Progress in Brain Research, Vol. 147
ISSN 0079-6123

CHAPTER 13

Dynamics and plasticity in developing neuronal networks in vitro

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Abstract: When dissociated cortical tissue is brought into culture, neurons readily grow out by forming axonal and dendritic arborizations and synaptic connections. These developing neuronal networks in vitro display spontaneous firing activity from about the end of the first week in vitro. When cultured on multielectrode arrays firing activity can be recorded from many neurons simultaneously over long periods of time. These experimental approaches provide valuable data for studying firing dynamics in neuronal networks in relation to an ongoing development of neurons and synaptic connectivity in the network. This chapter summarizes recent findings on the characteristics and developmental changes in the spontaneous firing dynamics. These changes include long-lasting transient periods of increased firing at individual sites on a time scale of days to weeks, and an age-specific repetitive pattern of synchronous network firing (network bursts) on a time scale of seconds. Especially the spatio-temporal organization of firing within network bursts showed great stability over many hours. In addition, a progressive day-to-day evolution was observed, with an initial broadening of the burst firing rate profile during the 3rd week in vitro (WIV) and a pattern of abrupt onset and precise spike timing from the 5th WIV onwards. These developmental changes are discussed in the light of structural changes in the network and activity-dependent plasticity mechanisms. Preliminary findings are presented on the pattern of spike sequences within network burst, as well as the effect of external stimulation on the spatio-temporal organization within network bursts.

Introduction

During early development neurons grow out by extending their dendritic and axonal arborizations, and connect to each other via synaptic specializations. Through these synaptic connections neurons communicate with each other via action potentials and, through activity-dependent mechanisms, form functionally connected networks (e.g., Ramakers et al., 1990; Fields and Nelson, 1991; Corner and Ramakers, 1992; Goodman and Shatz, 1993; Spitzer, 1995; Van Ooyen et al., 1995; Crair, 1999). Neuronal interactions within these connected networks become especially apparent during periods of synchronized firing in the network (network bursts). In developing neurons in culture, such synchronized bursts occur spontaneously, initiated and governed by spatial and temporal summation of synaptic events in the culture (Maeda et al., 1995) and not by pacemaker cells

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DOI: 10.1016/S0079-6123(04)47013-7

(Robinson et al., 1993). Interactions between the neurons in the network form the basis for patterns of firing activity, and it is generally believed that these patterns are strongly shaped by the structural properties of the network.

Developing neurons cultured on multielectrode arrays provide excellent experimental conditions for studying structural and functional development. They allow structural studies to be made concerning neurite outgrowth and synapse formation as well as functional studies of the firing characteristics of many individual neurons, simultaneously recorded from the many electrodes in the electrode array over full periods of developmental time (months). Our group integrates these approaches and studies the structural and functional development of cultured neurons (e.g., Van Ooyen, 1995; Baker et al., 1998; Ramakers et al., 2001; Corner, 2002; Van Pelt et al., 2004a, b). This chapter will focus only on the latter, by reporting results on the firing characteristics of neurons, especially during "network bursts," as recurrent events of strong neuronal interaction.

Activity-dependent mechanisms in neural development implicate a reciprocal relationship between the developing neuronal network and the emergent firing dynamics within the network (e.g., Van Ooyen et al., 1995). By these mechanisms neuronal networks develop meaningful dynamics in the presence of structured sensory information. Similarly, however, such reciprocal relationships will also operate in spontaneously active neuronal networks, not subjected to external stimulation. Especially during early development, spontaneous activity is expected to have an important structuring role (see for instance Corner, 1994; Corner et al., 2002). Also in cultured neuronal networks, showing robust levels of spontaneous activity, one may expect that the network develops under this reciprocal influence. A typical phenomenon in spontaneous network activity is the regular occurrence of short episodes of synchronized firing (*network bursts*). During these events many neurons cooperate through mutual interaction and built up these characteristic dynamic modes of network firing. The intense neuronal interaction during these network bursts is expected to provide special conditions for effectuating activity dependent mechanisms, making the internal structure of firing within network bursts an interesting topic of research.

Understanding of the mechanisms by which neuronal networks self-regulate their activities as they mature is essential for furthering our insights into the formation of neuronal networks and the characteristics of the emergent firing dynamics in these networks. For instance, comparing the firing dynamics in networks developed under spontaneous conditions and under stimulated conditions, will give a better view on the functional significance of synchronous network activities, which have been already implicated in synaptic transmission efficacy (Stevens and Zador, 1998), binding of distributed responses (Singer, 1999), learning (Thomas et al., 1998; Pike et al., 1999; Paulsen and Sejnowski, 2000) and memory consolidation (Steriade et al., 1993; Sejnowski, 1995).

In this chapter, we will present results of several longitudinal experiments on the development of primary cultures of rat cortical tissue, some of them previously published elsewhere (Van Pelt et al., 2004a, b). Emphasis is given to the patterns of spontaneous firing in the networks, in particular within network bursts, and their characteristic changes in the course of network development. Preliminary results of spontaneous bursting dynamics will be presented as they appear prior to and following a short period of electrically stimulation, respectively. The results will be discussed in the framework of active network connectivity shaping and the role of bursting in synaptic plasticity.

Spontaneous firing dynamics in cultured neuronal networks

Materials and methods

Experimental approaches are only briefly described. For a detailed description of the experimental setup, see Van Pelt et al. (2004a, b).

Multielectrode plates

Multielectrode plates were obtained from the University of Twente, Enschede, The Netherlands (Rutten et al., 2001). They consisted of 5 × 5 cm glass plates onto which a pattern of 61 electrically conductive lanes (material indium-tin-oxide or gold)

was deposited, running from two sides of the plate toward a central area where they ended in a hexagonal pattern of electrodes with a diameter of 12 μm, spaced 70 μm apart. In addition, commercial multielectrode arrays (HEXA MEAs) from Multi Channel Systems (Reutlingen, Germany) have been used, which include electrodes with diameters of 10, 20, and 30 μm. Electrode size determines how many neurons can contribute to the activity measured with the electrode. Multiunit contribution sharply goes down when electrode diameter is of the size of neurons' cell body (Van Pelt, 2004a). For rat cortical neurons, in combination with electrodes of about 10 μm, single unit activity has predominantly been recorded and, with additional spike amplitude discrimination, has resulted in single unit spike trains. These recording conditions are required to study temporal relationships of firing between individual electrodes, because multiunit activity smears out temporal preferences of individual neurons.

Cell cultures

Cell cultures have been prepared from the cortices of E18 Wistar rat fetuses (Ramakers et al., 1991, 1998, from which, after dissociation, a total number of 150,000 cells (50 μl cell suspension) were plated in a 7 mm round spot in the center of the multielectrode plate (MEP) coated with polyethylene-imine (PEI, Fluka, 10 mg/ml), using glass rings (inner diameter of 7 mm). After 1 h the rings were removed, and 10^6 cells in 1 ml of cell suspension were added to the culture chamber on the MEP (inner diameter 30 mm). For the inner area this resulted in a monolayer of cells in such a density that a surface area of, on the average, 200 μm^2 (corresponding to a 16 μm circle diameter) was available for each cell. At the time of plating, the cells themselves had a diameter of about 5 μm, which increased to about 15 μm by three weeks.

Neurons were cultured and recorded in 2 ml of glia conditioned medium (GCM) + 0.2% BSA containing 1.3 mM Ca^{2+} and 0.7 mM Mg^{2+}. The culture chambers were covered with a glass lid and firmly sealed with parafilm in order to prevent evaporation of the medium during the long-term recording period. Once a week, about 200 μl of the medium was replaced by 300 μl fresh medium. No further handling was applied during the entire recording period. These culturing conditions (see also Potter and DeMarse, 2001) ensured stable osmolarity as well as pH levels, with fluctuations of the former staying within 3% or less over a period of 29 days in vitro (DIV). After each experiment, the MEPs were cleansed by careful rinsing and then sterilized for 4 h at 140°C for reuse.

Spike discrimination and time stamping

Real time data reduction was essential to prevent data overload during longitudinal recordings, and was achieved by spike discrimination and real-time time stamping. Only when a spike was detected a data event was stored consisting of the site of activity and the momentary value of the time stamp clock, running at 10 kHz.

Spontaneous firing rates during development of cultured neuronal networks

A unique advantage of multielectrode array recordings is the possibility to record the firing activity of individual neurons over long periods of time, even months. Such periods cover the time over which cultured networks develop from isolated neurons into fully connected neuronal networks, thereby passing through phases of massive overproduction of synaptic connections and subsequent synaptic elimination and stabilization (for review see Corner et al., 2002). Longitudinal recordings have been obtained from a number of experiments and they have revealed characteristic details in the developmental changes in network firing activity.

Developmental changes in firing rates

Typically, spontaneous activity is first recorded at the end of the first week in vitro at a couple of sites of the multielectrode array. From then on, activity is recorded from an increasing number of sites, but with firing rates slowly increasing or decreasing over developmental time. In particular, it was surprising to find that individual sites showed one or a few periods, lasting a few days or weeks, in which their

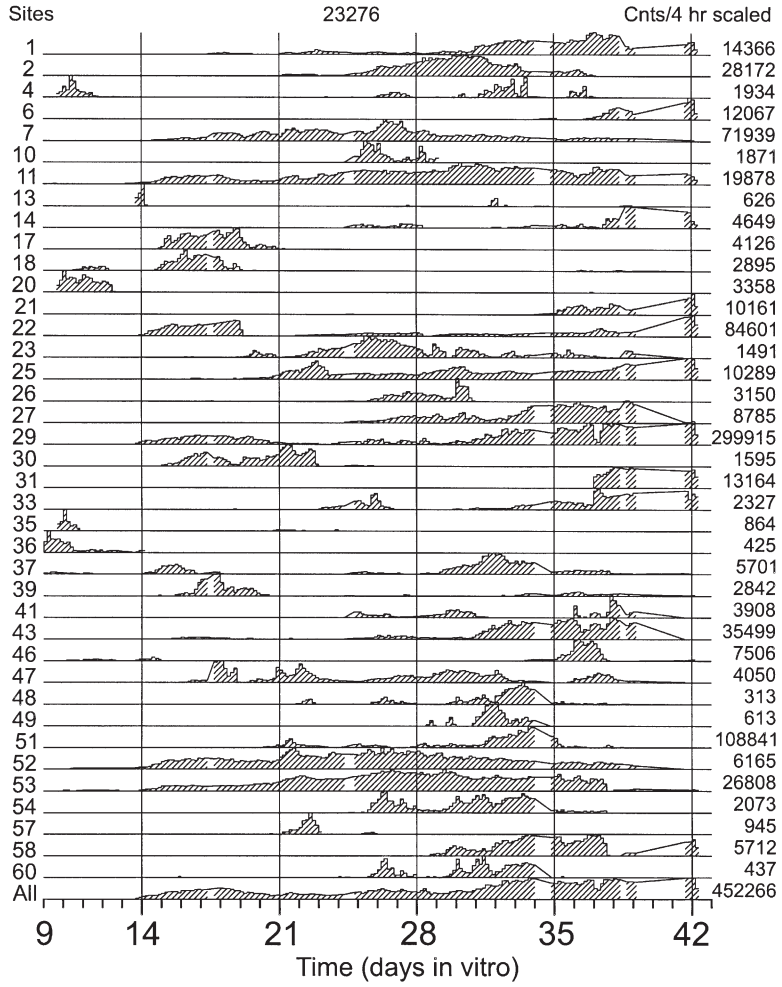

Fig. 1. Firing rates expressed as number of spikes per 4-h time bins at the individual sites of the multielectrode array for a whole period of recording up to 42 DIV. The numbers to the left of the panel denote the electrode numbers. The bottom trace shows the summed activity over all the sites. The firing rate traces are individually scaled, with the maximum rate per 4-h bin indicated on the right of the panel. In the interest of clarity, only sites with a mean rate exceeding 1 spike/min at some point in time are shown. The plot includes a few interpolated (nonhatched) episodes for which no data was available. (Adapted from Van Pelt et al. (2004a).)

firing rate was drastically increased. An example is given in Fig. 1, showing the mean firing rate per 4-h time bins at all the active recording sites, from the beginning of the 2nd week in vitro when activity has clearly shown up, up to the beginning of the 7th week in vitro, when this experiment was arbitrarily stopped but the network still continued with ongoing firing activity. Individual sites also appeared to show large differences in the maximal firing rates experienced during the full developmental period (scale numbers at the right of the figure). Despite the large differences in the firing rates among sites and developmental time points, as were also observed in other longitudinal experiments, summed activity over all the sites (such as in the bottom trace in Fig. 1) has not shown a clear or reproducible dependency on developmental time, which may be surprising in view of the drastic developmental structural changes in the network by the outgrowing neurons and the overshoot in synapse numbers.

Firing patterns at time scales of seconds — regular occurrence of synchronized network bursts

Spontaneous firing activity in cultured neuronal networks displays a characteristic time structure with the regular occurrence of short phases of synchronized firing (network bursts) as the most prominent phenomenon (e.g., Maeda et al., 1995; Kamioka et al., 1996; Jimbo and Robinson, 2000; Jimbo et al., 2000; Segev et al., 2001; Corner et al., 2002, Van Pelt et al., 2004a, b). During such network bursts the number of active sites as well as the firing rates at the active sites are increased. Typical examples are given in Fig. 2 in which the 18 DIV panel illustrates a recurrent pattern of firing with each repetition including a phase of low level firing activity, a short phase of synchronized firing, and a silent network recovery phase. The 30 DIV panel also shows a recurrent pattern of firing, but here the network bursts are not preceded by a phase of low firing rates but have a prompt initiation. These differences reflect characteristic developmental changes in the pattern of network firing.

Detection of networks bursts — time alignment

To facilitate the analysis of network bursts a procedure was developed for the automatic detection of network bursts in an ongoing stream of spikes. This detection is based on the property that during network burst both the number of active sites and their firing rates are increased. This means that the product of number of active sites and total spike

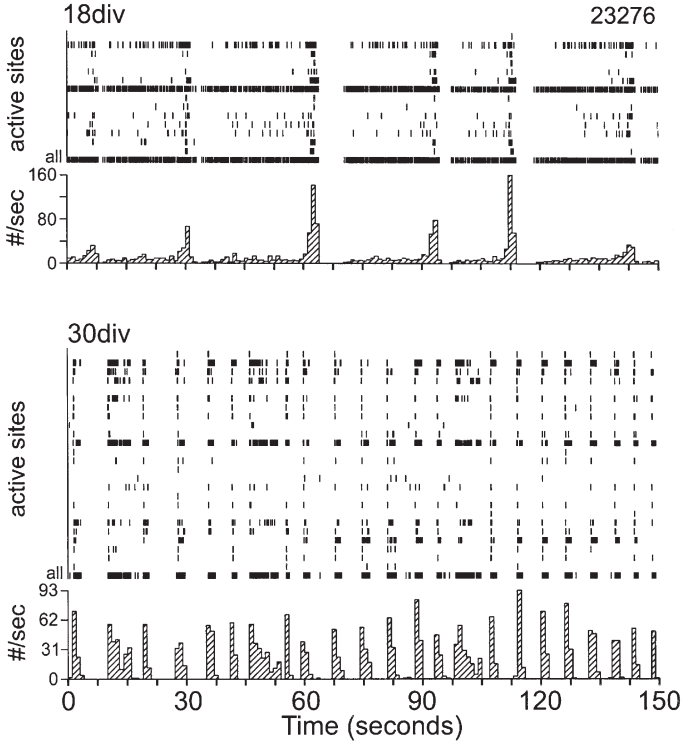

Fig. 2. Spike timings at the individual recording sites and total network firing rates during 150 s time periods. The samples are drawn at 18 and 30 DIV from preparation #23276. The figure illustrates the timing of spikes from the individual active sites as well as a trace for the summed activity, indicated by "all". The firing rate trace displays the time course of the total number of network spikes per second. The examples illustrate the repetitive pattern of network spiking with active and silent phases, the occurrence of network bursts (short episodes of intense and synchronous firing) within the active phases, the differential contribution of individual sites to the active phase of each repetitive period, and the developmental changes in the repetitive firing patterns (illustrated here for the 3rd and 5th week in vitro). (Adapted from Van Pelt et al. (2004a).)

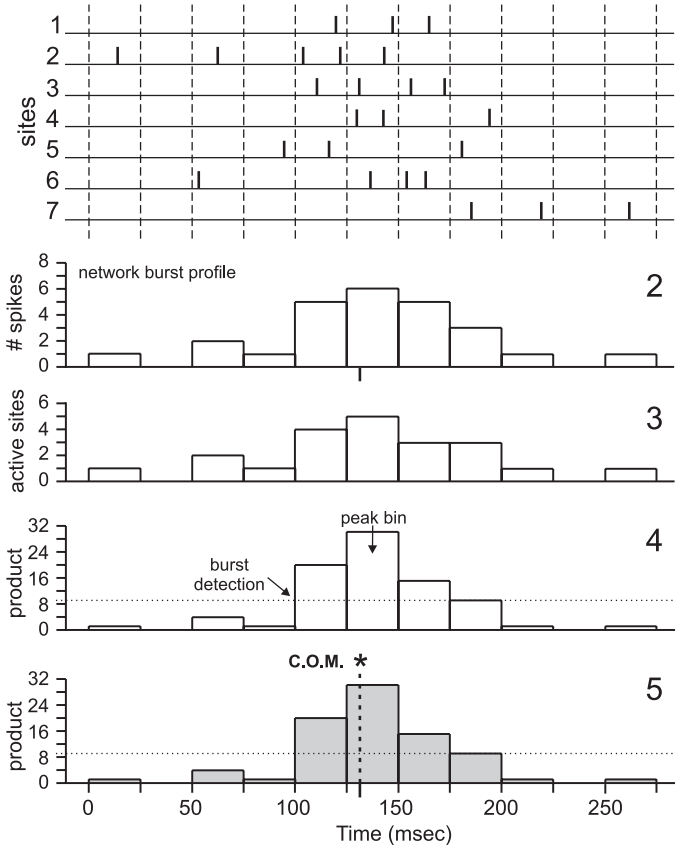

Fig. 3. Illustration of the procedure for burst detection. The upper panel displays an arbitrary pattern of spikes at 7 sites. Panels 2 and 3 display the histograms for the number of spikes and the number of active sites, respectively, counted for a time division of 25 ms. Panel 4 displays the product of the spike count and the active-site count. A "burst" is detected when this product exceeds an arbitrarily chosen value of 9, as indicated by the arrow. The time bin with the maximal product value (peak bin) is taken as the center bin of the burst. The center time of the burst is finally calculated as the center-of-mass (C.O.M.) point of the product distribution over the bin range center +/− 5 (panel 5), and indicated by a small line underneath the spike count histogram (i.e., network burst profile).

count, as evaluated in short time bins, peaks significantly during network bursts. An arbitrary chosen value of 25 ms for the time bins turned out to give good practical results. The recorded multi-electrode spike trains were subsequently scanned for this product function. It turned out that an arbitrarily chosen value of 9 was well able to distinguish network bursts (exceeding in their product function this value) from the low level firing periods in between. Following the detection of a network burst the maximal product bin was searched among the adjacent bins, while the *center time of a burst* was calculated as the center-of-mass time point for the product distribution evaluated within a window of 5 bins left and right of the center bin. This center time point is indicated by a mark underneath the network burst profile (panel 2 in Fig. 3).

Spatiotemporal pattern of firing within network bursts

Variability in the spatiotemporal patterns of firing in individual network bursts

During network bursts the activation of the network is dominated by a cooperative mode of excitation in which neurons keep themselves in an active state through intense mutual interaction (excitation and inhibition). These interactions are reflected in the

precise time points of spiking of the participating neurons and, thus, by the spatio-temporal pattern of spiking within the network. Evidently, only an extremely small portion of this pattern becomes visible for the neurons recorded by the electrodes in the multielectrode array. The precise structure of these spatio-temporal patterns depends on many factors, such as the *network burst initiation sites* (i.e., which neurons have spontaneously driven the network bursts), the *flow of activity* through the network (determined by the specific connectivity pattern of the network), the *momentary states* of the neurons (such as their membrane potentials and states of refractoriness), and the time taken by neurons to *propagate activity* (synaptic delays, dendritic integration times). Individual network bursts can thus be expected to show considerable variation in their spatio-temporal patterns of firing, as indeed has been observed in all of the recordings. An example of such variation is given in Fig. 4 showing the precise time points of spiking for the active sites during three network bursts.

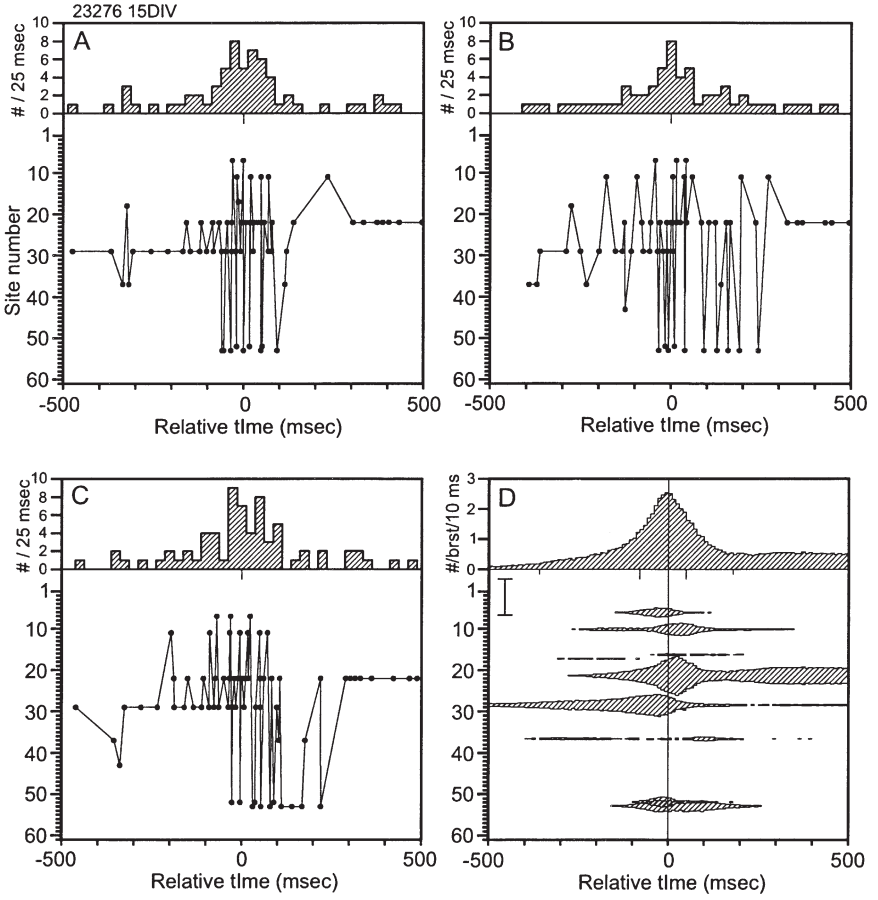

Fig. 4. (A, B, C) Three network bursts from preparation #23276 at 15 DIV. The upper part of each panel displays the total firing rate as the number of spikes per time bin of 25 ms. The lower part of each panel displays the exact timing of the individual spikes (dots) for each of the sites. Consecutive spikes are connected by thin lines in order to indicate the temporal order of the individual spikes. The center of each network burst is indicated by a small line drawn underneath the total firing rate plot. These centers are used as alignment time marks for the summation of consecutive network bursts. (D) Firing rate profiles obtained after summation of detected network bursts during a period of 4 h of recording. The upper part of the panel displays the total firing rate in number of spikes per 10 ms time bins. The lower part illustrates the firing rates for the individual sites, plotted as bars symmetrically around the horizontal lines. The scale bar denotes a firing rate of 1 spike per 10 ms per burst.

Probabilistic structure of spatio-temporal firing patterns in network bursts

Although much variation is present in the individual network bursts, a certain degree of constancy in the underlying probabilistic process may also be expected, as a direct reflection of the constant factors in the network burst generating process. Especially the synaptic connectivity pattern in the network (topology, synaptic strength) might not vary strongly on the time scale of network bursts. Such constancy, in combination with preferential routing of the activity flow, would result in a stable pattern of temporal relationships among the active sites. Such underlying probabilistic structure becomes apparent when the burst-to-burst fluctuations are filtered out, which can be realized by averaging the spatio-temporal patterns of many consecutive network burst. For the required time-alignment in the summation, the center times of the network bursts have been used. The summation results in firing rate profiles for the individual sites within network bursts, and these profiles reveal the differential contribution of each site to the total activity in the network bursts.

The example shown in Fig. 4D illustrates how the firing rate profiles of individual sites can differ in maximal firing rate, time point of maximal firing, and in shape. For instance, the firing rate profile of site 22 shows its maximal firing rate about 30 ms after the burst center, followed by a strong reduction at about 130 ms after the burst center, and again a moderate increase. The averaged network burst firing rate profiles thus illustrate spatio-temporal characteristics of the firing dynamics in the network, when averaged over many realizations of the underlying probabilistic process. We will consider them as a ''blueprint'' of the firing dynamics. As discussed above, we hypothesize that these characteristics correlate mainly with stable or slowly changing network properties such as the synaptic connectivity pattern and the routing of activity through the network. Support for this hypothesis is given by the fact that the dynamic blueprints show only minor changes over periods of hours. In contrast, large changes in the dynamic blueprint are to be expected over longer periods, such as the periods of early network formation and exuberant synapse formation, and subsequent pruning of synapses, respectively, phases in which synaptic connectivity patterns change drastically.

Developmental changes in network burst firing rate profiles

Indeed, all longitudinal experiments have shown systematic changes in the network burst firing rate profiles (Van Pelt et al., 2004a, b), see example in Fig. 5. The most drastic changes concern the duration and shape of the network bursts. Network bursts increase their durations up to about the end of the 3rd week in vitro, and decrease their duration in the period thereafter. Especially the drastic shortening of the network burst onset phase at the end of the first month in vitro is a significant finding. It must be noted that these changes occur only gradually over time, and comparing network bursts over periods of several hours will reveal only small differences. The panels in Fig. 5 further illustrate that individual sites contribute in varying degrees to the network activity at widely different developmental time points, as was already pointed out in the overview plot of Fig. 1.

In order to analyze quantitatively the durations of network bursts they have been expressed in terms of burst half-widths, i.e., the time difference between the time points at which burst intensity passes half maximal values in up- and downward direction, respectively. The rising phase half-width is defined as the time difference between the time point at which the burst intensity profile reaches half-maximal values and the time point of maximal values. Figure 6A illustrates mean and SEM values of the network burst half-widths, as based on five longitudinal recordings. The rising phase half-width reaches peak values of almost 400 ms at 21 DIV, but decreases sharply after about 5 weeks in vitro to stable values of less than 50 ms.

Temporal relationships of firing within network bursts

Although network bursts represent episodes of cooperative and intensified firing within the neuronal network, individual neurons still display differences in the precise shape of their firing rate profiles, the precise time point of maximal firing rates, and in the

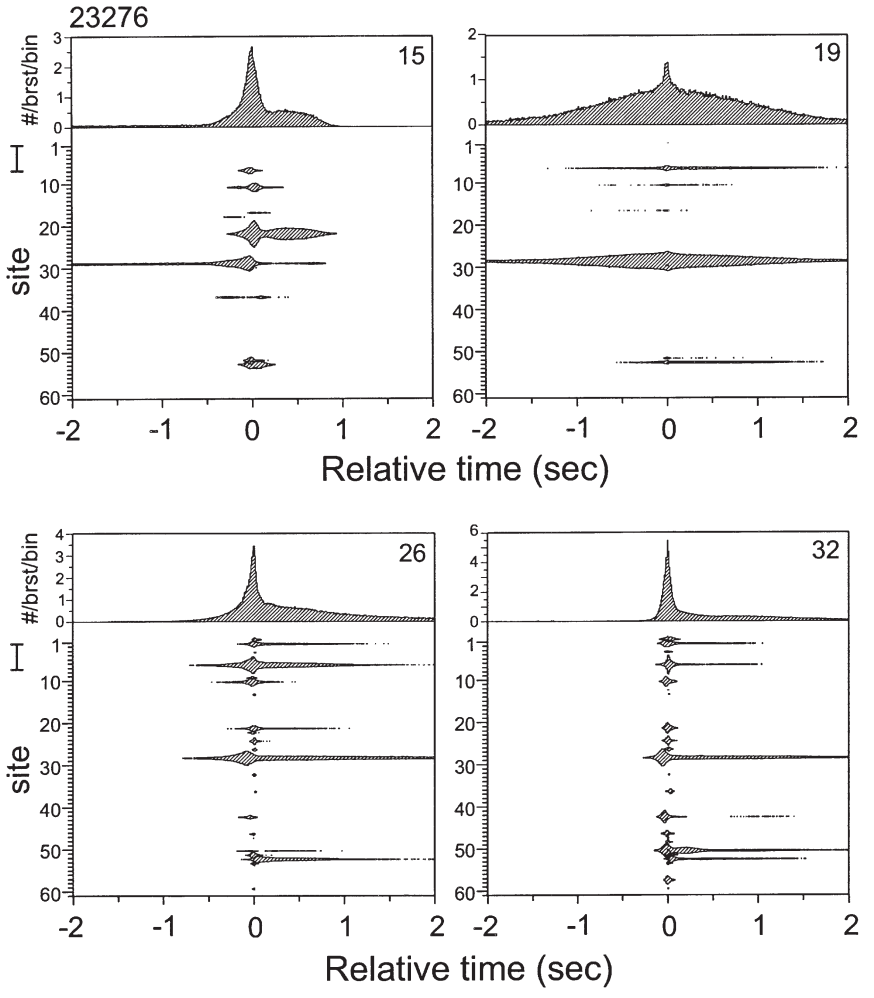

Fig. 5. Averaged network burst firing rate profiles at several time points during development of the cultured neuronal network. The network burst firing rate profiles in each of the panels were obtained by averaging the firing rate during time-aligned individual network bursts detected over a period of 4 h. Each panel displays (on the upper trace) for a time window of 4 s the averaged total network burst intensity as number of spikes per network burst per time bin (10 ms). The lower traces show the averaged firing rates at individual sites, with the scale bar to the left of the figure indicating a firing rate of 1 spike per site per network burst per time bin. The bars are plotted symmetrically around their horizontal axes (note that frequencies smaller than 0.02 spikes per site per network burst per time bin have been omitted from plotting). The figure illustrates the evolution of the firing rate profiles within network bursts, during the development of the neuronal network in vitro. (Adapted from Van Pelt et al. (2004a).)

maximal firing rate level. These differences are clearly illustrated in Fig. 7, in which three panels of Fig. 5 are plotted at a finer time scale, with each panel showing the average of network bursts detected during a recording period of 4 h. When compared with successive 4 h periods the profiles display only slight changes (Van Pelt et al., 2004a, b), but when compared over longer periods of development significant changes become apparent, such as recruitment of new active sites, silencing sites, changes in maximal firing rate, changes in the duration of firing. Also the temporal order of firing among individual sites may change but surprisingly, many neuron pairs maintain their temporal order of firing.

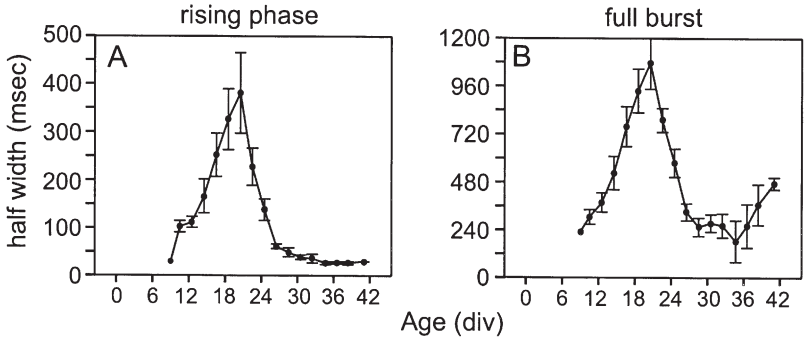

Fig. 6. Half-width of rising phase (A) and full network bursts (B) during network development in vitro. Half-width values are calculated from averaged network burst intensity profiles obtained by summation of all network burst encountered on each day. The rising phase half-width indicates the time difference between the time point at which the burst intensity profile reaches half-maximal values and the time point of maximal values. The full burst half width indicates the time difference between the time points at which burst intensity passes half maximal values in up- and downward direction, respectively. The figure summarizes the mean and SEM values per day for *five* longitudinal experiments. (Adapted from Van Pelt et al., 2004a.)

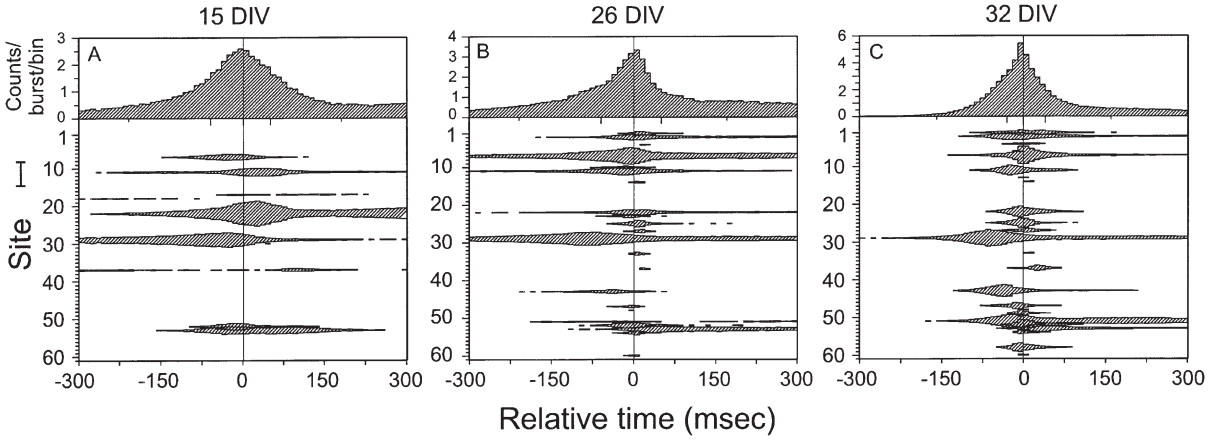

Fig. 7. A–C. Total firing rate profile and spatio-temporal organization of network bursts in preparation #23276 at (A) 15, (B) 26, and (C) 32 days in vitro. The figures are similar to those in Fig. 5 but plotted at a higher time resolution with a time window of 600 ms. For further legend information see Fig. 5. The figure illustrates how each site contributes to a network burst in a highly specific way, both in amplitude and time point of maximal firing, thus displaying a clearcut temporal order of firing. Panel C also illustrates the drastic shortening of the network bursts after about 4 weeks in vitro, while maintaining temporal order among the sites.

Network burst firing rate profiles thus reflect sensitively how individual neurons participate in the cooperative mode of firing. It is tempting to assume that these differences find their origin both in the excitability properties of the individual neurons and in their synaptic connectivity within the network. As has been emphasized above, total firing rate profiles show remarkable stability over short term periods and, indeed, this also concerns the temporal relationships of individual neuron firing within the network bursts. This also suggests stability in the routing of electric activity through the network, implicating stability in the connectivity in the network.

Correlation between structure and function in developing neuronal networks in vitro

By the end of the 3rd WIV neocortical cell cultures have passed through a period of delayed

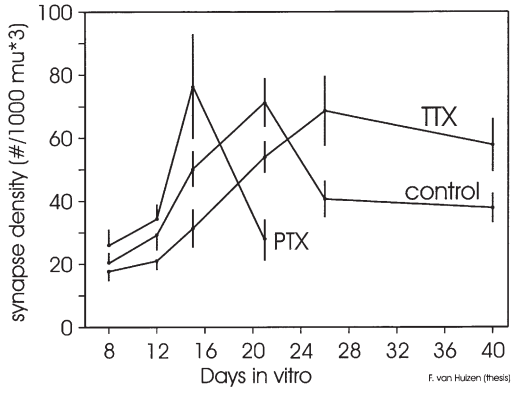

Fig. 8. Transient overproduction of synapse numbers during development of dissociated cerebral cortex cells in vitro. Chronic blockade of activity (TTX) largely prevents synapse elimination, whereas intensification (PTX) accelerates the elimination process. After Van Huizen (1986) (Van Huizen et al., 1985, 1987a, b; see also Van Pelt et al., 1996).

pharmacological development of synaptic inhibition relative to excitatory neurotransmission (Ramakers et al., 1994), and, in addition, have reached peak values for the numerical densities of dendritic spine as well as shaft synapses (putatively excitatory and inhibitory, respectively) (Van Huizen, 1985, 1986). The 4th WIV coincides with a "pruning" of dendritic spine, but not shaft, synapses to a much lower plateau level (see Fig. 8), further shifting the balance of synaptic excitation and inhibition strongly in favor of the latter.

It is remarkable that the time point of maximal synapse number appears to coincide with the time at which network bursts attain their longest durations, and that the period of strong reduction in synapse number coincides with the period in which network bursts develop extremely short rising phases. The morphological developments could contribute to these parallel physiological changes. For instance, GABAergic disinhibition of 3-week-old cortical cultures induces patterns of short intense bursting, characteristics for the very immature networks (Ramakers et al., 1990; Corner and Ramakers, 1992), thus suggesting that increasing network inhibition has the effect of prolonging the initially short but intense bursts. The simultaneous increasing density of excitatory (i.e., spine) synapses (Van Huizen et al., 1985) would be expected to prolong the burst durations even more. One may also speculate that the massive overproduction of synapses results in a network with an unstructured connectivity. Such networks could give rise to prolonged modes of cooperative firing and also to a degree of frustration in the internal propagation of activity. On the one hand, increasing connectivity will provide excitatory drive to an increased number of target neurons, resulting in a more widespread excitation in the network. On the other hand neurons will also receive inhibitory connections, which will oppose the unbridled circulation of activity. Unstructured divergence and convergence in the flow of activity might be one of the reasons for network bursts to be so prolonged.

In the subsequent period of synapse reduction, network connectivity may become tuned to the particular firing dynamics in the network through activity dependent mechanisms. This tuning process would eliminate the frustration in the firing dynamics, by adapting network connectivity so as to maximally support the flow of firing through the network with an optimized pattern of divergent and convergent connections. Such a tuned network then will show an increased efficiency in the spread of activity through the network, thus accounting for the rapid onset phase in network bursting. In this period, also the balance between excitation and inhibition will be changing since synaptic pruning primarily concerns the dendritic spine (excitatory), but not shaft (inhibitory) synapses (Van Huizen, 1985, 1986). The observation that in organotypic cortical explants the oldest cultures studied showed a pronounced lengthening, rather than shortening, of each burst upon exposure to GABAergic receptor blocking agents (Corner et al., 2002) suggests that, at this developmental time point, the changed balance between excitation and inhibition may also be involved in the abrupt termination of the bursts and the development of the short burst onsets.

Van Huizen (1986) showed that the pattern of overshoot in synapse numbers depends strongly on the state of activation of the network (Van Huizen et al., 1985, 1987a, b). Thus, chronically inhibiting GABAergic drive in the network resulted in an accelerated pattern of overshoot, whereas silencing the network resulted in a slower ascent to maximal synapse numbers, and the lack of a subsequent

pruning phase. Networks formed under these experimental conditions may be expected to display network burst patterns correlating with their altered connectivity, and such experiments should be able to test the above mentioned hypotheses.

Spike-time dependent synaptic plasticity — implications of temporal relationships within network bursts

One of the activity-dependent mechanisms underlying the reciprocal interaction between network structure and network activity is spike timing dependent synaptic plasticity (e.g., Zhang et al., 1998; Song et al., 2000). According to this mechanism, synapses strengthen when presynaptic firing precedes postsynaptic firing but weaken when the sequence is reversed. This "plasticity" mechanism thus implements causality, and could be an important mechanism for tuning the connections in the network to the temporal flow of firing. By this tuning process, the connectivity in the network comes to optimally support the patterns of network firing, resulting in increased stability of the dynamic modes of firing. If this mechanism operates from the earliest phase of network development, network connectivity would start to become tuned already from the first appearance of cooperative network firing, which might explain the already early stability of temporal patterning of firing within network bursts. It could also implicate that the network readily develops a principal connectivity pattern that is maintained during the phase of rapid increase in synapse numbers, and through which also a principal pattern in the dynamics is maintained despite the disruptive effects of the many unstructured connections being formed during this phase of massive overproduction of synapses. This could explain the observation that many neuron pairs maintain their temporal relationships of firing throughout the entire period of network maturation (Van Pelt et al., 2004a). (Such a view resembles the patterning of water channels when developing from an initially flat but tilted landscape. Upon first water drain, initial randomly formed microchannels readily grow when they are functional in the downward waterflow and by this positive feedback between waterflow and channel size will develop into major stable and efficient streams in the channel network).

Effect of stimulation on network burst spatiotemporal patterns of firing

All the data presented thus far concern spontaneous activity in the cultured networks and one may speculate that under these spontaneous conditions network structure and activity have developed in mutual consistency through activity-dependent mechanisms. This consistency is broken when the network is electrically stimulated, and one may expect that such stimulation would disrupt the stability of the network burst firing rate profiles. Preliminary experiments in 11 day old cultures indeed revealed differences in spontaneous network burst firing rate profiles measured immediately before and after a one hour stimulation session (Fig. 9). Some sites became silent, while others became active, and the rise time of the network bursts was strongly reduced, a phenomenon that under spontaneous conditions is expected to occur only after about 3 weeks in vitro. Clearly, after the stimulation session, network bursts have changed in a manner never observed under spontaneous conditions in such a short period of time.

The modulation of functional connectivity by patterns of electrical activity has been demonstrated by Marom and his group (Shahaf and Marom, 2001; Marom and Shahaf, 2002; and Marom et al., this volume), who found that the correlation of firing between neuron pairs became enhanced when a series of evoked patterns of activity was stopped whenever both neurons became activated. In a subsequent experiment, the number of stimulations needed to evoke a pattern with both neurons active, was strongly reduced. Apparently, stopping the stimulation after the last selected pattern allows the network to update the connections involved in that last pattern. It is remarkable that a single activity pattern has such a measurable "imprinting" effect on network connectivity. It actually suggests that network connectivity is continuously being subjected to modulating influences that stabilize under further quiescent conditions. The question arises as to what effect spontaneous network bursts have on network

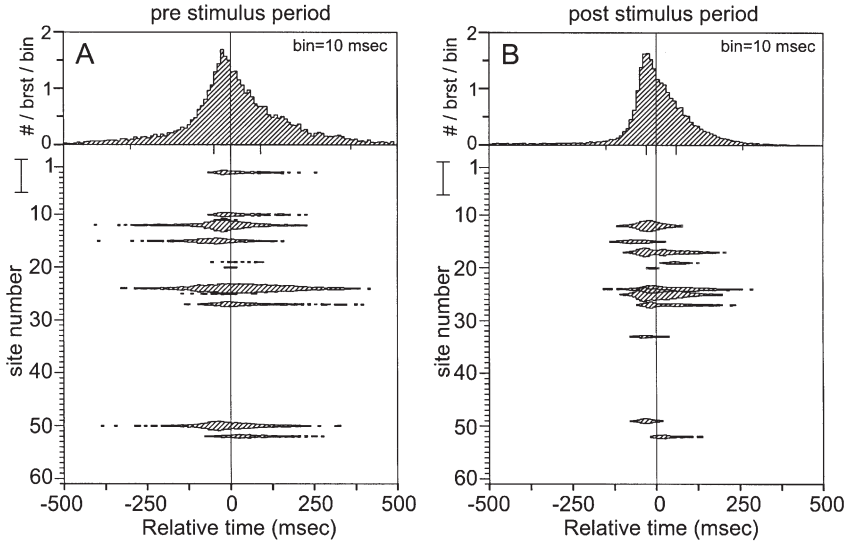

Fig. 9. Effect of electrical stimulation on spontaneous network burst firing rate profiles measured in cultures of 11 DIV. The firing rate profiles have been averaged over spontaneous network bursts detected during a 121 min period (A) before and a 70 min period (B) after a 90 min period of periodic electrical stimulation of one of the sites in the network. The firing rate profiles are the averages of (A) 377 and (B) 989 spontaneous network bursts. Changes induced by the stimulation concern the shortening of the leading edge from a rising phase half-width of 50–30 ms, a falling phase half-width of 90–60 ms, disappearing activity at sites 2, 10 and 50, and initiating activity at sites 17, 33, and 49 and changes in peak firing rates for several sites.

connectivity when such synchronized events are followed by periods of low level firing. One may speculate that the repetitive occurrence of spontaneous network bursts and the stable probabilistic structure of firing within them provide especially favorable conditions for tuning correlations of firing during these bursts into stable network connections.

Both the mechanisms of spike-timing dependent synaptic plasticity and the imprinting conditions of Marom indicate the putative importance of the spatio temporal firing patterns of individual bursts and, in particular, the stability of spike sequences. An impression of such spike sequences within network bursts is given in Fig. 10. Here, spikes were assigned to time bins of 10 ms, and sequence lines were drawn for all pairs of active sites between successive time bins (continuous lines; dotted lines for nonsuccessive time bins, when spanning empty time bins). Figures 10B–D illustrate the spike sequences as detected in the three network bursts of Fig. 4. Figure 10E illustrates for each site its most frequent sequence from one time bin to the next, as counted in all of the 227 network bursts detected in a period of 1 h at 15 DIV in preparation #23276. As expected, sequence numbers peak in the center of the burst, but the panel also shows the preferred sequences in the center area of the burst, where almost all sites have their most frequent sequence toward site 22, as well as the different pattern in the period just before, when sites can have their most frequent sequence also toward site 29. It is a topic of further research to develop the appropriate statistical framework for evaluating the observed sequence frequencies.

Functional role of spontaneous network bursts

In the foregoing, spontaneous network bursts have been given the functional role of increasing the consistency between network connectivity and patterns of firing. When such a consistency is broken after a period of strong stimulation, subsequent spontaneous network bursts could again exert this functional role such that the induced changes in connectivity and firing dynamics become integrated and a new consistency is being established. One might argue that weak stimulation induces only slight effects, which readily may be overruled by the

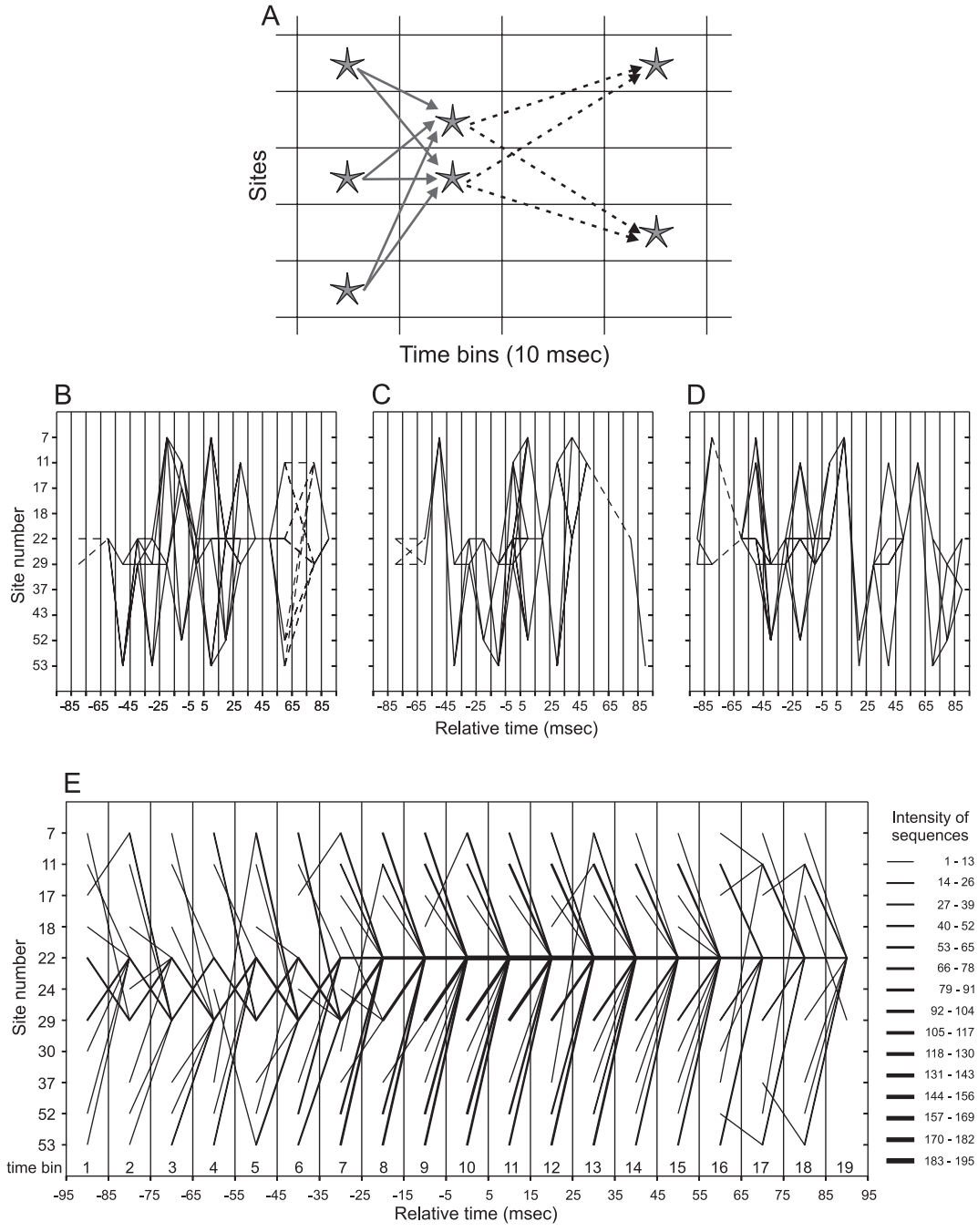

Fig. 10. Spike sequences within network bursts. (A) Schematic illustration of spike sequences in a single burst, defined as all possible sequences between spikes recorded in different time bins (of 10 ms) with continuous lines for sequences between adjacent time bins, and dotted lines for sequences between nonadjacent time bins, thus spanning silent time bins. (B–D) Spike sequences between successive time bins in three different network bursts aligned with their time center at relative time zero (10th time bin). The sequence plot is restricted to a time window of 195 ms around time center. (E) Number of sequences detected for all network bursts detected in a period of 1 h at 15 DIV in preparation #23276. For each active site only its most frequent sequence is plotted.

principal patterns of firing, thereby not changing the dynamic repertoire of firing. Such expectation can be tested by comparing network burst structure immediately after a period of stimulation and after a longer period of recovery. Preliminary experiments indeed tend to support this hypothesis.

Synchronized burst firing is a phenomenon not restricted to cultured dissociated neuronal networks but also occurring in organotypic slices and even in the intact nervous system. Especially during slow wave sleep spontaneous network bursting is a prominent recurrent phenomenon. It is tempting to assume that these network bursts play similar roles in enhancing consistency between network connectivity and dynamics after a period of wakefulness, when sensory experiences have broken network consistency. When spatio-temporal firing patterns in the intact brain are seen as memory entities this could support the idea that during slow wave sleep memories are consolidated by the recurrent spontaneous network bursts (e.g., Nadasdy, 2000; Steriade and Timofeev, 2003).

Summary

Network spike bursts are robust recurrent phenomena in spontaneous activity in dissociated cortical neuronal networks in vitro. They represent episodes of strong electrical interaction and synchronized firing of an increased number of neurons. Although the intrinsic spatio-temporal pattern of firing is highly variable among individual network bursts, the underlying probabilistic pattern appears to be highly stable over long periods of time. Gradual but significant changes have been shown during network development, indicating the sensitiveness of the spatio-temporal pattern of firing to structural changes in the network. Preliminary findings have indicated that the spatio-temporal profile of spontaneous network bursts can change significantly, when recorded immediately after a period of external stimulation. It is suggested that the stable temporal relationships of firing among neurons in network bursts provide optimal conditions for effectuating synaptic plasticity mechanisms, giving spontaneous network bursts an important functional role in enhancing consistency between network connectivity and its dynamic repertoire of firing.

Acknowledgments

Part of the work has been supported by the Earth and Life Sciences Foundation (grant #805-39.162) of the Netherlands Organization for Scientific Research and by the EU (grant # IST-2001-33564, Neurobit).

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