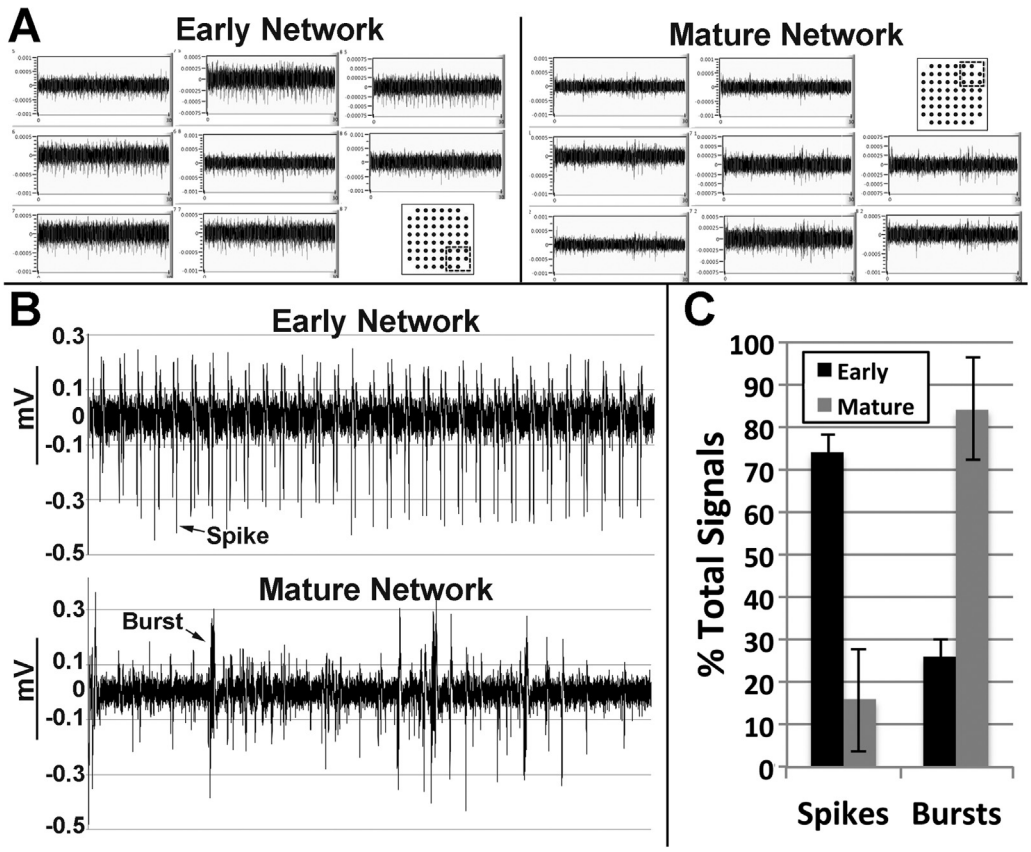

Fig. 1. Cultures display complex bursts following maturation. Panel A presents representative 30-s recordings of 8 channels from an early and a mature culture (9 and 42 days after plating, respectively; the portion of the total 60 recording channels presented is highlighted for each network by a dotted line in the accompanying diagrams). Panel B presents the average of all channels from the networks presented in panel A. A representative spike and burst are indicated (arrows). Panel C presents quantification of the mean percentage (±standard error of the mean) of total signals (defined as from baseline by ≥0.25 mV) that were spikes (separated from others by a minimum of 0.7 s) ≥mV or bursts (clusters of ≥3 spikes within 0.7 s prior to returning to baseline; Zemianek et al., 2012b) of the averages of all channels from 6 early and 5 mature cultures.

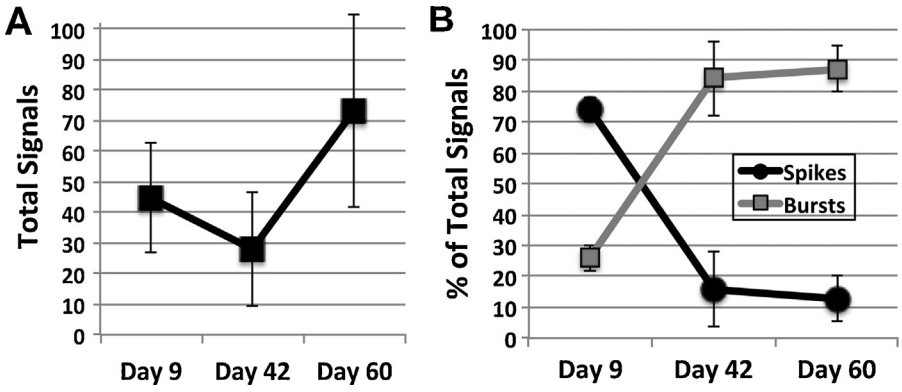

Fig. 2. Cultures continue to display complex bursts during aging. Panel A presents quantification of total signals as defined in Section 2 and in the legend to Fig. 1 in early, mature and aged cultures (9, 42 and 60 days after plating, respectively. No change in total signals was observed between early and mature cultures ($p < 0.16$; 6 early and 5 mature cultures) but a significant increase was observed between 42 and 60 days after plating ($p < 0.04$; 5 mature and 3 aged cultures). Panel B presents quantification of the percentage of total signals classified as spikes and bursts as defined in Section 2 and in the legend to Fig. 1; while the relative percentage of bursts significantly increased between days 9 and 42 ($p < 0.04$; see also Fig. 1), no change in the relative percentage of spikes *vs.* bursts was observed from 42 to 60 days after plating ($p < 0.71$).

predominant signal types change during and following maturation, (3) signal predominance is dependent upon inhibitory activity, and (4) certain signals preferentially follow others in a non-reciprocal manner. These findings indicate that complex signaling is an emergent property of neuronal networks.

2. Methods

Cortical neurons harvested from day 15 C57BL/6 mouse embryos were dissociated and plated at 100 cells/mm^2 in laminin-coated, standard glass multi-electrode arrays (MEAs) from Multichannel Systems (Reutlingen, Germany) and maintained in B27-supplemented Neurobasal medium (Invitrogen, Carlsbad, CA) as described (Serra et al., 2008, 2010). Homogenates from multiple embryos were combined prior to dispensing into MEAs. Sacrifice of pregnant females was carried out under procedures approved by our Institutional Animal Care and Use Committee.

MEAs were placed in a MEA-1060-INV amplifier (Multichannel) and synaptic activity recorded *via* a DT9814 data acquisition system (Data Translation; Marlborough, MA). Signals were recorded over 30 s intervals. Prior studies using combinations of excitatory and inhibitory neuronal antagonists have confirmed synaptic origin of signals (Serra et al., 2008). Data files (30 s in length) for each channel were analyzed manually with computer-based clustering algorithms (described