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Chronic electrical stimulation of cultured hippocampal networks increases spontaneous spike rates

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ABSTRACT

We chronically stimulated hippocampal networks in culture for either 0, 1 or 3 h/day between 7 and 22 days in culture in an effort to increase spontaneous spike rates and to give these networks some portion of external stimuli that brain networks receive during their formation. Chronic electrical stimulation of hippocampal networks on multi-electrode arrays (MEAs) increased spike rates 2-fold after 3 weeks of culture compared to cultures that received no external stimulation prior to recording. More than 90% of the spikes for all experimental conditions occurred within bursts. The frequency of spikes within a burst increased with time of stimulation during culture up to 2-fold higher (90 Hz) compared to networks without chronic stimulation. However, spontaneous overall spike rates did not correlate well with the amount of stimulation either as h/day or proximity to the limited number of stimulation sites due to shorter burst duration with 3 h/day stimulation. The results suggest that chronic stimulation applied during network development recruits activity at 50% more electrodes and enables higher rates of spontaneous activity within bursts in cultured hippocampal networks.

1. Introduction

The overarching goal of in vitro networks of cultured neurons is to model network behaviour of the brain. In our systematic approach to improving live neuronal network activity on multi-electrode arrays (MEAs), we have shown that network responses can be improved by patterned substrates to more precisely locate neuronal soma over recording electrodes (Corey et al., 1991; Chang et al., 2006), added astroglia to improve network dynamics (Boehler et al., 2007) and a response-optimized, synapse-enhancing culture medium (Brewer et al., 2008, 2009). Customarily, neural network cultures are placed in an incubator and allowed to develop spontaneous activity over a period of several weeks without external stimulation (Thomas et al., 1972; Gross, 1979; Pine, 1980; Droge et al., 1986; Van Pelt et al., 2004; Chiappalone et al., 2006; Wagenaar et al., 2006; Brewer et al., 2009). A key reason for low spontaneous spike rates of such cultures may be caused by a sleeping or catatonic network which receives no external stimulation. Without external stimulation, Vogt et al. (2005) found that less than 10% of patched pairs of neurons were connected by excitatory transmission onto an excitatory neuron. During development, the brain receives large amounts of external stimulation critical to developing excitatory synapses (Turrigiano and Nelson, 2004). The effect of chronic electrical stimulation on spontaneous neuronal firing rates of dissociated hippocampal cultures during network development has not been widely studied, although directed training effects have been reported (Eytan et al., 2003; Stegenga et al., 2009).

This work was motivated by the network studies of Potter (Wagenaar et al., 2005) who showed that burst dynamics can be greatly modulated by repeated high-frequency stimulation, but chronic stimulation over many days was not investigated. We chose a paired-pulse paradigm of stimulation with 50 ms interval for optimal presynaptic activation (Zucker and Regehr, 2002; Hama et al., 2004; Mori et al., 2004). We pose two alternative hypotheses applied to cultured networks: (1) paired-pulse stimulation over long periods (chronic stimulation), will reduce spontaneous bursting, but increase overall spike rates similar to acute high-frequency stimulation (Wagenaar et al., 2005); or (2) chronic paired-pulse stimulation will increase spontaneous spike rates possibly within a burst. Here we report a major effect of chronic stimulation on spontaneous spike rates and burst dynamics during 3-weeks of network development in vitro.

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2. Methods

2.1. Neuron culture

E18 rat hippocampal cells were plated at 500 cells/mm^2 on poly-D-lysine coated MEAs in NbActiv4^{TM} medium (Brewer et al., 2008, 2009) (BrainBits, Springfield, IL). Every 4–5 days, 1/2 of the culture medium was removed and replaced with the same volume of fresh medium up until the day of recording. At the time of recording at 3 weeks, the culture was comprised of about 67% neurons and 33% astroglia (Brewer et al., 2008). The results reported here were from 5 arrays for each of the 3 conditions of stimulation. The 5 arrays were the result of 4 different animal dissections/condition.

2.2. Multi-electrode arrays (MEA) and recording

The MEA's from Multichannel Systems (MCS, Reutlingen, Germany) consist of 59 TiN electrodes with diameters of 30 μm and spacing of 200 μm; one other electrode was used as ground. The spontaneous activity on the MEA's was measured using an MCS 1100x amplifier at 40 kHz sampling with a hardware filter of 8–3000 Hz at 37 °C under continuous flow of hydrated, sterile 5% CO_2, 9% O_2, balance N_2 (AGA, Springfield, IL). Electrical stimuli were delivered by a stimulus generator (MCS STG2004). A Zebra strip (Fujipoly America, Carteret, NJ) was used on arrays to reduce noise and a Teflon membrane (ALA Scientific, Westbury, NY) was used to reduce evaporation and chances of contamination during stimulation. MCRack software was used to record for 3 min followed by a high-pass software filter of 200 Hz. Offline data analysis was performed in MATLAB (The Mathworks, Natick, MA). Spikes were analyzed within a 2 ms search window for their peak-to-peak amplitudes and detections were noted whenever the peak-to-peak amplitude exceeded 11 times the noise standard deviation (roughly equivalent to a threshold of 5.5 standard deviations above zero-to-peak amplitudes). Spikes occurring within a 1 ms refractory period were discarded. Both timestamp and amplitude were saved (Maccione et al., 2009).

2.3. Chronic stimulation

Stimulation trains for chronic stimulation included groups of 30 μA paired pulses (50 ms ISI; biphasic, 100 μs/phase duration, positive first) with a wait of 5 s between pairs. This level consistently led to at or near 100% (maximal) activation (commonly 15 μA was the threshold) in related work in our laboratory with low density cultures and 30 μm diameter electrodes. This mode and level are consistent with reports in the literature (e.g. Eytan and Marom, 2006). Constant current stimulation has the advantage of consistent charge injection with variable electrode impedance, and stimulation is theoretically believed to be consistently correlated with current. We note, however, that there are competing reasons that lead others to find constant voltage stimulation preferable (Wagenaar et al., 2004). A paired-pulse paradigm with delay of 50 ms was chosen as a minimum number of stimuli that could avoid short-term plasticity while enhancing the probability of postsynaptic potentials (Soleng et al., 2004). Wait times between pulses of 1 s and 5 s between 2 pairs of pulses were empirically chosen as minima that avoided desensitization. Arrays were chronically stimulated for either 0, 1 or 3 h(s)/day at 7, 11, 12, 14, 18, 19, and 21 days in vitro. On the day of recording, arrays that did not receive any chronic stimulation were initially recorded for 3 min to obtain spontaneous activity. Arrays that had received chronic stimulation were chronically stimulated for 1 h or 3 h, then recorded for 3 min. An automatic stimulation program was created using Microsoft Visual C++ 2008 that stimulated the entire top half of the MEA (30 electrodes) in a pseudorandom sequence, the same for every array, every time; electrodes were not preselected for activity. Recorded arrays received 0, 2100, or 6300 stimuli/day × 8 days during culture development. The pseudorandom design avoided stimulation of any two adjacent electrodes consecutively. The bottom half of the array never received direct stimulation from an adjacent electrode and served as an unstimulated condition within the array. The experimental setup resulted in electrodes in the top half of the array being stimulated by one of 2, 3, 4, 5, 7, or 8 adjacent electrodes.

2.4. Burst detection

The activity of each electrode trace was searched for bursts using the method of Chiappalone et al. (2005). A burst was defined as a sequence of at least 5 spikes with inter-spike interval less than 100 ms. Burst analyses were conducted with MatLab software with a burst criterion of ≥0.4 bursts/min.

2.5. Statistics

Statistical analyses were performed by Student's *t*-test with a cutoff at $p < 0.05$ for significance. All graphs display means and S.E.

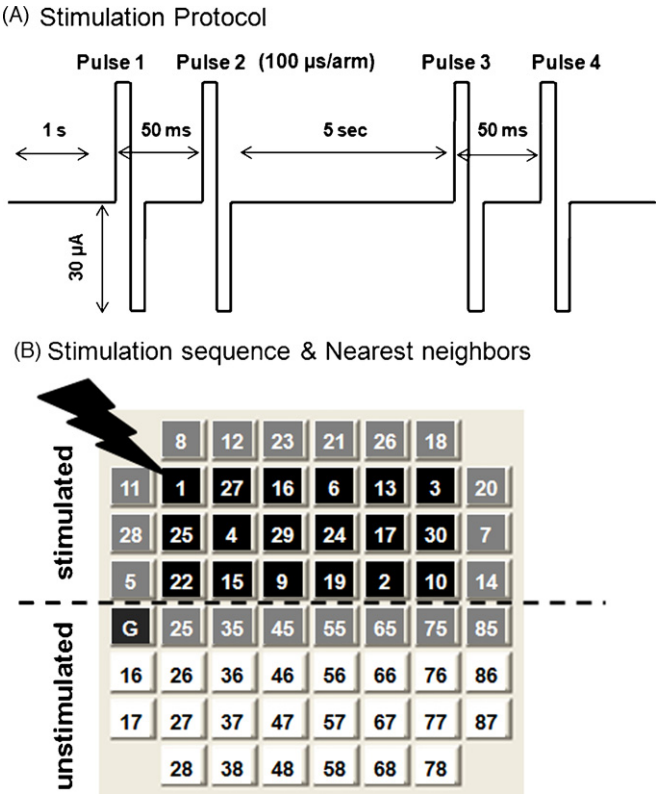

Fig. 1. Chronic stimulation protocol applied to determine effect of external stimuli on spike rates during time in culture. (A) A train of two paired pulses, 50 ms between single pulses in a pair, 5 s apart was delivered to a single electrode, followed by switching to a second electrode 1 s later. (B) The switching was repeated to each of 30 electrodes in top half of array. This process was repeated for either 1 or 3 h/day for a total of either 2100 or 6300 pulses per array/day over 8 days. The program switches channels in a pseudorandom sequence after each train to avoid stimulation of any two adjacent electrodes consecutively and to minimize short-term plasticity. Black squares represent electrodes with 5, 7, or 8 neighbours in close proximity during stimulation. Gray squares represent electrodes with 2, 3, or 4 neighbours in close proximity and white squares represent electrodes with zero neighbours in close proximity.