Stimulation Induced Transitions in Spontaneous Firing Rates in Cultured Neuronal Networks also Occur in the Presence of Synaptic Plasticity Blocker KN93

Linda van der Heiden, Ildiko Vajda, Jaap van Pelt, and Arjen van Ooyen

Abstract Spontaneous firing activity in dissociated rat cortical tissue cultured in vitro shows highly stable firing rates over periods of hours. Recently, it was shown that a short period of low-frequency electrical stimulation induces significant and lasting changes in these firing rates. Now, it is shown that these changes also occur in the presence of the synaptic plasticity blocker KN93 in the culture medium. Apparently, the changes in firing rates after a short period of low-frequency stimulation do not depend on CaMK-II mediated synaptic plasticity.

Keywords Cultured networks · Spontaneous firing · Stable firing rates · Attractor dynamics · State transitions

1 Introduction

When dissociated rat cortical tissue is brought into culture, neurons readily grow out by forming axonal and dendritic arborizations and synaptic connections, and display spontaneous firing activity from about the end of the first week in vitro. This activity typically consists of alternating periods of synchronized (network bursts) and of largely uncorrelated activity. Mean firing rates at the individual sites of a multi-electrode array appear to be stable over periods of hours, changing only on developmental time scales, [1–4]. Recently, Vajda et al. [5] showed that a short period of low frequency (LF) electrical stimulation induces significant and lasting changes in these mean firing rates. The possible role of synaptic plasticity in these activity changes has now been investigated by repeating the experiments in [5] in the presence of the plasticity blocker KN93 in the culture medium. KN-93 prevents both pre- and postsynaptic CaMK-II activation, necessary for LTD and LTP induction, respectively [6–8]. The present experiments confirm the results of [5], also in the presence of KN93.

L. van der Heiden (✉)
Department of Integrative Neurophysiology, Center for Neurogenomics and Cognitive Research, VU University Amsterdam, 1081 HV Amsterdam, The Netherlands

R. Wang, F. Gu (eds.), *Advances in Cognitive Neurodynamics (II)*,
DOI 10.1007/978-90-481-9695-1_24,

2 Methods

Cell cultures on multielectrode arrays – Dissociated E18 rat neocortical neurons were cultured on planar multi-electrode arrays (MEAs) from MultiChannel Systems e.g., [1]. The preparation of the cultures was similar as in [5]. MEAs had either a so-called Hexagonal configuration with mixed 30, 20 and 10 μm electrode diameters or an 8 × 8 configuration with 10 μm micrometer diameter.

Pattern of electrical stimulation – The low-frequency (LF) electrical stimulation protocol, as shown and explained in Fig. 1, was derived from [5]. During the present experiments the culturing medium has been replaced by the recording medium Hepes ($N = 16$) or by Hepes + 10 μM KN93 ($N = 11$).

Comparison of mean firing rates at individual sites between two periods - Mean firing rates at individual sites in two periods were compared using a scatter plot as shown in Fig. 2 [5]. Data points at the diagonal indicate sites with equal firing rates for both periods. Dashed lines indicate 3 standard deviation (3σ) boundaries assuming a Poisson distributed spike train. The relative deviation *rd* of a data point from the diagonal is expressed by the ratio of its distance from the diagonal and the 3σ distance. The mean relative deviation *mrd*, of all data points in the scatterplot, thus provides a measure for the amount of scatter around the diagonal. The expected *mrd* value for a Poisson distributed spike train is equal to 0.27 [5]. For each experiment, the following periods of spontaneous activity were compared: (a) the 1st and 2nd half of the period before stimulation (Fig. 2, first column), b the 2nd half of the period before stimulation and the 1st half of the period after stimulation (Fig. 2, central column), and c the 1st and 2nd second half of the period after stimulation (Fig. 2, right column).

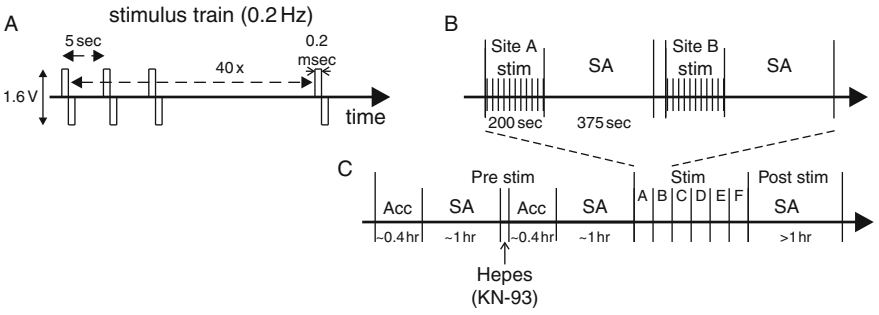

Fig. 1 **a** Pattern of low-frequency stimulation, consisting of a train of 40 bipolar pulses of 1.6 V (peak–peak) of 0.2 ms width (*single phase*), delivered with 5 s intervals (0.2 Hz). **b** Each pulse train is followed by a period of 375 s for recording spontaneous activity (SA) in the network. **c** Pulse trains are successively applied to six different electrodes in the multielectrode array. At the start of the experiment and after replacing culturing medium by recording medium Hepes (without or with KN93) a period of accommodation (Acc) is included. Both before and after the period of stimulation spontaneous activity is recorded for a period of at least 1 h

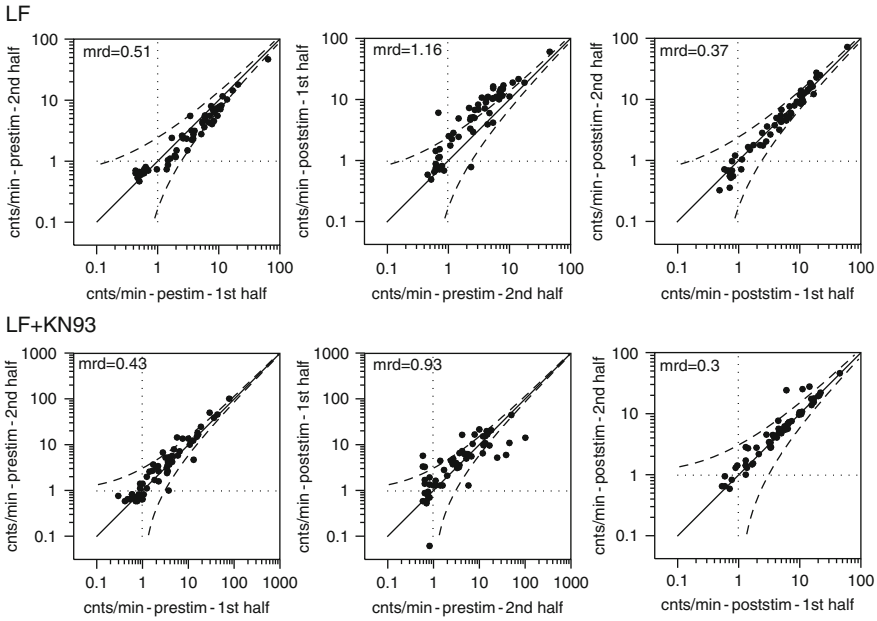

Fig. 2 Scatter plots comparing mean firing rates at individual sites between two periods. Compared are the 1st and 2nd half of the pre-stimulus (prestim) period (*left column*), the 2nd half of the prestim period with the 1st half of the post-stimulus (poststim) period (*central column*), and the 1st and 2nd half of the poststim period (*right column*). Data points at the diagonal indicate sites with equal firing rates in both periods. *Dashed lines* indicate 3σ (standard deviation) boundaries assuming a Poisson distributed spike train. The relative deviation *rd* of a data point from the diagonal is the ratio of its distance from the diagonal and the 3σ distance. The mean of all *rd* values in a scatterplot (*mrd*) is displayed in the upper *left* corner of each panel

3 Results

Scatterplots of low frequency (LF) stimulation experiments – Fig. 2 shows and describes examples of scatterplots for the three types of comparisons (columns) of firing rates (pre–pre, pre–post, post–post) in the absence (top row – LF) and in the presence of KN93 (bottom row - LF+KN93).

Analysis of mrd values. A summary of the *mrd* values is shown in Fig. 3, as well as the statistical comparisons between the different groups, as labeled by "a", "b", and "c", indicating the different periods in the firing rate comparisons (see scheme in Fig. 3). In both the LF and the LF+KN93 experiments, the "b" group of *mrd* data has significantly higher mean values than the "a" or "c" groups. This is not the case in the right panel of control experiments, in which group "b" compares firing rates in periods separated by a control period of the same duration as a stimulation period (data from experiments without or with KN93). The LF-results confirm the finding in [5], that low-frequency stimulation induces significant and lasting changes in the spontaneous firing rates in the network. Importantly, the LF+KN93 experiments

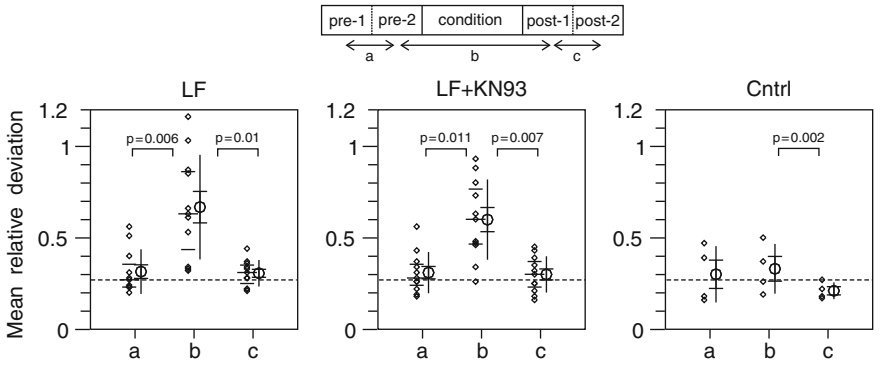

Fig. 3 Mean-relative-deviation (*mrd*) values in the absence (LF) and in the presence (LF+KN93) of the plasticity blocker KN93, and for the control experiments (Cntrl). Individual *mrd* values are shown along with their median and quartiles, as well as the mean, the SD intervals and the standard error in the mean intervals. The *p*-values are obtained from using the Kruskal Wallis test. The *dashed lines* indicate the *mrd* value of 0.27 as expected from fluctuations in firing rates in a Poisson distributed spike train

show that the plasticity blocker KN93 does not prevent these changes to occur. The dashed lines in Fig. 3 indicate the *mrd* value of 0.27 for the fluctuations in firing rates as expected for a Poisson distributed spike train. The *mrd* values of the test comparisons "a" and "c" appear to be close to this theoretical expectation, underscoring the stability in firing rates during a period of spontaneous activity.

4 Discussion

The present results confirm the findings of [5], that a period of low-frequency electrical stimulation induces significant and lasting changes in the pattern of spontaneous firing rates, and also demonstrate them in the presence of the plasticity blocker KN93. Apparently, these activity changes do not depend on CaMK-II mediated synaptic plasticity, suggesting that they are not caused by changes in synaptic efficacies. Other intrinsic network properties may have changed as well by low-frequency stimulation. However, the activity changes may also be of a dynamical nature, i.e., caused by state transitions in the firing dynamics state space, without structural changes in the neuronal network. Further research is needed to substantiate this interesting hypothesis.

References

1. Van Pelt, J., Wolters, P.S., Corner, M.A., Rutten, W.L.C., Ramakers, G.J.A.: Long- term characterization of firing dynamics of spontaneous bursts in cultured neural networks. IEEE-TBME **51** (2004) 2051–2062.

2. Segev, R., Baruchi, I., Hulata, E., Ben Jacob, E.: Hidden neuronal correlations in cultured networks. Phys. Rev. Lett. **92** (2004) 118102.
3. Chiappalone, M., Bove, M., Vato, A., Tedesco, M., Martinoia, S.: Dissociated cortical networks show spontaneously correlated activity patterns during in vitro development. Brain. Res. **1093** (2006) 41–53.
4. Le Feber, J., Van Pelt, J., Rutten,W.L.C.: Latency-related development of functional connections in cultured cortical networks. Biophys. J. **96** (2009) 3443–3450.
5. Vajda, I., Van Pelt, J.,Wolters, P., Chiappalone, M., Martinoia, S., van Someren, E., van Ooyen, A. Low-frequency stimulation induces stable transitions in stereotypical activity in cortical networks. Biophys. J. **94** (2008) 5028–5039.
6. Lisman, J., Schulman, H., Cline, H.: The molecular basis of CaMKII function in synaptic and behavioural memory. Nat. Rev. Neurosci. **3** (2002) 175–190.
7. Stanton, P.K., Gage, A.T.: Distinct synaptic loci of Ca2+/calmodulin-dependent protein kinase II necessary for long-term potentiation and depression. J. Neurophysiol. **76** (1996) 2097–2101.
8. Ninan, I., Arancio, O.: Presynaptic CaMKII is necessary for synaptic plasticity in cultured hippocampal neurons. Neuron **42** (2004) 129–141.