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SPATIO-TEMPORAL FIRING IN GROWING NETWORKS CULTURED ON MULTI-ELECTRODE ARRAYS.

Van Pelt J, Wolters PS, *Rutten WLC, Corner MA, Van Hulten P, Ramakers GJA.

Graduate School Neurosciences Amsterdam
Netherlands Institute for Brain Research
Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands

*Institute for Biomedical Technology (BMTI)/Faculty of Electrical Engineering, University of Twente, Enschede, The Netherlands.

j.van.pelt@nih.knaw.nl W.L.C.Rutten@el.utwente.nl m.corner@nih.knaw.nl
g.ramakers@nih.knaw.nl

Summary

During the development of cultured neurons into a synaptically connected network, spontaneous activity emerged towards the end of the first week in vitro: thereafter, individual neurons experienced transient periods of elevated firing rates, lasting from days to weeks. At the second to minute level, network activity was organized in typical temporal patterns with active phases separated by periods of quiescence of a few seconds. Active phases included short periods of intense synchronous firing at many sites (*network bursts*). Network bursts show drastic broadening in the 3rd and 4th week in vitro, while losing a clear center of activity. Subsequently, they reestablish an even more sharper profile in time. Phase relationships appeared to be highly stable over the full recording period of 6 weeks. Thus after the 4th week in vitro network activity develops an increased precision of spike timing within network bursts.

Introduction

Many mechanisms involved in neurite outgrowth and network formation are activity dependent (Van Ooyen, 1994; Ramakers et al., 1998). A reciprocal influence is thus expected to exist between the development of neuronal form, function and connectivity on the one hand, and neuronal and network activity on the other hand (Van Ooyen, 1994). It is hypothesized that this interrelationship contributes, possibly in a correlated way, to the large variations found in both morphology and electrical activity among neurons in a neuronal network. Testing this hypothesis at the level of individual neurons requires the monitoring of their morphological and electrophysiological development during network formation. In the present study the spontaneous firing activity of many individual neurons has been studied longitudinally for a period of 6 weeks in vitro, during which the isolated neurons grow out into a synaptically connected network.

Methods

A combination of long-term culturing techniques and multi-electrode arrays has been used to enable longitudinal recordings of spontaneous firing activity. Neurons were obtained from the cortices of E18 Wistar rat fetuses and, after dissociation, plated in the center of the multi-electrode plate (MEP) coated with polyethylene-imine (PEI, Fluka, 20 microg/ml). The details of the culturing conditions can be found in Ramakers et al. (1991, 1998). The culture chambers were sealed with a glass cover in order to prevent evaporation of the medium during the long-term recording period. Once a week, a fraction of the medium was refreshed, but no further handling was applied during the entire recording period of 6 weeks.

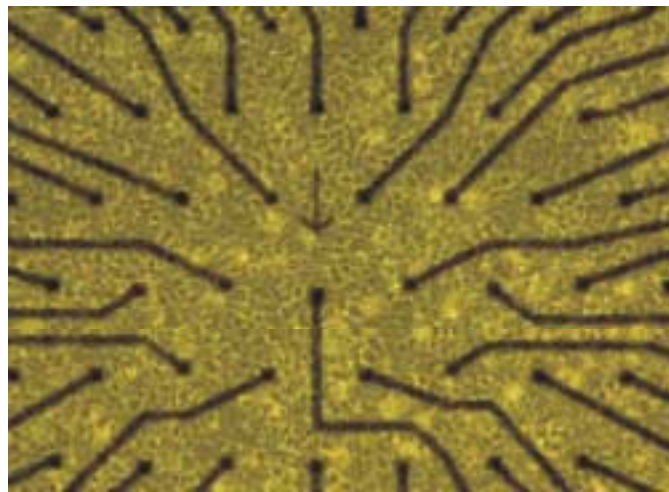


Figure 1. Inner area of the multi-electrode array covered with a monolayer of dissociated rat cortical cells

Multi-electrode plates (MEPs) were obtained from the Institute for Biomedical Technology (BMTI) / Electrical Engineering Faculty at the University of Twente, Enschede, The Netherlands. The MEPs consist of 5 x 5 cm glass plates onto which a pattern of 61 electrically conductive lanes were etched, running from two sides of the plate towards a central area where they ended in a hexagonal pattern with a mutual spacing of 70 micron and a lane width of 10 micron (see Figure 1), a pattern similar to the layout used by Meister et al. (1994). MEPs were produced with either transparent indium tin oxide (ITO; see Gross et al. 1985) or with gold as the electrically conductive medium. The MEPs were covered with an insulation layer consisting of a "sandwich" of silicon oxide, silicon nitride and silicon oxide layers (ONO, total thickness 800 nm). The electrode tips were de-insulated (diameter 12 micron) using reactive ion etching. The electrode tips were platinized in order to reduce their impedance to less than 1 Mohm (at 1 kHz, Buitenweg et al. 1998).

Over a period of 43 days *in vitro*, action potentials from 60 electrode sites were continuously recorded by the time of their occurrence using a time stamp clock running at 10 kHz. Data files were saved at the end of each day or weekend. Spike shapes and noise levels were routinely checked.

Results

Firing rates. An overview of the spontaneous network activity during the development of the dissociated neurons into a connected network is shown in Figure 2. Firing rates are displayed as counts per 4 hour periods. The scaling numbers indicate that the maximum firing rates per trace recorded during the full period. They appear to differ considerably between the recording sites. The Figure also shows that firing rates are not more or less constant but change slowly over time. Especially, each recording site appears to display one or a few periods of high firing rates lasting a couple of days up to several weeks.

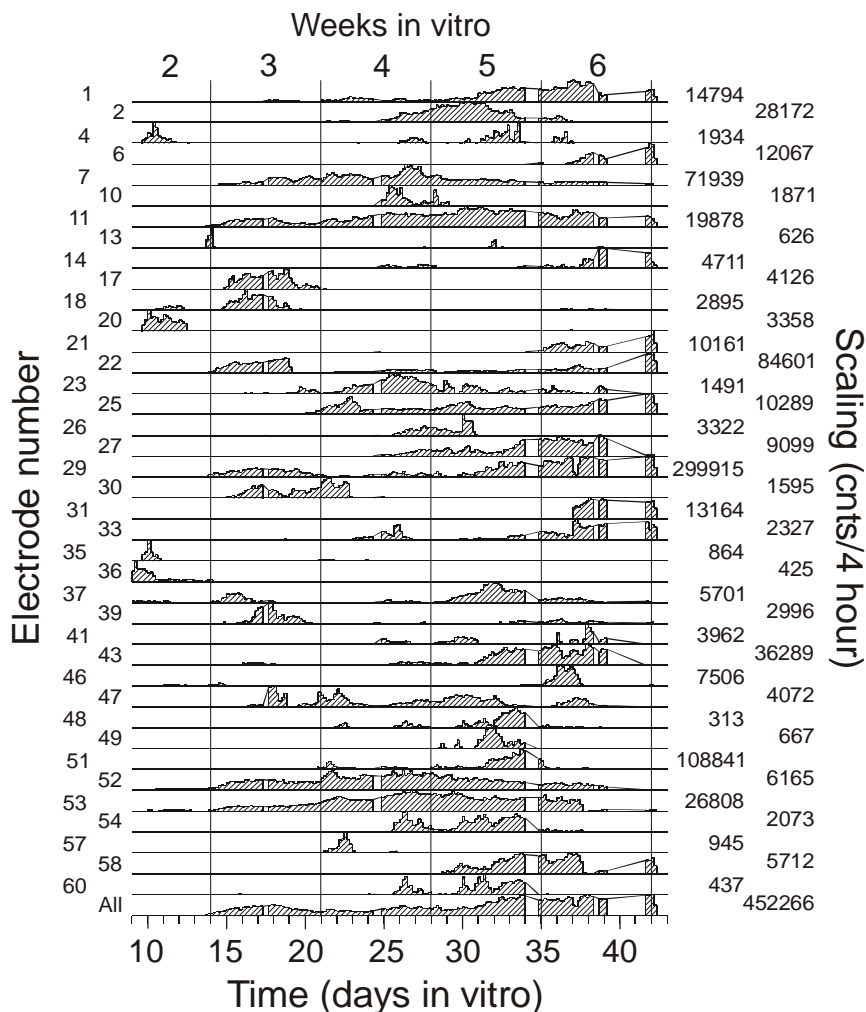


Figure 2. Firing rates in counts per 4 hour time bins at the individual electrode sites of the multi-electrode array for the entire recording period from 9 to 43 days in vitro. The lowest trace shows the total activity at all the sites. The firing rate traces are individually scaled, with the maximum rate per time bin indicated at the right side of the figure. Electrodes from which less than 300 spikes per time bin at the maximum are recorded are not included. The figure includes some empty periods, resulting from interruptions in the recordings.

Network bursts. At the time scale of seconds, network activity appears to be organised in an ongoing pattern of alternating active and quiescent phases (Figure 3). During active periods, showing irregular firing at different sites, short phases of intense synchronized network activity occur (*network bursts*)

appearing in Figure 3 as high intensity bins. Note, that the bin size is 100 msec, indicating that *network bursts* have a duration of smaller than or about 100 msec, except in the 20 DIV and 25 DIV traces.

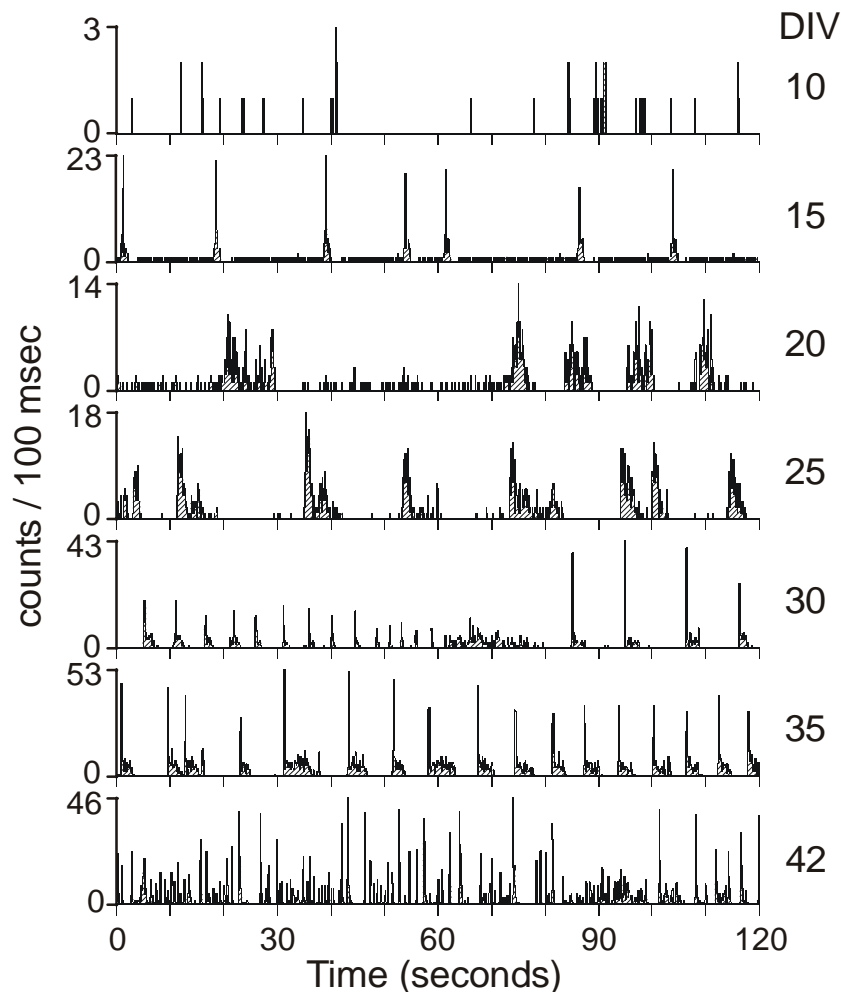


Figure 3. Network firing rate traces in counts per 100 msec time bins. The samples of 120 seconds are taken at the indicated days *in vitro* from the time point of the start of the culture.

Spatio-temporal structure of firing within network bursts. The precise timing of the individual spikes within network bursts is shown in Figure 4. The spatio-temporal patterns of spiking within individual network burst is highly variable, but their probabilistic structure becomes clear when accumulating many network bursts, as is shown in the 3rd column of Figure 4. The total firing rate profile within network bursts appears to undergo clear developmental changes. From 15 DIV to 25 DIV, network bursts broaden, while losing a clear center of maximal activity. After this phase, they redevelop a sharper profile in time and, at 35 DIV, become even shorter than at 15 DIV. The firing rate profiles at the individual sites show clear phase relationships. For instance, maximal firing rate at site 29 precedes maximal firing rate at site 21. This phase relationship is found at 15, 25 and 35 DIV indicating their stability over long periods of development.

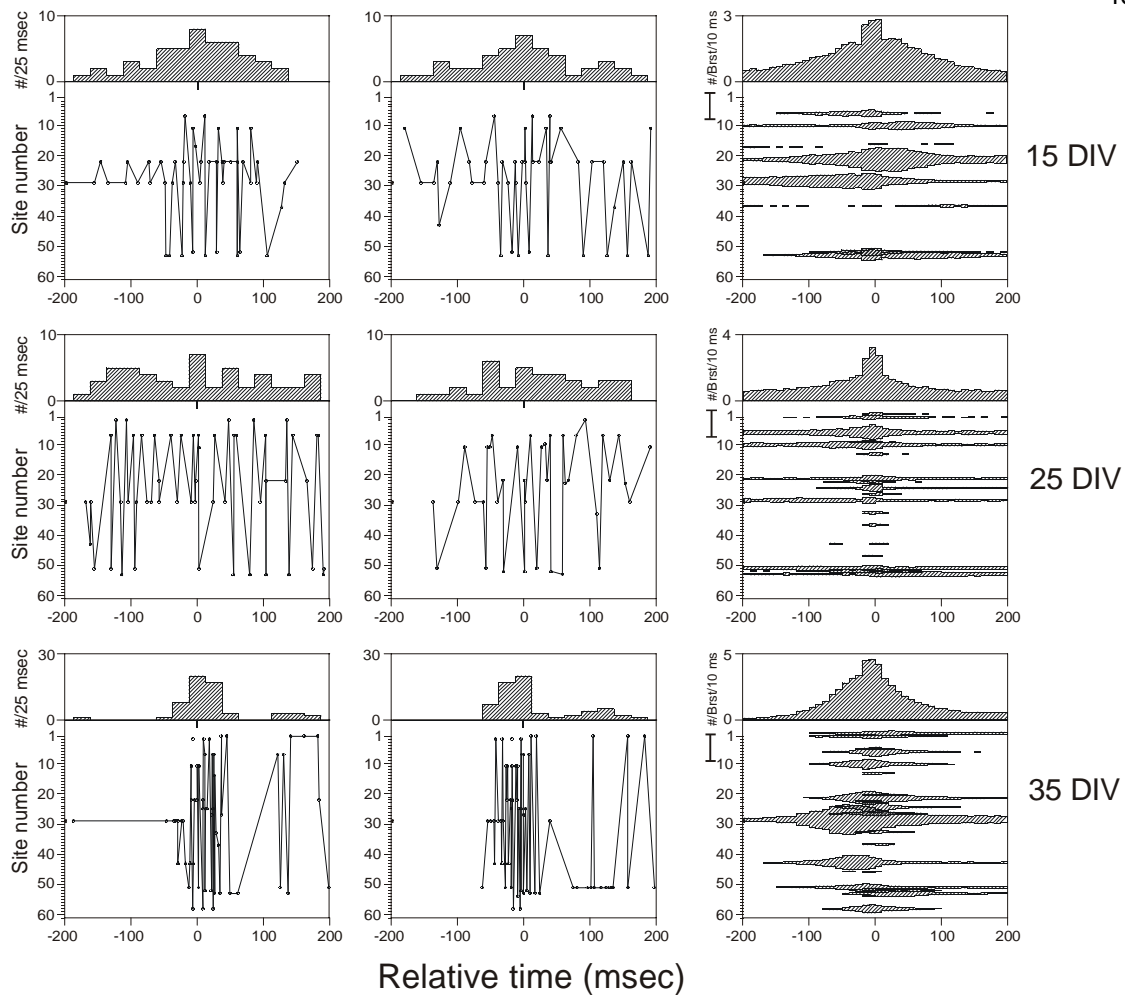


Figure 4. Spatio-temporal patterns of spontaneous firing during network bursts. The first two columns show individual network bursts. The last column shows the averaged firing rates, calculated by accumulating the firing patterns within all the networks bursts, occurring in a period of 2 hours. The bursts have been obtained from recordings at 15 DIV (first row), 25 DIV (second row) and 35 DIV (third row). The lower part in each panel of the first two columns displays the precise time and site of each action potential within a single network burst, indicated by small dots. Consecutive spikes are connected by thin lines to emphasize their temporal order. The upper part in each panel displays the total firing rate within a single network burst in 25 msec time bins. The lower part of the panels in the right column displays the firing rate at the individual sites, and the upper part the total firing rate, both calculated for 10 msec time bins. The lower part is scaled according to the scale bar indicating 1 spike per bin per network burst.

Conclusion

The present experiments have shown the feasibility of long-term recordings of firing activity in neuronal networks, cultured on multi-electrode arrays. During network development firing activity undergoes significant changes, becoming apparent at different time scales. At a coarse timescale, individual sites appear to show transient increases of firing rates, lasting for periods of days or weeks. At a time scale of seconds, network activity is organized in alternating active and quiescent periods. Within active periods, short phases of high activity occur during which many sites cooperate in a synchronized firing mode, called *network bursts*. The spatio-temporal pattern of firing within network bursts also showed developmental changes, with a substantial broadening up to about 25 DIV and a significant shortening at 35 DIV. These developmental changes in spontaneous network firing may be the functional

consequences of the many structural changes occurring when isolated neurons develop into a synaptically connected network. How structural and functional development are related is a question that cannot be understood without the help of computational and modeling tools, as being developed within the area of Neuroinformatics. The present findings contribute to the necessary experimental data for such explorations.

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