BEHAVIORS FROM AN ELECTRICALLY STIMULATED 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 are chosen as a neurobiological system quite appropriate for long-term recording.

In this paper we analyze 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 make microelectrode arrays (MEAs) a valuable tool for electrophysiology [2], namely: a) they are non-invasive and therefore, under appropriate conditions, they can register the electrophysiological activity of neurons for a long period of time (i.e., from several minutes up to several hours) and b) allow a multi-site recording [7]. Electrical activity from the network is simultaneously recorded by 8 electrodes. Thus it is possible to analyze changes induced by the electrical stimulation and 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 ranges from asynchronous spiking to organized patterns in the form of "bursting". A burst is 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 is in the scale of seconds or even minutes. In other words, from the point of view of the signal patterns, we deal with time sequences where episodes in the range of seconds (bursts) are 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 is to show that is 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 is 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 is 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 is 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 is 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(™) AT-MIO device, used for generating electrical stimuli.

C. Signal Processing

Signals collected from a microelectrode array have typical amplitudes in the range of 0.1-0.4 mV and are 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 recognizes a potential of action when the difference between the maximum and the minimum, of the considered window (bins of 5 msec.) of the signal, is 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 is shifted along the processed signal and when a spike cluster is detected a graph is shown to the user. This graph represents the time of occurrence of spike clusters and their amplitudes in arbitrary units, which represent 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 are 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 is a useful step to define a reference state for further analyses.

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

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

A high concentration of K^+ is added to culture medium. This is 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 continues post stimulus behavior, while after two hours of incubation it is observable that the spinal neurons deliver 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 is higher than the previous one: neurons seem to recover their synchronized spontaneous activity, in term of burst duration (fig.2) and ISI (fig.6), but some changes inside burst can be observed: ISI is now mostly ranged between 5 and 7,5 msec., as shown in fig. 8, 9. Moreover it may observable that the new state is characterized by more and longer burst (fig.1, 2) with lower IBI (fig.3) than control condition.

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

IV. DISCUSSION AND PROSPECTS

In this study we have examined how a large number of synaptic pathways are affected by electrical and tetanic stimulation, with the aim to characterize how a complex spinal cord network respond to an external input and whether learning will occur. Our result gives some insight into the changes that happen inside in the network, in particular we find that electrical stimulation produces long lasting changes in the neurons, which are measured as changes in the number of extra cellular spikes recorded from the system.

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

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

This can be explained, in the activity-dependent modification terms as a LTD (Long-Term-Depression) [8]. Another possibility might be that electrical stimulation produces totally release of synaptic neurotransmitters. After the incubation, network increases its activity (fig.1, 2,3,6). This fact can validate the hypothesis of high release of neurotransmitters and confirm that electrical stimulation produce a long lasting but reversible effect on neural network dynamics. The tetanic stimulation induces depression of activity, but network is still synchronized in terms of events (fig.7). This depression is less drastic than previous one, so it is possible to note that network is delivering stimulation frequencies sensitive; in other words it seems to mean that it may 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) represent the current state of the network, so these behaviors may underline whether learning is occurring and how the network is encoding new features.

Moreover we observe that in all the experimental phases the network shows time synchronized activity among all considered sites. It seems to mean that induced, by the electrical stimulation, modifications are not a local effect, but refer 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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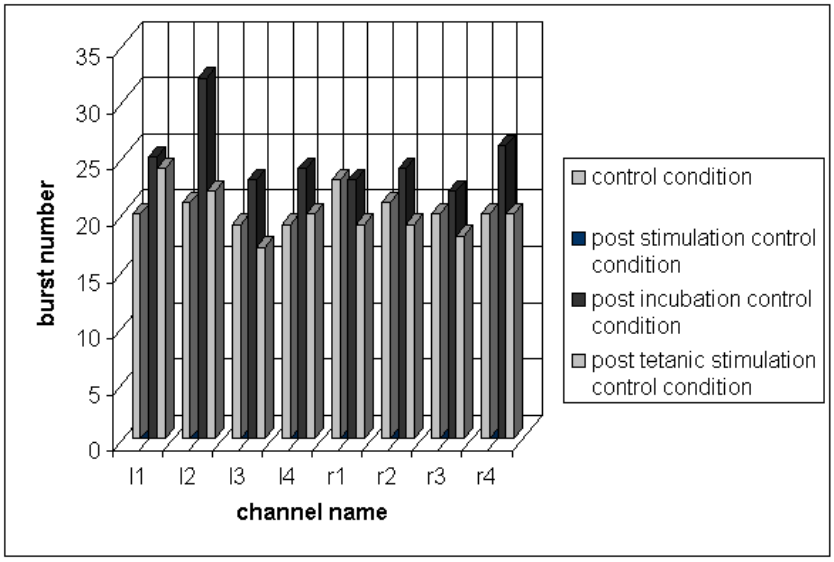

Fig.1 Thanks to electrical stimulation is possible to change the network activity in term of number of bursts.

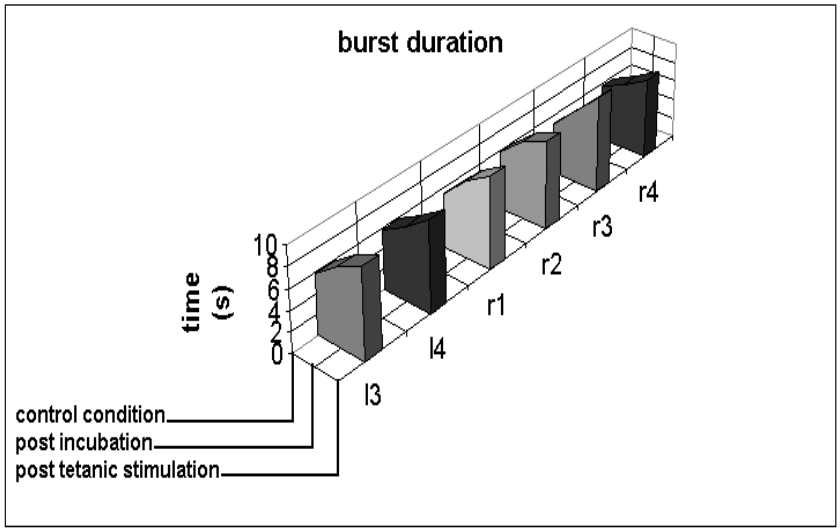

Fig.2 During the three phases observed the burst duration increases.

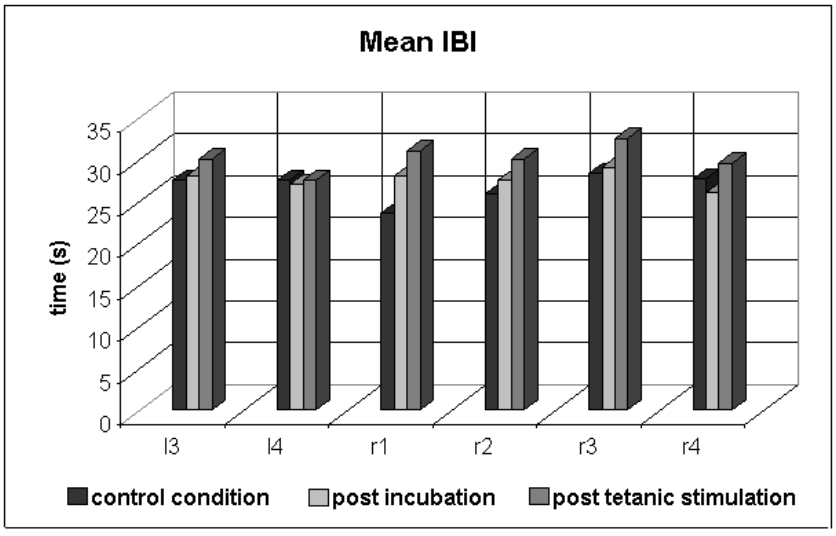

Fig.3 The Mean Inter Burst Interval is a parameter to indicate the intensity of network activity.

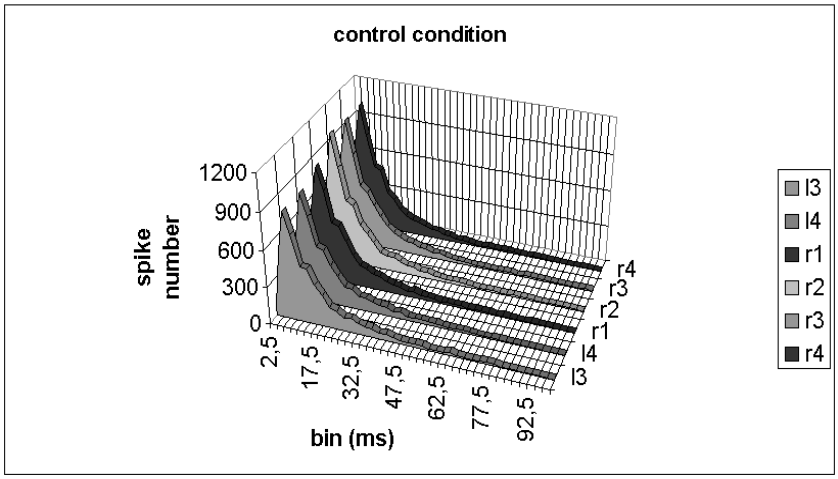

Fig.4 The Inter Spike Interval histogram is useful to define the level of the synchronization in the network behavior.

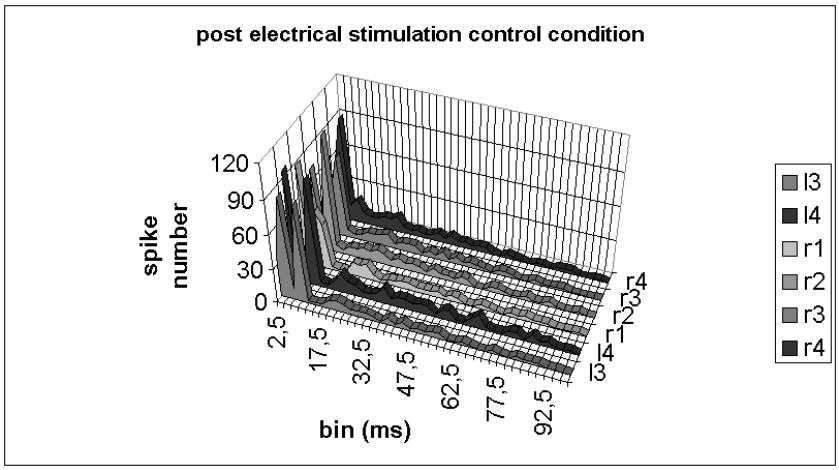

Fig.5 After the electrical stimulation the activity decreases and the ISI histogram becomes very different from the control condition one.

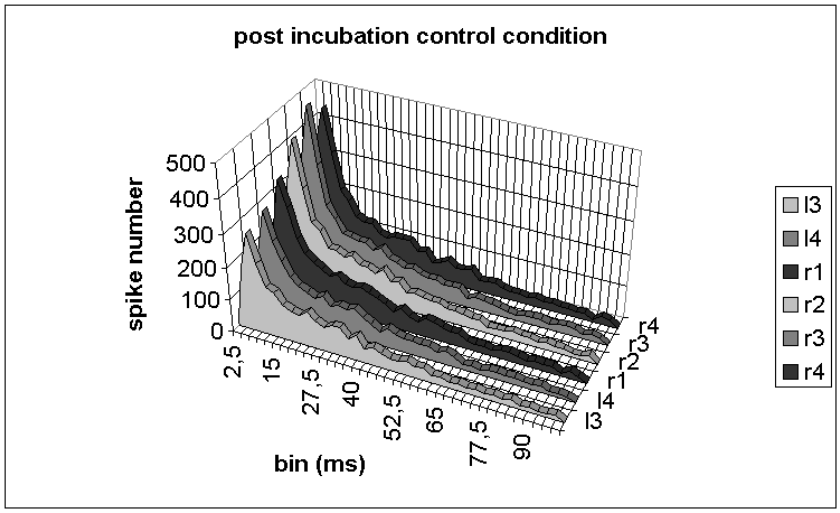

Fig.6 After two hours of incubation the spinal neurons deliver a spontaneous activity with a different level of synchronization observable by a different shape of the ISI histogram.

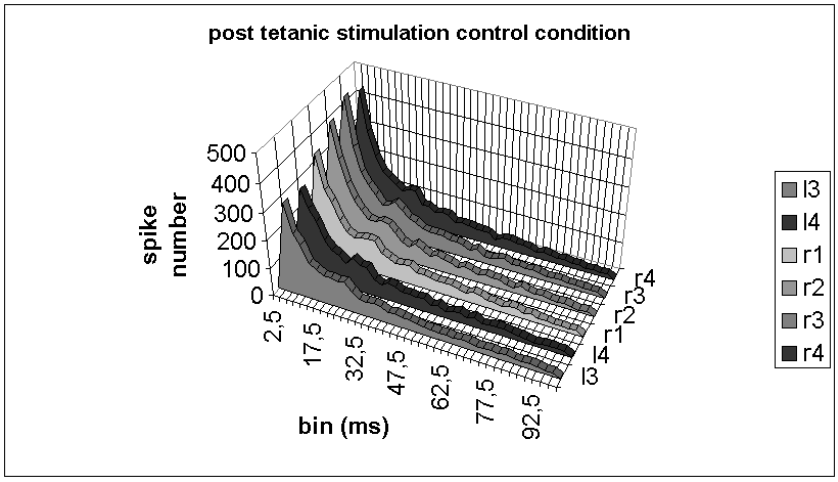

Fig.7 This figure shows that after the titanic stimulation the shape of the ISI histogram is very similar to the post incubator one.

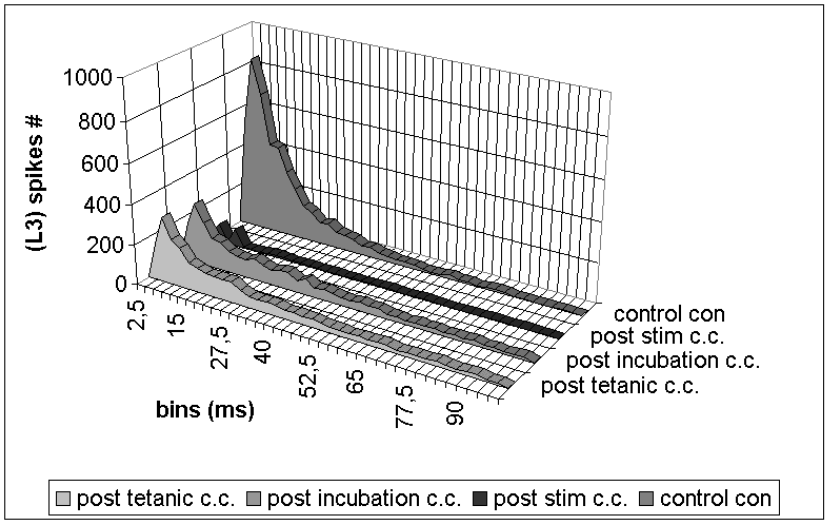

Fig.8 During the experiment the shape of the ISI histogram changes in all channels.

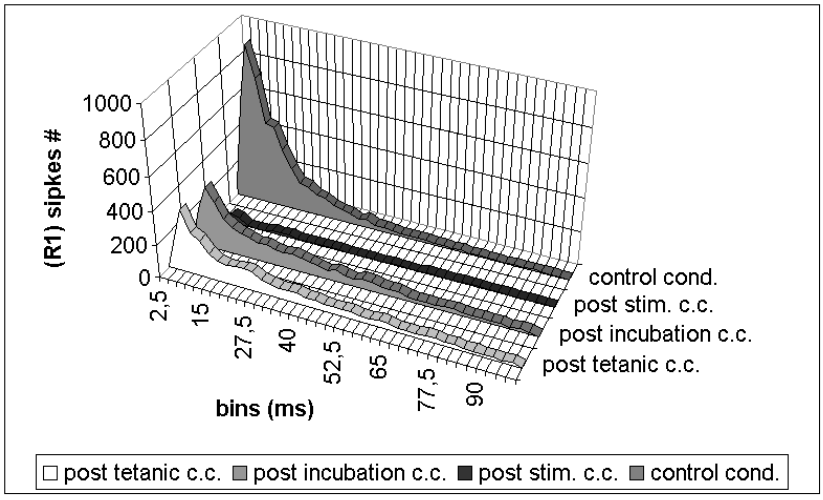

Fig.9 The ISI histogram trend in the channel R1 is very similar to the channel L3 one