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Longterm stability and developmental changes in spontaneous network burst firing patterns in dissociated rat cerebral cortex cell cultures on multielectrode arrays☆

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Abstract

Spontaneous action potentials were recorded longitudinally for 4–7 weeks from dissociated rat occipital cortex cells cultured on planar multi-electrode plates, during their development from isolated neurons into synaptically connected neuronal networks. Activity typically consisted of generalized bursts lasting up to several seconds, separated by variable epochs of sporadic firing at some of the active sites. These network bursts displayed discharge patterns with age-dependent firing rate profiles, and durations significantly increasing in the 3rd week in vitro and decreasing after about 1 month in vitro, when they evolved into short events with prompt onsets. These findings indicate that after about a month in vitro these cultured neuronal networks have developed a degree of excitability that allows almost instantaneous triggering of generalized discharges. Individual neurons tend to fire in specific and persistent temporal relationships to one another within these network bursts, suggesting that network connectivity maintains a core topology during its development.

Keywords: Spike-train analysis; Spontaneous spike generation; Neuronal networks; Cell culture; Cerebral cortex

A striking aspect of developing neuronal networks is their tendency, in vitro as well as in situ, to discharge spontaneously in the form of repetitive polyneuronal bursts of activity (for review see refs. [1,2]). These studies have suggested that the patterns of spike discharges are strongly site-specific and dependent on the stage of development. The present investigation aims at verifying these impressions in a quantitative manner. Multielectrode arrays were used for continuously monitoring the spontaneous action potential activity of neonatal rat neocortex neurons (also see ref. [3]) over a period of many weeks during maturation in vitro. The recorded data has been analyzed quantitatively for the patterns of burst firing at all recording sites throughout network development. The results establish unequivocally that: (1) developing cortical networks in vitro display consistent burst discharge patterns with age-dependent firing rate profiles and mean durations which increase significantly during the 3rd week in vitro and strongly decrease in the weeks thereafter; and (2) individual neurons at all ages tend to fire in specific and persistent temporal relationships to one another within these generalized network bursts.

Dissociated E18 rat neocortical neurons were cultured on planar multi-electrode plates, and monitored for their spontaneous firing activity during their development into synaptically connected networks. The 60 electrodes, with 12 micron diameters, were arranged in a hexagonal pattern with a mutual spacing of 70 micron (for technical details see refs. [5,7]). Action potentials were detected by amplitude discrimination and stored as time stamps for off-line analysis. Most of the spike trains concerned single-unit activity, with stable amplitudes and waveforms, and showing refractory periods of 2–3 msec in their interspike interval distributions throughout the culture period. The pattern of spontaneous firing was characterized by regular occurrences of network bursts, i.e. short periods of synchronous firing at many of the recording sites.

During off-line analysis, network bursts were automatically detected by means of an algorithm based on the product of total network firing rate and number of active sites. The time point at which this product was maximal was taken as the center of a network burst [7]. Statistical estimates for the patterns of firing within network bursts

☆ The paper honors Manfred Zimmermann on the occasion of his 70th birthday.

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were obtained by averaging the firing activity of all network bursts within a period of 4 h, aligned according to their time centers. Profiles of the averaged firing rates in successive 10 msec time bins were subsequently calculated for all the individual sites in the multielectrode array, as well as for the total network activity. These total network firing-rate profiles were then characterized by the half-width of their rising and falling phase (i.e. the difference between the central time point and the time points at which the network burst firing rate was half-maximal at the rising and the falling phase, respectively). For five cultures which remained highly active over an extended period (4–7 weeks in vitro), the results of these calculations were printed out sequentially for consecutive 4 h epochs. Visual inspection and statistical analysis of these plots form the basis of the present report.

All cultures showed spontaneous action potential discharges from about the beginning of the 2nd week in vitro. Network bursting during this early phase of maturation was typically preceded by a ramp-like build-up of activity at one or several sites, culminating in a widespread discharge lasting on the order of 1–2 s, and ending abruptly with a generalized cessation of spontaneous firing (Fig. 1A). Higher time resolution plots illustrate how the individual sites contribute to the pattern of firing within network bursts (Fig. 1B). Successive network bursts appear to be quite similar in their spatial and temporal pattern of firing. This becomes evident from the averaged firing rate profiles, obtained by summing all network bursts over a period of 4 h (Fig. 2).

Plotted with 4 h intervals, the three panels each illustrate that the firing rate profiles change very little over periods of many hours. Over longer periods of time, however, network bursts slowly undergo major changes in their total firing rate profile, as illustrated in Fig. 2 for 14, 18, and 29 DIV, respectively, at which time points the differences were maximal. The initially short and slightly skewed bursts (lasting ca. 1–2 s) evolved during the 3rd week in vitro into long-lasting bursts of about 6 s with almost symmetrical firing rate profiles. At still later ages, network bursts shortened down to about 200 msec. These highly significant developmental changes (Kruskal–Wallis one-way analysis of variance) are depicted by the half-width of the rising and falling phase in Fig. 3. While at earlier days in vitro network bursts are preceded by a period of low firing rates at a few sites, after a month in vitro they have developed into promptly triggered events without noticeable pre-burst firing, with an extremely short rising and falling phase of about 30 msec half width (Fig. 3), and often followed by a low intensity after-discharge at a few sites (Fig. 2).

Many sites appear to fire at preferred phases within these bursts. Some sites contribute to the onset phase, others support activity in the later phase, while still others fire predominantly during the central phase of the network bursts (Fig. 1B). Some sites contributing to the onset phase also support the low level firing in the period before burst onset, while some sites supporting the later phase of the network bursts also continue their firing in a post-burst phase of after discharges. For instance, in Fig. 2 at 14 DIV, site 29 contributes to the onset phase, site 48 to the central phase and, with site 12, to the later phase. Site 54 fires only at the end of the burst. Bursts detected 8–12 and 16–20 h

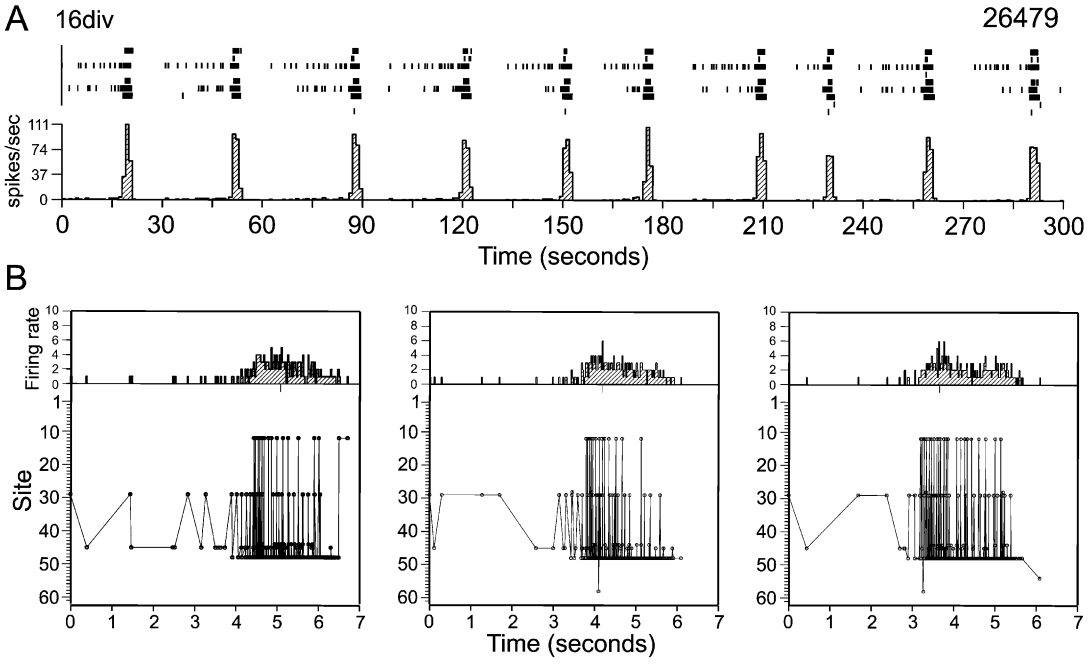

Fig. 1. Repetitive network bursting pattern at 16 DIV in preparation #26479. (A) Typical 5 min. stretch of spontaneous firing at all active recording sites, along with a firing rate plot (spikes/s) of the total recorded activity. (B) Expanded plot of three network bursts, showing the precise spike timing (dots) at the individual sites, as well as the time profile of the overall firing rate.

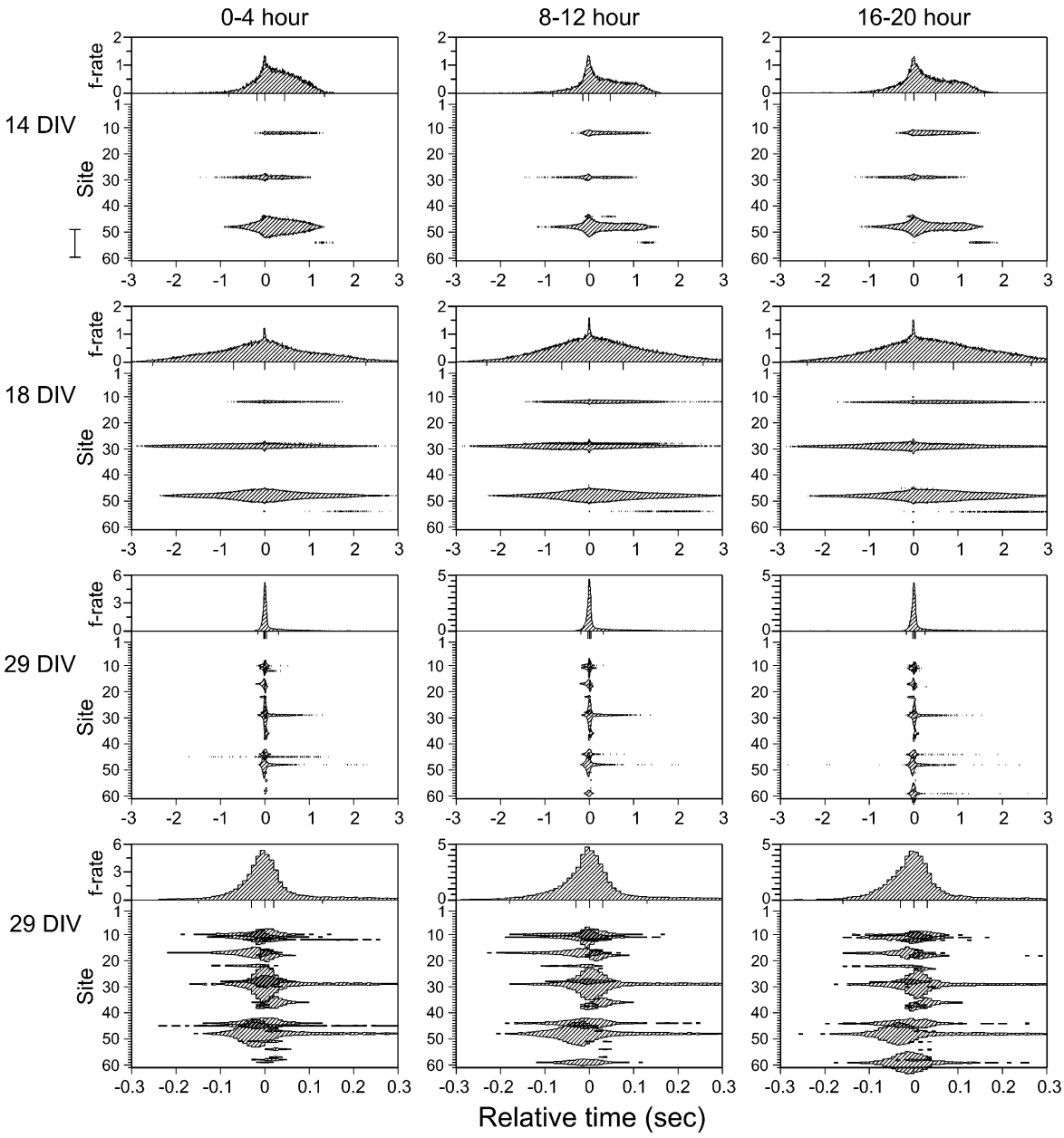

Fig. 2. Averaged firing rate profiles (number of spikes/10 msec time bins) of total network activity (upper part) and of individual sites (lower part, and scaled according to the scale bar denoting 1 spike/10 msec per burst) across a full day of continuous recording in preparation #26479 at three different ages in vitro, and with the 29 DIV panels also displayed at higher time resolution (4th row).

later show similar phase preferences, demonstrating that neurons maintain their temporal relationships of firing over a full day in vitro. This was also found at 18 DIV, when network bursts have broadened significantly. Even at 29 DIV, when network bursts have significantly been shortened and more sites were active, some of the mentioned sites still maintain the same temporal relationships.

The present study has thus made it clear that neurons in cultured neocortical networks differ greatly not only in their mean firing levels but also in their preferred timing for spike discharges during periods of synchronized network activity. The stability of interneuronal phase relationships throughout the period of development in culture indicates that the connectivity within the network maintains a highly conserved topology, an unexpected finding for networks which are undergoing profound maturational changes [1,2,4]. Previously, stable phase relationships between spontaneously firing pairs of cultured neurons had been demonstrated for much shorter periods [6] and it was shown in addition that the strength of such interconnections is activity dependent. It is therefore quite possible that the age-dependent changes observed in the present experiments could in part have been due to the prominent ongoing spontaneous activity, as suggested earlier by cross-sectional studies under comparable culture conditions [1,2,4].

At the same time, the technique of uninterrupted

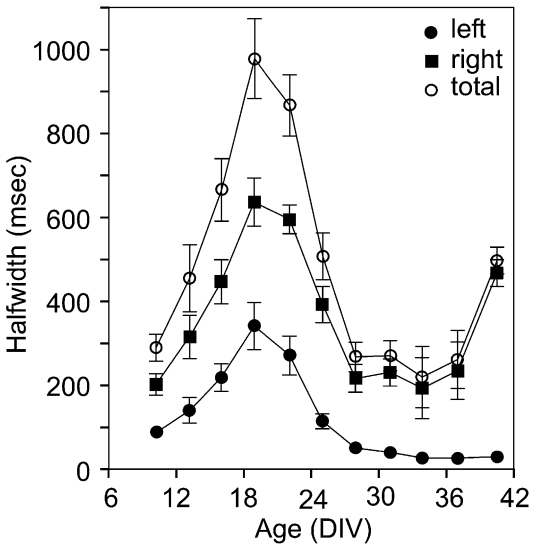

Fig. 3. Mean duration in milliseconds of network bursts at different ages in vitro, as calculated for five longitudinal experiments on the basis of half-width values of the averaged amplitude/time profile, obtained by summation of all detected bursts on each day of recording: 'left' – rising phase to the 'center' of the burst (see Fig. 1); 'right' – falling phase from the center; 'total' – total burst.

longitudinal recording from many sites simultaneously has made possible the demonstration that, in the course of network maturation, many neurons undergo progressive quantitative shifts in their contribution to generalized burst discharges, with respect both to their precise timing and to their overall amount of spiking. Equally striking is the observation that units which, at early stages, putatively trigger network-wide bursts by virtue of a ramp-like increase in firing level, in later life are largely silent during the intervals leading up to the next burst. Conversely, many neurons which at early stages abruptly cease discharging spontaneously for several seconds following each network burst are no longer inhibited in this respect later on. It is interesting in this respect that Jimbo et al. [3] report that an early component, characterized by precise interneuronal timing and very little jitter, can be distinguished in the response of older cortical cultures (30 days in vitro) to electrical stimulation, which could mean that the network has by then attained a degree of excitability such that any adequate stimulus, whether generated intrinsically or applied, will result in almost instantaneous triggering of a stereotyped generalized discharge.

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