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Short communication

Long-term recording on multi-electrode array reveals degraded inhibitory connection in neuronal network development

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Abstract

Spontaneous neuronal activity plays an important role in development. However, the mechanism that underlies the long-term spontaneous developmental change of cultured neuronal networks *in vitro* is not well understood. To investigate the contribution of inhibitory and excitatory connections to the development of neuronal networks, dissociated neurons from an embryonic rat hippocampal formation were cultured on a multi-electrode array plate and spontaneous activities were recorded by multi-channel system. These spontaneous activities were compared to bicuculline-induced firings, which were recorded by 60 electrodes simultaneously from 1 to 14 weeks *in vitro* (WIV). The phenomena showed that the spontaneous firing activities changed from an initial pattern of synchronized bursts to a later pattern of high frequency random spikes. The bicuculline-induced firing activities transformed from a pattern of synchronized bursts throughout all active sites in 3 WIV, to a pattern of local synchronized or random spikes appearing in the intervals of synchronized bursts after 11 WIV, while the firing rate hardly changed. Kynurenic acid, a broad-spectrum glutamate receptor antagonist, blocked all activities while CNQX inhibited only the local synchronized or random spikes. These suggest that the inhibitory connection was age-dependent degraded *in vitro* and the developmental spontaneous firing pattern was built by the homeostatic balance of the excitatory-inhibitory connection networks. Long-term cultures on MEA provided a useful tool to measure the relationship between spontaneous developmental change and pharmacological influence *in vitro*.

Keywords: Neuronal network; Development; Multi-electrode array; GABA-A receptor

1. Introduction

The maintainance of brain functions depends on spontaneous neuronal activities, which play a pivotal role in many aspects of neural development, including neuronal migration, differentiation, and connection patterning (Goodman and Shatz, 1993; Yvert et al., 2004; Van Pelt et al., 2005). The changes in spontaneous activities during development of the central nervous system have been investigated considerably *in vivo* and *in vitro* (Ritter and Zhang, 2000; Legrand et al., 2004; Pierrefiche et al., 2004; Wong et al., 2005). Random spikes and correlated burst activities that have been detected in hippocampal slices and cultures strengthened the idea that particular firing patterns are features of specific periods during postnatal development (Hiroyuki Kamioka et al., 1996). Nevertheless, the mechanism that underlies these developmental changes remained poorly understood.

Synchronous bursts can be induced in cultures of dissociated cells from an embryonic rat hippocampus by disinhibition through blocking GABA-A receptor, which is a major functional receptor of GABA in central nervous system (Papatheodoropoulos and Kostopoulos, 2002). In the developing hippocampus, inhibitory connections are thought to form and mature sequentially in various ways. For instance, GABAergic synapses can switch from GABA-mediated depolarization to hyperpolarization (Ritter and Zhang, 2000; Fujiwara-Tsukamoto et al., 2004; Lujan et al., 2005). However, the contribution of inhibitory connection to changes in the firing pattern during long-term development *in vitro* is unknown.

To monitor the developmental changes in the neuronal firing pattern, we utilized a multi-electrode array (MEA) that could continuously monitor the spontaneous activities of neuronal

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networks over multiple weeks (Egert et al., 1998; Potter and Demarse, 2001; Van Pelt et al., 2005). Such a simple model system provides an efficient platform to reveal the cellular mechanisms underlying the developmental phenomena and to facilitate the evaluation of pharmacological test substances. To explore the contribution of inhibitory and excitatory connections during development, we used multi-site recordings on cultured hippocampal neuronal networks, and compared bicuculline-induced firing patterns with their spontaneous counterparts. This approach enabled us to track the developmental changes in the firing pattern that are dependent on inhibitory connections in the whole synaptic network rather than in a limited sample of paired cells.

2. Methods and materials

2.1. Cultured neuronal networks

The dissociated hippocampal neuronal network was cultured, according to a modified version of the protocol described elsewhere (Potter and Demarse, 2001). In brief, hippocampal tissues were taken from an embryonic day (E) 17–18 Wistar rat and dissociated by trituration after digestion with 0.125% trypsin (Gibco). Cells were plated on laminin and poly-D-lysine (Sigma) coated MEA with a density of 1×10^7 cells/mm^2. The culture medium consisted of Dulbecco's modified Eagle's medium (DMEM) (Gibco) containing 10% heat-inactivated horse serum (Hyclone), and 2 mM Glutamax (Gibco). Semi-medium was exchanged twice a week. Recordings were carried out after 3–5 days *in vitro* (DIV).

2.2. Pharmacological protocols

Each measurement was comprised of three recordings, control, test substance and second control, in order to exclude possible systematic baseline trends in electrophysiological activity during long-time measurements. After recording, bicuculline was washed away by changing the medium three times.

2.3. Recording system

Electrical activities were recorded with a square array of 60 substrate-embedded titanium nitride electrodes, 30 μm in diameter, with 200 μm spacing (Multi Channel Systems, Germany). After 1200× amplification, signals were sampled at 50 kHz using a multi channel systems data acquisition card, controlled through MC_Rack software. The threshold for spike detection for each channel was set to five standard deviations (S.D.s) of average noise amplitude during 500 ms at the beginning of each measurement.

Activities recorded by microelectrode arrays have typical amplitudes in the range of 30–200 μV. Each signal was the result of a time-spatial summation of the activities from a few cells, so it could provided information of the network 'unit' related to that recording site. No attempt was made here to discriminate or sort spikes collected by one electrode.

2.4. Data analysis

The threshold for spike detection for each channel was set to five standard deviations of average noise amplitude during 500 ms at the beginning of each measurement. The spontaneous spike rate was averaged over the whole recording time (300 s), and the mean value was averaged over all electrodes. Burst analysis was executed by Neuroexplorer software, designed with 100 ms as the max interval to start or to stop, three spikes as the minimum in one burst. Cross-correlogram was calculated from 300 s of spikes recorded from single electrode to another one from the same MEA, designed with 5 ms as the bin. For comparisons between experimental stages, a cross-correlation constant was designed as the maximum of the cross-correlogram, and was executed by the Neuroexplorer software.

3. Results

Hippocampal neurons began neuritic outgrowth within several hours after plating and, within a few days, formed a neuronal network with numerous functional synaptic connections. Spontaneous activities became obvious as early as 5 DIV and were recorded from 5 to 90 DIV. Fig. 1 shows the spontaneous spikes measured simultaneously in 60 electrodes at 15 DIV, and the inset shows details on spikes and bursts. Firing varied much among different sites of the network. For instance, at some sites the firing occurred in random spikes with small amplitudes, while at the other sites it occurred in spikes and bursts. Comparisons of the timing of the spikes and bursts recorded from different electrodes on the same MEA revealed that the network fired in local synchrony as shown in Fig. 1.

To access the changes in firing patterns in cultured neuronal networks during bicuculline treatment, we compared spontaneous activities with activities during pharmacological treatment. The spontaneous firing rate and amplitude was stable during the short-term (300 s) (Fig. 1). Removal of GABAergic inhibition with bicuculline led to synchronized bursts throughout all active sites. Comparison to the timing of the bursts from different sites suggested that there was a very small variability in the burst frequency value among the electrodes treated with bicuculline. As illustrated by the cross-correlograms in Fig. 1, there was a high correlation between electrodes in the presence of the drug. The spike frequency was 18.9 ± 0.4 Hz in comparison to the control frequency of 2.4 ± 0.2 Hz (Fig. 1F). Under the disinhibitory treatments, there was a high variability in spike rates over the whole network, proving that these drugs actually changed the status of the network, causing perturbations in firing activity. There were some specific style oscillations in the firing, shown as little wave crests in the auto-correlograms around the maximum value. At the same time, the amplitude of spikes increased from 23.1 ± 0.3 to 52.4 ± 0.5 μV, indicating that the gain in spike rate did not result from splitting the intrinsic spikes.

To detect developmental changes in firings, the spontaneous activities were recorded during three developmental stages of the cultured neuronal networks, respectively. In Fig. 2, the examples show the activities recorded from four electrodes of one MEA. During 3 WIV, highly synchronized bursts on all