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Accelerated establishment of mature signaling patterns following stimulation of developing neuronal networks: "learning" versus "plasticity"

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ABSTRACT

Neuronal networks established on micro-electrode arrays provide useful models for synaptic plasticity. Whether or not this represents a facet of learning is debated since ex vivo networks are deprived of organismal interaction with the environment. We compared developmental signaling of such networks with and without stimulation with a prerecorded synaptic signal from another mature culture as a model of sensory input. Unstimulated networks displayed a developmental increase in individual signals that eventually declined, yielding a pattern containing organized bursts of signaling. Minimal stimulation, to model the onset of sensory input hastened the onset of developmental signaling. However, the overall developmental pattern of stimulated networks, including the total number and type of signals as well as the length of this developmental period, was identical to that of unstimulated networks. One interpretation of these findings is that ongoing plasticity may be essential to establish an appropriate platform for learning once sensory input ensues.

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1. Introduction

Learning in situ is contextual, and modulates behavior. As such, it invokes specific pathways, and in doing so, encompasses specific receptors and multiple synaptic alterations (Descalzi et al., 2012; Goosens and Maren, 2002; Izquierdo and Medina, 1997; Mitsushima et al., 2011). One critical aspect of neuronal activity that underlies learning is synaptic plasticity, which involves establishment, strengthening, weakening, and degradation of synapses (Bruel-Jungerman et al., 2007; Buonomano and Merzenich, 1998; Caroni et al., 2012; Chen and Buonomano, 2012; Malinow and Malenka, 2002; Maren and Baudry, 1995; Neves et al., 2008; Owen and Brenner, 2012; Poirazi and Mel, 2001; Wang et al., 1997; Winnubst and Lohmann, 2012).

Neuronal networks established on micro-electrode arrays (MEAs) provide useful models for some of these phenomena, since they develop functional networks that transmit synaptic signals over considerable distances (Serra et al., 2008a; Wagenaar et al., 2004). Moreover, they can be subjected to controlled stimulation and alterations in their signaling profile can be monitored (Bakkum et al., 2008; Brewer et al., 2009; Chao et al., 2005; Jimbo et al., 1998; Madhavan et al., 2005; van Pelt et al., 2004a,b; Wagenaar et al., 2004; Zemianek et al., 2012).

While cultured neuronal networks clearly display plasticity, a fundamental concern remains as to whether or not the use of an isolated neuronal network, deprived of organismal interaction with the environment, can be utilized as a model of bona-fide "learning." and whether or not phenomena observed in such networks can be compared to modifications that observed in situ. To address this distinction, we compared the signaling profile generated by developing neuronal networks over an extended period with and without stimulation with a synaptic signal that was recorded from another mature culture. We utilized a minimal stimulation regimen with the intent of modeling the impact of the first sensory input. This approach provided us the ability to compare the developmental profile of "naïve" networks (i.e., those that received no external signaling) with the profile displayed by the identical networks receiving a model of sensory input during the same developmental period.

2. Materials and methods

Dissociated cortical neurons from day 18 C57BL/6 mouse embryos were plated on poly-D-lysine/fibronectin-coated, MEAs (Multichannel Systems, Reutlingen, Germany) in B27-supplemented Neurobasal medium (Invitrogen, Carlsbad, CA) (Serra et al., 2008a). Sacrifice of the pregnant female was carried out under procedures approved by our Institutional Animal Care and Use Committee. Glia were allowed to proliferate as prior studies demonstrated that they improved signaling (Serra et al., 2010).

Neuronal networks were recorded for 30 s periods via a DT9814 data acquisition system (Data Translation; Marlborough, MA) using a MEA-1060-INV amplifier (Multichannel). Signals were quantified manually and with an algorithm that distinguishes bona-fide activity from baseline disturbances (Serra et al., 2008a; Wagenaar et al., 2005; Zemianek et al., 2012). Data were captured at 3.125 kHz simultaneously

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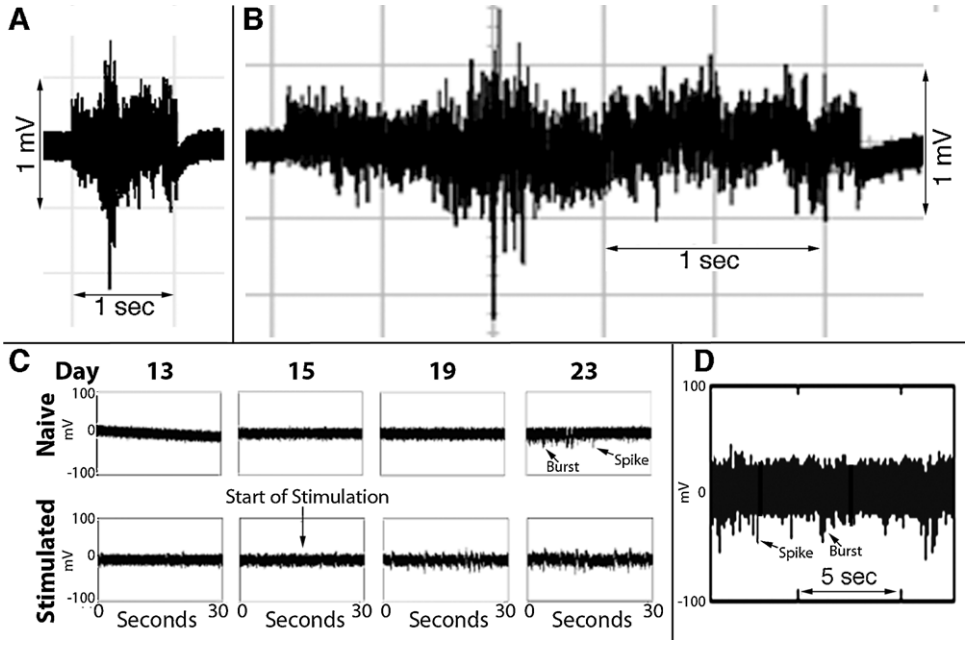

Fig. 1. Stimulation hastens the appearance of signals in developing neuronal networks. (A) Cultures were stimulated with this short segment of a spontaneous synaptic signal recorded from a mature culture. (B) An expanded view of the stimulatory synaptic signal is presented. (C) Panels present representative recordings from naïve and stimulated cultures as indicated. All recordings in each series are from the same electrode in the same culture. Note the initial appearance of signals at day 23 in naïve cultures, and the earlier appearance of signals in stimulated cultures. The initiation of stimulation at day 15 is indicated. A representative burst and spike are indicated. Note the more rapid appearance of signals in stimulated versus naïve cultures (day 19 versus day 23). (D) An expanded representative image of signals generated by cultures is presented, with a representative burst and spike indicated.

accessing 16 channels and 12.5 kHz accessing 4 channels; the latter was used to validate the use of undersampling for the purpose of observing more channels. Prior studies using combinations of excitatory and inhibitory neuronal antagonists have confirmed synaptic origin of signals in these cultures (Serra et al., 2008a,b, 2010; Serra and Shea, 2009).

Spontaneous signals and signals resulting from stimulation were classified as "spikes" if they appeared as individual signals separated by other signals by a minimum of 0.7 s, and "bursts" if they consisted of clusters of at least 3 spikes within a 0.7 s period prior to returning to baseline (Serra et al., 2008a; Zemianek et al., 2012).

Many studies utilizing external network stimulation utilize a relatively high-amplitude, biphasic "spike" or series of such spikes (Brewer et al., 2009; Jimbo et al., 1998; Wagenaar et al., 2004, 2005, 2006), coupled with the use of a distal "bath ground" electrode. This regimen results in simultaneous stimulation of virtually the entire culture (Zemianek et al., 2012 and references therein). Herein, in our attempt to model the onset of sensory input, we instead set out to stimulate localized regions of the networks, and to utilize a physiological signal. To achieve these goals, networks were stimulated a single time per stimulation session with a 1 s segment of a spontaneous signal recorded from a mature culture (Fig. 1A) applied to an individual electrode with an adjacent electrode utilized as a ground. This localized loop restricted spread of the signal among synaptically connected clusters of neurons, and was previously demonstrated to induce robust, complex responses (Zemianek et al., 2012). Prior studies from our laboratory and others typically utilized cultures 1–2 months after plating, with the rationale that the neuronal network had stabilized by then (Kamioka et al., 1996; Pasquale et al., 2008). Herein, we set out to monitor the nature of signals during this developmental period. We therefore monitored spontaneous signaling in dense cultures (Serra et al., 2010) earlier than usual (5 days after plating), and applied a stimulation of 1 s in duration to a single electrode of alternate cultures every 2nd day starting on day 15 (prior to the observation of routine spontaneous signals), for a total of 14 stimulations over 28 total days, resulting in continuous monitoring of spontaneous signaling patterns for a total of 41 days. This minimal stimulation regimen (a single stimulation every 2nd day), coupled with localized application within the network, was utilized as a model of the onset of sensory input during development, as opposed to the more robust and continuous input characteristic of an established nervous system. The same stimulation and ground electrodes were utilized for all stimulations. A minimum of 3 naïve and 3 stimulated cultures were recorded at each interval.

3. Results

Spontaneous, irregular spikes were observed within the first week after plating (Serra et al., 2008a). In naïve cultures, streams of relatively continuous spikes and bursts regularly appeared at 20 days after plating (Fig. 1B), persisted at a relatively high frequency for 15 days, then declined (Fig. 2). Bursts did not decline but remained constant for the entire observation period (Fig. 2). Since over time the number of spikes decreased while bursts persisted, this eventually resulted in an overall prevalence of bursts.

Additional cultures were stimulated every second day with a prerecorded synaptic signal from a mature culture (Fig. 1A). In stimulated cultures, spikes and bursts appeared more quickly (15 days after plating) than in naïve cultures, and, notably, after the first stimulation (Fig. 1B). This suggests that stimulation hastened the appearance of both signal types. Spikes declined in stimulated cultures at day 25 (Fig. 2). Bursts remained at similar levels throughout the observation period in naïve cultures. In stimulated cultures, bursts also remained constant for the entire observation period. Notably, the total number of bursts in stimulated cultures was comparable to that of naïve cultures.

Superimposing the pattern of spikes in naïve and stimulated cultures on the same axis (Fig. 2) reveals that stimulation not only hastened the appearance of spikes but also hastened their temporal decline. The overall similarity of the developmental pattern of spikes in naïve and stimulated cultures was further highlighted by shifting the respective curves so that the initiation of the temporal increase in spikes for both naïve and stimulated cultures coincided (Fig. 2, bottom panel). While there are differences in the respective trendlines for each curve, densitometric analyses revealed that the areas under each curve coincided by 75%. This variation is identical to the variation observed among individual stimulated cultures ($76.5 \pm 11.7\%$, mean $\pm$ standard error, $n = 6$ cultures). In addition, there was no significant difference in the total number of signals displayed by naïve versus stimulated cultures over the 41 day observation period ($p < 0.42$, $n = 3$ naïve versus 7–9 stimulated cultures), nor was there any change in the percentage of signals classified as spikes or bursts ($p < 0.34$ and 0.52, respectively; Fig. 3). These findings indicate that the developmental pattern was hastened by stimulation but that this pattern was not altered either in total signals or in the type of signals.

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