CONTROL OF BURSTING IN DISSOCIATED CORTICAL CULTURES ON MULTI-ELECTRODE ARRAYS

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We are investigating whether often repeated firing patterns can rewire the connections in a neuronal culture as many reports of spike-timing dependent plasticity suggest. The goal of this work was to seek a stimulation protocol that reduces the amount of spontaneous or uncontrolled activity relative to induced spikes, by the continuous application of stimulation patterns to cultured rat cortical networks. Cultures controlled in this manner will then be used in the study of stimulus-induced plasticity and information processing in these distributed systems [Potter-2001]. Also, by reducing spontaneous activity or enhancing our level of control over the network activity we can study learning in animats (simulated animals) [DeMarse-2001].

Dissociated cultures of cortical neurons in vitro exhibit complex, spontaneous bursts of activity. These bursts or "barrages" are sudden increases in spike frequencies that occur simultaneously on a large fraction of recorded cells in the culture [Gross-93, Kamioka-96, Jimbo-99]. During experiments involving persistent stimulation, we noticed that the number of spikes in these barrages dominates the activity, potentially swamping out the effects of stimulation.

Pharmacological methods can be used to reduce barrages. Commonly, the concentration of extracellular Mg^{2+} is increased to reduce the frequency of spontaneous activity [Tateno-99]. When we increased concentration of Mg^{2+} above 2mM, the number of uncontrolled barrages was reduced, but this was accompanied by a decrease in the overall spontaneous activity of the dish. Also, the dose-response curves of these experiments were not repeatable across different cultures. Since Mg^{2+} blocks NMDA channels, which are involved in synaptic plasticity, this method seems contradictory to our goals.

Instead of a pharmacological approach, frequent electrical stimulation might be a more natural way to reduce bursts in these cultures. It has been shown that increasing the fraction of endogenously active cells in steadily firing cultures causes a decrease in bursting [Latham-2000]. To test whether electrical stimulation could serve the same role as increasing the number of endogenously active cells, we applied continuous stimuli at different frequencies. At low stimulus frequencies (less than 1Hz) on single electrodes, the barrages tend to synchronize with the stimuli. At frequencies higher than 20Hz, the number of evoked responses and overall activity of the dish was reduced. At stimulation frequencies from 1Hz - 20Hz, two distinct phases were observed. In the short early phase (the first few minutes of stimulation), there is a transient suppression of the bursts, which then reemerged later and persisted for the remaining duration of stimulation (maximum recording period was 12 hours). This reappearance of bursts, may have been caused by the desensitization of the neurons near the stimulated electrodes.

To test this hypothesis, we stimulated *multiple* electrodes sequentially, each at a low frequency of 1Hz, but at a combined frequency of 7Hz or more. This resulted in a decrease (by about 60%) in the number of uncontrolled barrages, and suppressed their re-emergence. In contrast, the number barrages evoked by stimulation increased, suggesting that we reduced the desensitization and have greater control over the activity. Also, these evoked responses were observed at electrodes which, when probed individually, produced only few precisely timed responses [Wagennar-2003], suggesting that the context of stimulation on other electrodes maybe relevant.

This reduction of the contribution of uncontrolled barrages to the total firing rate of the culture is a promising step towards the goal of influencing the structure of the developing network by imposed activity patterns.

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