ELECTRICAL STIMULATION CAN INDUCE CHANGES IN DYNAMICS OF A SPINAL CORD NEURONAL NETWORK CULTURED ON MICROELECTRODE ARRAYS

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Abstract-**The spontaneous electrophysiological activity of neural networks seems to play an important role in the Central Nervous System (CNS) developing, subsequent maturation and learning. Learning a new behavior is an exploration process that involves the modulation and the formation of association set between stimuli and responses. Here we analyze how the electrophysiological activity of cultured spinal cord neurons (14 DIV) from the chick embryo is affected by electrical stimulation. Active neurons show a typical high frequency activity pattern called burst. Induced changes in the patterns of electrophysiological activity are described.**

Keywords **- microelectrode array, spinal cord network, burst activity, electrical stimulation, network dynamics.**

I. INTRODUCTION

It is well known that neuronal networks in the developing spinal cord are spontaneously active. Evidence is also accumulating that such activity plays an important role in the maturation of the networks [5].

Cultured spinal neurons from the chick embryo were chosen as a neurobiological system quite appropriate for long-term recording.

In this paper we analyzed the signals generated by neurons dissociated from the embryo spinal cord in terms of electrophysiological activity, by taking advantages of arrays of planar microelectrodes [6].

Two main features made microelectrode arrays (MEAs) a valuable tool for electrophysiology [2], namely: a) they were non-invasive and therefore, under appropriate conditions, they could register the electrophysiological activity of neurons for a long period of time (i.e., from several minutes up to several hours) and b) allowed a multi-site recording [7]. Electrical activity from the network was simultaneously recorded by 8 electrodes. Thus it was possible to analyze changes induced by the electrical stimulation and to correlate the activity between different channels (i.e., different area in the neural network), in order to define the dynamics of the network.

Electrophysiological rhythmic activity mostly ranged from asynchronous spiking to organized patterns in the form of "bursting". A burst was characterized by a rapid sequence of several spikes separated from each other by a few ms (Inter Spike interval, ISI) and the interval among bursts was in the scale of seconds or even minutes. In other words, from the point of view of the signal patterns, we dealt with time sequences where episodes in the range of seconds (bursts) were separated by periods of silence in the tens of seconds range (Inter Burst interval, IBI).

It should be underlined that, because of a number of sources of biological variability, the signals resulting from one specific experiment show a similar pattern but not a one to one reproduction of other experiments. The main aim of this work was to show that was possible to reversibly modify the neurons spatio-temporal dynamics by electrically stimulating the neural network.

II. METHODOLOGY

Dissociated neurons were obtained from the spinal cord of chick embryos after 7 – 8 days of incubation, according to a modified version of the protocol described in [4]. Neurons were seeded on microelectrode arrays covered with adhesion promoting molecules (Polylisyne, laminin).

Electrophysiological signals were recorded after 20 days in vitro (DIV), which need to allow the formation of synaptic contacts among the cells.

A. Experimental protocol

In order to investigate the role of the activity-dependent modification of synaptic strength in the developing Central Nervous System, an electrical stimulation protocol was used (Jimbo and Kawana, 1998) [8]. The experimental protocol consisted of the following steps:

1) Control Condition, corresponding to the spontaneous activity in culture medium (Neuro Basal medium, Sigma).
2) Electrical Stimulation Test, single pulse stimulus (amplitude: 0.3-0.1V, lasting 100μsec) delivered for 2 minutes at 0.5Hz.
3) Post Electrical Stimulation Control Condition.
4) High K^+ Condition, corresponding to the activity under the effects of K^+, added at the final concentration of 9mM.
5) Resting, the network was left 2 hours in the incubator to verify healthy condition of neurons.
6) Post Incubator Control Condition.
7) Tetanic Stimulation, delivered by trains of stimuli (each ones of 0.3 - 0.1V, lasting 100μsec, at 20Hz) each train at 0.2Hz.
8) Post-Tetanic Stimulation Control Condition.

B. Microelectrode Array and measurement system

The array used for our experiments was made of glass with 60 gold microelectrodes. An experimental set up, based on the microelectrode array and constituted by the following functional elements, was developed:

1) Microelectrode array, which was itself an interface between the biological and the electrical environment;
2) Faraday Cage, to avoid electromagnetic interference;
3) 8 channels amplifier and filtering stage (gain=1000);
4) Long term acquisition instrumentation: Digital Tape Recorder (BioLogic DTR-1802) with a maximum of 8 recording channels at the sampling frequency of 12kHz and GPIB Interface. Each channel was connected to the MEA via the amplification and filtering system. For an easier identification, we named them according to the labels on the DTR display: L1, L2, L3, L4, R1, R2, R3, R4.
5) Oscilloscope for real time monitoring of signals;

6) System for network electrical stimulation (MUX, Stimulating Interface, and Isolator);
7) PC for data management, equipped with National Instruments(TM) AT-MIO device, used for generating electrical stimuli.

C. Signal Processing

Signals collected from a microelectrode array had typical amplitudes in the range of 0.1-0.4 mV and were embedded in biological and thermal noise ranging from 10μV up to 40μV peak to peak. To extract spike features we processed data by an ad hoc algorithm of peak-detection, which recognized a potential of action when the difference between the maximum and the minimum, of the considered window (bins of 5 msec.) of the signal, was higher than peak-to-peak set threshold. Moreover, to investigate burst patterns [1], we developed an algorithm for their automatic detection, utilizing pre-processed data by the peak-detection algorithm. A window was shifted along the processed signal and when a spike cluster was detected a graph was shown to the user. This graph represented the time of occurrence of spike clusters and their amplitudes in arbitrary units, which represented the sum of spikes amplitudes that belong to the same cluster.

While in spiking-processing we extracted the number of spikes vs. bins, the extracted features for each burst detected were the following:

- Time of occurrence (msec)
- Burst Duration (msec)
- InterBurst Interval – IBI (msec), defined as the time length between the end of a burst and the beginning of the next one.
- Burst amplitude (arbitrary units)

III. RESULTS

Chicken spinal cord dissociated neurons were cultured on the substrates and the experiment was done after 14 DIV (days in vitro) cultures.

Firstly we processed the spontaneous activity, control condition, by means of a bursting (fig.1, 2,3) and ISI (fig.4, 8,9) analyses. This was a useful step to define a reference state for further analyses.

It should be observe that the electrophysiological pattern in control condition was about the same for all the channels: bursts, lasting about 5,6 seconds, characterized by about 26,6 seconds of IBI. Synchronization of network could be appreciated observing the ISI behaviors (fig.4), which were about the same for each channel and shown that two spike were mostly time separated by less than 10 ms.

After the electrical stimulation phase, the spinal neurons responded with significantly different signals, in which bursting seemed to be suppressed (fig.5, 8,9), as shown by Jimbo et al. [9].

A high concentration of K^+ was added to culture medium. This was done to block intra cellular K^+ natural flux to the extra cellular sites and to increase the excitability of network depressed by electrical stimulation. Initially the network continued post stimulus behavior, while after two hours of incubation it was observable that the spinal neurons delivered a spontaneous activity (fig.1, 2,3) characterized by IBI of about 27,6 sec, higher (fig.3) than control condition one, with burst duration of about 7.4 sec (fig.2). In this experimental phase, the spontaneous activity was higher than the previous one: neurons seemed to recover their synchronized spontaneous activity, in term of burst duration (fig.2) and ISI (fig.6), but some changes inside burst could be observed: ISI was now mostly ranged between 5 and 7,5 msec., as shown in fig. 8, 9. Moreover it could be observable that the new state was characterized by more and longer burst (fig.1, 2) with lower IBI (fig.3) than control condition.

The tetanic phase shown that the electrical stimulation induced a new depression of activity, both in terms of bursting (fig.1, 2,3) and spiking (fig.7, 8,9), which was less drastic than previous, post electrical stimulation, depression.

IV. DISCUSSION AND PROSPECTS

In this study we had examined how a large number of synaptic pathways were affected by electrical and tetanic stimulation, with the aim to characterize how a complex spinal cord network could respond to an external input and whether learning would occur. Our result gave some insight into the changes that happened inside the network, in particular we found that electrical stimulation produced long lasting changes in the neurons, which were measured as changes in the number of extra cellular spikes recorded from the system.

First of all we observed that the spontaneous activity of network shown a high level of synchronization (fig.4).

After the electrical stimulation, the network changed behavior, in which bursting seemed to be depressed (fig.1, 5).

This could be explained, in the activity-dependent modification terms as a LTD (Long-Term-Depression) [8]. Another possibility could be that electrical stimulation produced totally release of synaptic neurotransmitters. After the incubation, network increased its activity (fig.1, 2,3,6). This fact could validate the hypothesis of high release of neurotransmitters and confirmed that electrical stimulation produce a long lasting but reversible effect on neural network dynamics. The tetanic stimulation induced depression of activity, but network was still synchronized in terms of events (fig.7). This depression was less drastic than previous one, so it was possible to note that network was delivering stimulation frequencies sensitive; in other words it seemed to mean that it could be possible to induce different kind of activity-dependent changes according the exposed frequencies of electrical stimulation. The difference among these responses and the frequencies of these patterns (the IBI between patterns and ISI, between events, inside patterns) represented the current state of the network, so these behaviors could underline whether learning was occurring and how the network was encoding new features.

Moreover we observed that in all the experimental phases the network shown time synchronized activity among all considered sites. It seemed to mean that induced, by the electrical stimulation, modifications were not a local effect, but referred to a complex spatio-temporal network dynamics.

As very recently suggested in the literature [11,3,10], this kind of approach will open new opportunity for study in learning in-vitro and for using bio-artificial neural networks as new possible information processing tool [10].

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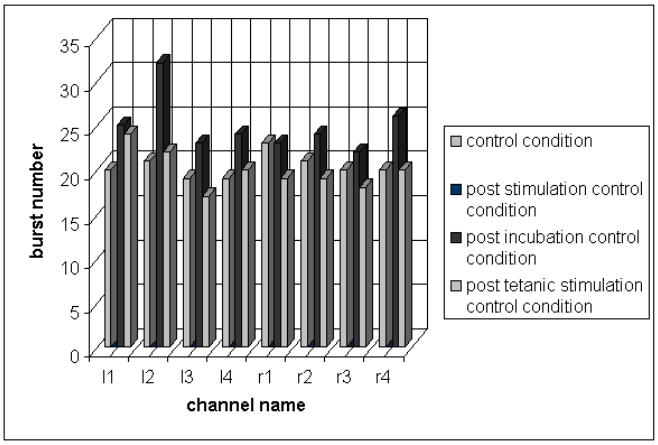

Fig.1 Electrical stimulation changed network activity in term of bursts.

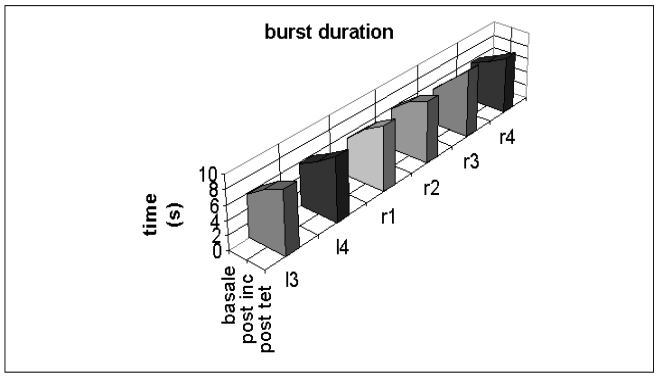

Fig.2 During the three phases observed the burst duration increased.

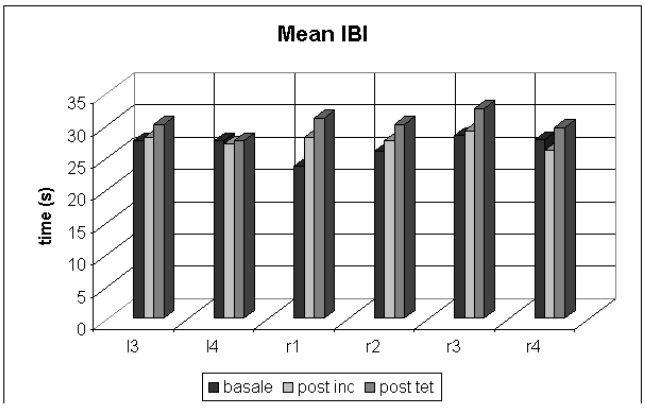

Fig.3 Mean Inter Burst Interval indicated the intensity of network activity.

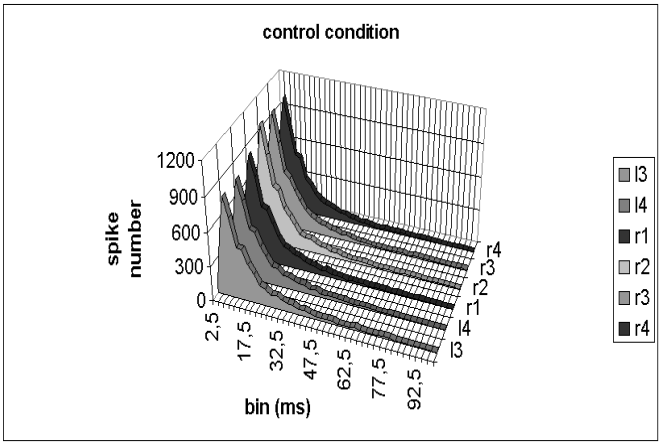

Fig.4 Inter Spike Interval histogram defined the level of the synchronization in the network behavior.

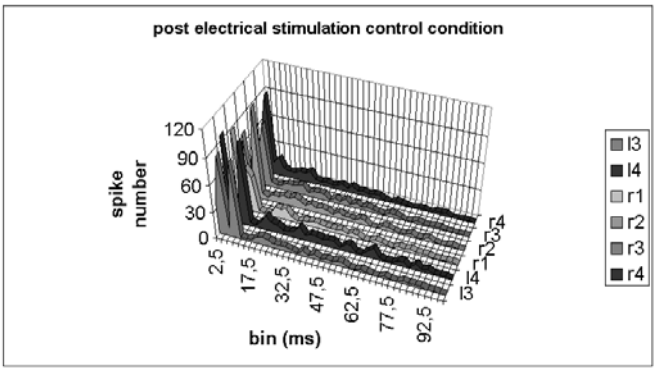

Fig.5 After the electrical stimulation network activity decreased and ISI histogram became different from control condition one.

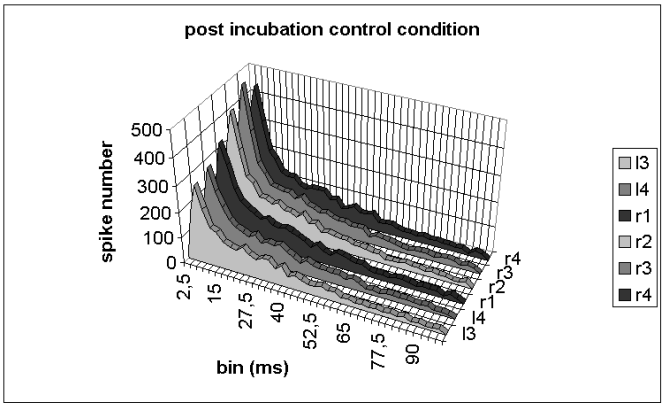

Fig.6 After two hours of incubation the spinal neurons delivered spontaneous activity with different level of synchronization observable by a different shape of ISI histogram.

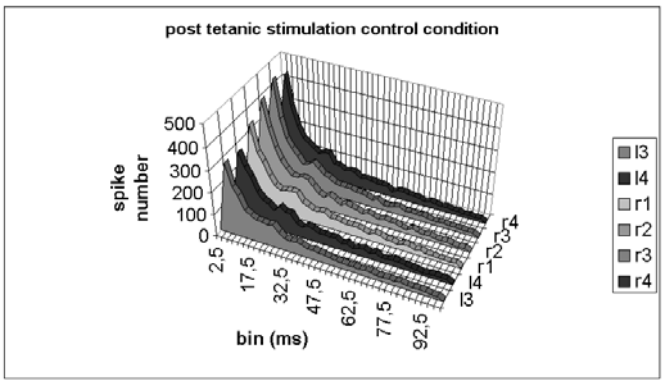

Fig.7 After tetanic stimulation the shape of ISI histogram was similar to post incubator one.

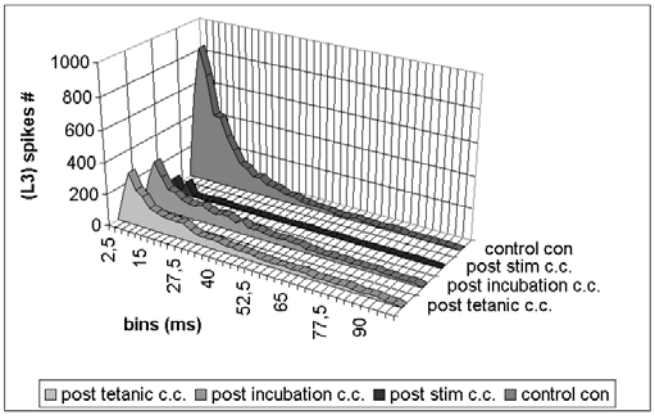

Fig.8 During the experiment, ISI histogram shape changed in each channel.

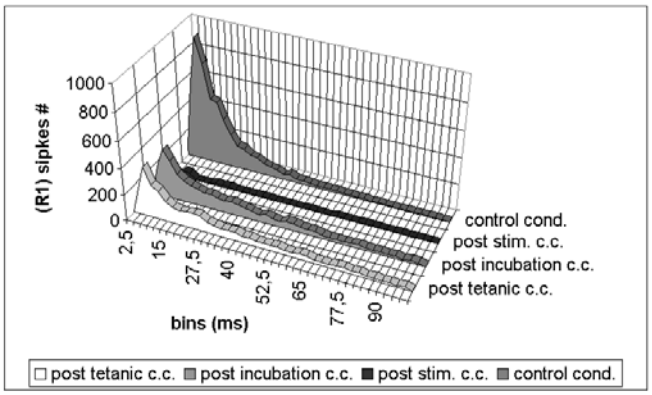

Fig.9 Channel R1 ISI histogram trend was similar to channel L3 one.