Effects of low-frequency stimulation on spontaneous firing dynamics in dissociated cortical cultures on multi-electrode arrays

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Spontaneous firing rates have been recorded in dissociated rat cortical neuronal networks cultured on multielectrode arrays before and after a period of electrical stimulation. The question was studied whether low-frequency electrical stimulation induces changes in spontaneous firing rates in the period thereafter. It was found that a period of low-frequency stimulation has significant and lasting effects on the spontaneous firing rates at the individual electrodes in the array and, thus, on the total firing rate at all the electrodes. The changes include significant increases and decreases in the spontaneous firing rates, as well as the activation of initially silent neurons, and the silencing of initially active neurons. These findings demonstrate that low-frequency stimulation protocols, as used in the literature for testing the effect of high-frequency tetanic stimulation protocols in plasticity studies, may by themselves induce changes in such cultured cortical neuronal networks.

1 Introduction and background

Plasticity studies in cultured neuronal networks usually include tetanic stimulation protocols of high frequency electrical pulses to induce plastic changes in the network (e.g., Maeda et al., 1998, Jimbo et al., 1998, 1999; Tateno & Jimbo, 1999). The tetanic stimulation is generally preceded and followed by low-frequency, low amplitude test stimuli in order to assess the changes in the stimulus-response characteristics of the network induced by the tetanic stimulation. Ideally, such test stimuli should only probe the input/output characteristics of the network without changing the firing characteristics of the network themselves. In the present study we have investigated whether test-stimuli-like stimulation patterns through planar MEA electrodes induce changes in the spontaneous firing rates of cultured rat cortical neuronal networks.

2 Cell cultures, stimulation protocol and data analysis

Cortical cells from embryonic (E18) rats were dissociated and plated on 60 electrode planar MEAs (100000 cells per plate) (Ramakers et al., 1991, 1998) of the HEXA-MEA type (MCS, Reutlingen, Germany) with electrodes of different diameters (10, 20 and 30 µm, respectively). Experiments were performed on 20 cultures in total (from 7 rats) of ages between 10 and 54 days in vitro. The stimulation protocol (Fig. 1) comprised single pulse trains applied sequentially to 6 different 30 µm diameter electrodes. A pulse train consisted of 40 biphasic rectangular voltage pulses (positive first, 200 microsecond per phase and 1,5V peak-to-peak) at 0.2 Hz. Previous experiments showed that the stimulation voltage threshold for evoking a spike differed from culture to culture (unpublished results). The minimum threshold however was never lower than 1.5 V peak-to-peak which we considered to be a moderate voltage. Spontaneous extracellular spike signals were recorded according to the procedure described in Van Pelt et al. (2004).

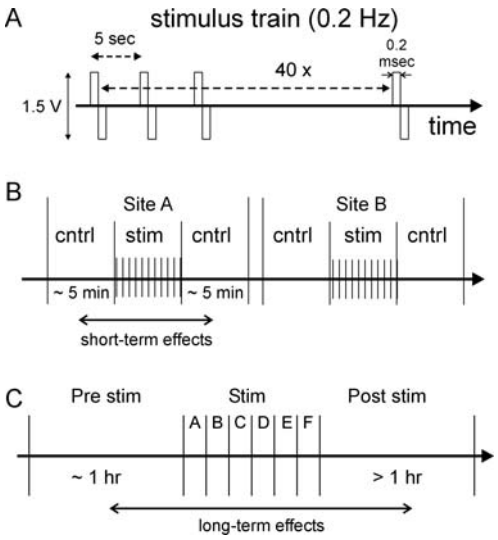

Fig. 1 Low-frequency stimulation protocol. A stimulus train of 40 bipolar pulses at 0.2 Hz is given sequentially to 6 different 30 µm diameter electrodes of a MCS-HEXAMEA. Each train and the whole stimulus session is preceded and followed by a period of recording of spontaneous firing activity.

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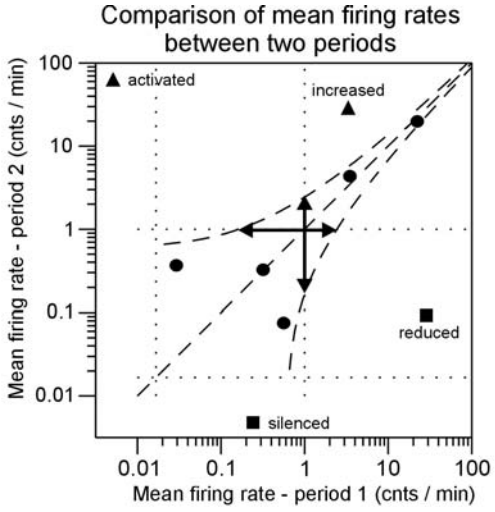
Spontaneous activity was recorded prior to and following each pulse train for about five minutes, and prior to and following the total stimulation session for at least one hour. Firing rates at the individual electrodes and summed over all the electrodes were compared in the periods before and after individual pulse trains for short period effects, and in the periods before and after the total stimulation session for long period effects. Scatter plots were constructed to visualize changes in firing rates on individual sites (Fig. 2).



Error intervals estimated from Poisson process statistics:

$$3\sigma - range = \pm 3\sqrt{N} = \pm 3\sqrt{MFR \times period(\min)}$$

Fig. 2 Example of a scatter plot of mean firing rates at individual sites for comparison between two periods. The error intervals are drawn assuming Poisson process counting statistics. The data points illustrate sites with Poisson distributed fluctuations (circles), sites with increased firing rates (triangles), and sites with decreased firing rates (squares).

These scatter plots allow visual comparison between the firing rates in the two periods. Equal firing rates in both periods result in data points on the diagonal. However, even under stationary conditions statistical fluctuations result in data points scattering around the diagonal. For the ease of interpretation dashed lines are drawn to mark a region around the diagonal equal to three standard deviations (3SD) based on the assumption that the spikes in the spike train originated from a Poisson process. Then, data points within this region denote mean firing rates that do not differ significantly between both periods. Data points outside this region however denote significant different mean firing rates between the two periods. Data points close to the axes indicate the activation of initially silent sites or the silencing of initially activated sites.

3 Results and conclusions

In addition to the comparison of the mean firing rates in the periods before and after a period of stimulation, comparisons were made to test the stationarity of firing during both periods. To this end both periods were divided into two half periods followed by a comparison of the mean firing rates between first and second half of both periods and a comparison between the second half of the pre-stimulus period with the first half of the post-stimulus period. An example of such a triple comparison is given in Fig. 3C. The left and the right panel in Fig. 3C show that all the data points are within the 3SD area bounded by the dashed lines indicating that the scatter around the diagonal does not exceed the 3SD levels, as expected for a Poisson process generated spike train. The middle panel in Fig 3C illustrates the scatter of mean firing rates when comparing the 2nd half of the pre-stimulus period with the 1st half of the post-stimulus period. This panel shows data points outside the 3SD area illustrating that the period of electrical stimulation has had significant effects on the mean firing rates. The observation that the post-stimulus period again showed stationary firing indicates that the stimulus-induced changes were lasting over the post-stimulus period. The applied low-frequency stimulation thus had significant and lasting effects on the total firing rates of the network. These changes included both significant increases and decreases in total firing rates, which were caused by increases and decreases of firing rates of individual sites, and by activation of initially silent sites, and silencing of initially active sites (see also the spike plot in Fig 3A). Similar results were obtained in all the experiments done.

Fig. 3B displays the firing rates per second during the pre-stimulus and the post-stimulus period. These plots clearly indicate an ongoing alternation between low-level firing and the occurrences of synchronized network bursts. The mean firing rate for such spike trains then depends on the firing rate during low-level and during network burst firing and the frequency of network bursts. Although the assumption of a single Poisson process generated spike train for the scatter estimation is an oversimplification, the observed data points in the left and right panel of Fig. 3C nevertheless obey the scatter area surprisingly precisely.

These findings demonstrate that low-frequency moderate-amplitude stimulation has a major and a lasting impact on the spontaneous firing dynamics in cultured networks. Thus, the assumption that low frequency stimulation for testing network responses does not interfere with network firing properties is not confirmed by our results. These findings point to the necessity of control experiments (e.g. stability of baseline) in plasticity experiments that make use of test stimuli to probe connection strength.

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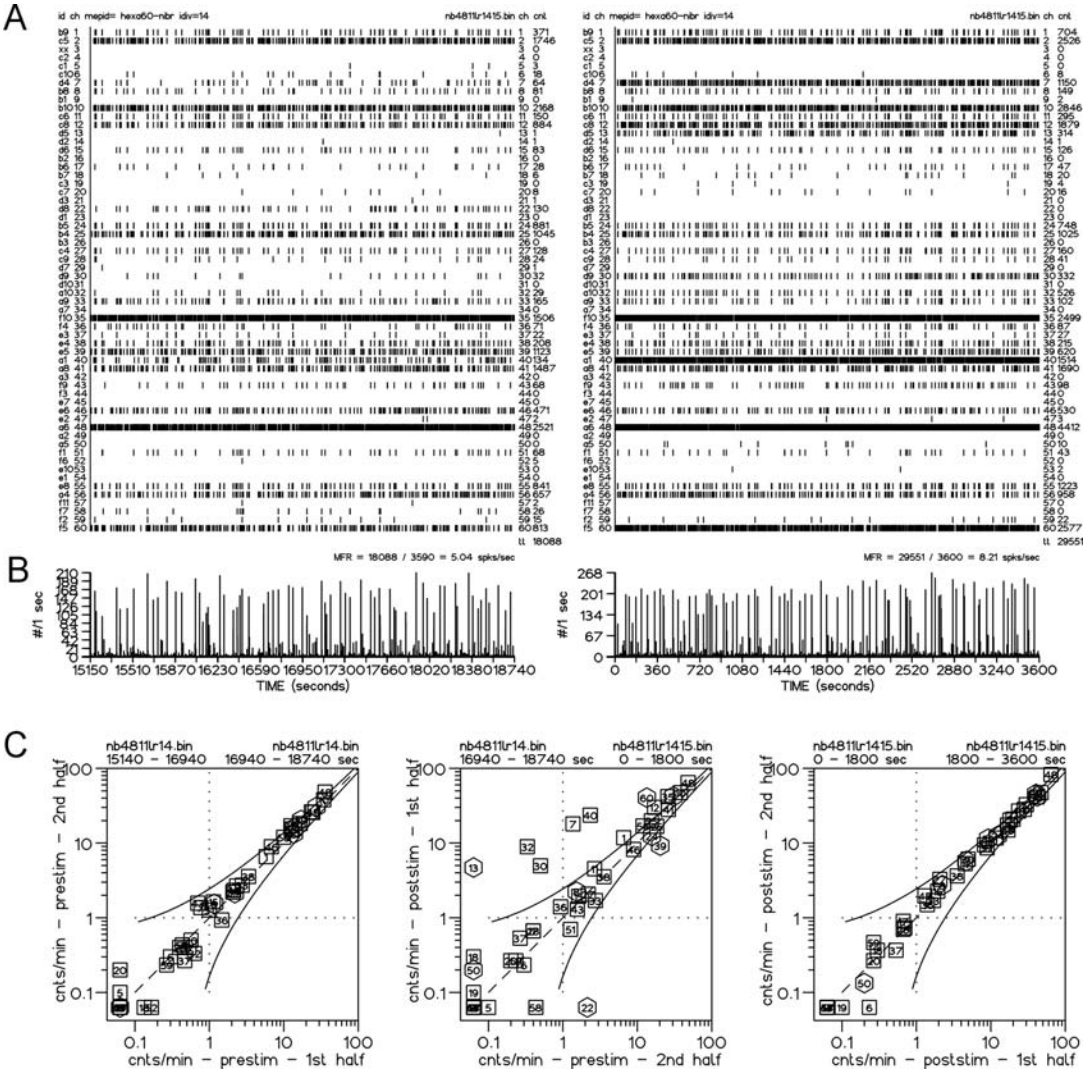

Fig. 3 – Comparison of spontaneous firing activity before and after a period of low-frequency stimulation, (A) the spike traces at the individual sites for one hour period, just preceding (left panel) and following the stimulation period (right panel), (B) the firing rates with 1 sec time bins, and (C) the scatter plots for comparison of the two periods, with the left panel comparing first and second half of the pre-stimulus period, the central panel comparing second half of the pre-stimulus period with the first half of the post-stimulus period, and the right panel comparing first and second half of the post-stimulus period.

Acknowledgement

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