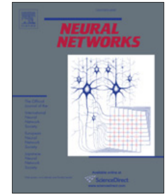
Neural Networks 21 (2008) 1232–1237

Contents lists available at ScienceDirect

Neural Networks

journal homepage: www.elsevier.com/locate/neunet



Spatio-temporal motifs 'remembered' in neuronal networks following profound hypothermia

Liel Rubinsky [a,*], Nadav Raichman [b], Jacob Lavee [c], Hanan Frenk [a], Eshel Ben-Jacob [b]

[a] Department of Psychology, Tel-Aviv University, Tel-Aviv 69978, Israel
[b] School of Physics and Astronomy, Raymond & Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Tel-Aviv 69978, Israel
[c] Department of Cardiac Surgery, Sheba Medical Center, Tel Hashomer 52621, Israel

ARTICLE INFO

Article history:
Received 1 November 2007
Received in revised form
6 March 2008
Accepted 14 June 2008

Keywords:
Low temperature
Neuronal cultures
Microelectrode arrays
Synchronized bursting events

ABSTRACT

Surgical procedures using hypothermic temperatures have been linked to complications such as seizures, impaired mental development and impaired memory. Although there is some evidence that the profound hypothermia (<12 °C) used in these procedures may be contributing to these neurological impairments, skepticism remains because of lack of evidence from experimental studies isolating the effects of hypothermia on neuronal networks. In order to attain a better understanding of profound hypothermia effects on neurons during surgical procedures, we applied cold to a cultured *in-vitro* neuronal network. The typical pattern of activity of such cultures is in the form of synchronized bursts, in which most of the recorded neurons fire action potentials in a short time period. In most cases, the bursting activity shows one or more repeating precise spatio-temporal patterns (motifs) that are sustained over long periods of time. In this experimental study, neuronal networks grown on microelectrode arrays (MEA) are subjected to profound hypothermia for an hour and the collective dynamics of the network as a whole are assessed. We show, by using a similarity analysis that compares changes in the time delays between neuronal activation at different burst motifs, that neuronal networks survive total inhibition by profound hypothermia and retain their intrinsic synchronized burst motifs even with substantial generalized neuronal degeneration. By applying multiple sessions of cold, we also show a marked monotonic reduction in the rate of burst firing and in the number of spikes of each neuron after each session.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Low temperatures are a common tool used to protect the brain during traumatic injury, patient recovery and surgical procedures (Kumral et al., 2001; McIntyre, Fergusson, Hebert, Moher, & Hutchison, 2003; Sessler, 2001). Their specific therapeutic effects in reducing cerebral injury associated with hypoxia, ischemia, inflammatory and traumatic injury are well documented (Barone, Feuerstein, & White, 1997; Muller et al., 2004).

In particular, profound hypothermia (temperatures below 15 °C) have played a preferential role in protection of the brain during many cardiovascular surgical procedures (Deleon et al., 1998; Gillinov et al., 1993). However, there is a controversy as to the possible detrimental effects with the use of profound hypothermia on cortical functions following these surgeries (Deleon et al., 1998). Although many studies validate neuroprotection by profound hypothermia during surgical situations (Gillinov et al., 1993; Haka-Ikse, Blackwood, & Steward, 1978; Sailhamer et al., 2007), other studies show significant neurological complications such as impaired mental and memory development in patients undergoing surgeries using such low temperatures (Deleon et al., 1998; Khaladj et al., 2006). Several studies link these neurological complications to swelling, cytoplasmic voculation and cytoskeletal disruption of the neurons caused by hypothermia (Emery & Lucas, 1995).

Nevertheless, identifying profound hypothermia's effects on neurological functions *in-vivo* has been difficult (Deleon et al., 1998), suggesting a need for a better controlled experimental system that allows the isolation of hypothermia as a variable.

In order to overcome these difficulties and assess the effect of profound hypothermia on neurons and neuronal networks, we have developed an *in-vitro* system that allows the study of the effects of low temperature on neuronal cultures (Rubinsky et al., 2007). In our experimental setup we cultivate neuronal networks on microelectrode arrays (MEA) and subject them to hypothermic parameters. MEAs enable high-resolution temporal

* Corresponding address: School of Physics and Astronomy, Raymond & Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Tel-Aviv 69978, Israel. Tel.: +972 3 640 7604; fax: +972 3 642578.
E-mail address: lielrubinsky@yahoo.com (L. Rubinsky).

0893-6080/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neunet.2008.06.008

recordings of external membrane potentials of several dozen cells simultaneously. Using methods based on similarity and clustering analysis (Raichman & Ben-Jacob, 2008), we are able to characterize the spatio-temporal relations between neurons and between regions in the network, which allow for an understanding of the collective dynamics of the network as a whole (Segev et al., 2002; Segev, Baruchi, Hulata, & Ben-Jacob, 2004).

Thus the current experiment was undertaken to assess the effects of profound hypothermia (∼12 °C) on neurons and neuronal networks in an experimental study similar to those found in surgical settings. By applying our methodology we are able to isolate the effects of profound hypothermia on an *in-vitro* neuronal culture by comparing the intensity, synchronized bursting patterns (motifs) and culture state before, during and after hypothermia. Here we report on the neuronal and network activity following one hour of profound hypothermia and subsequent cooling sessions. We show that multiple sessions of cold are marked by a monotonic reduction in the rate of burst firing and in the number of spikes of each neuron after each session. In addition we demonstrate the preservation of initial synchronized burst motifs even with a generalized neuronal degeneration.

2. Experimental procedures

2.1. Network preparation

The neuronal networks used in this study were prepared from dissociated cortical cultures of neurons and glia of Sprague Dawley rats in their 18 embryonic day (E18), prepared and maintained according to the protocol described in Segev et al. (2002) and grown on a microelectrode array (Multi Channel Systems, Germany). The MEA consists of 60 microelectrodes that are 30 μm in diameter and are arranged in a square grid with a distance of 200 μm between electrodes. Before plating, the MEA is first coated with a poly-*d*-lysine (PDL, Sigma p-7886, Israel) substrate and left overnight. A well for the liquid medium is constructed by gluing a glass cylinder with a diameter of 2 cm to the MEA surface surrounding the recording cites. The neurons are then plated into the well at a density of 2×10^6 cells/electrode on the PDL substrate. The culture is maintained in a culture solution of growth medium containing 25 ml Horse serum (Beith Haemek, Israel), 100 uL Gentamycin (Beith Haemek, Israel), and 10 ml Glucose 1 M to 464 ml of MEM (Beith Haemek, Israel) to which was added 125 uL of Glutamine (Beith Haemek, Israel). The culture is kept in an incubator at 37 °C with 5% CO_2 and 95% humidity. Cultures are incubated for a minimum of 10 days to mature into self-organized connected and active neuronal networks. Twice a week 1 ml of medium is replaced.

2.2. Data acquisition

The electrical signals recorded from the neurons are local action potentials from cells that have formed capacitive coupling with the microelectrodes. At 10 days *in-vitro*, the network is placed in an external recording chamber that maintains the same physiological conditions as those found in the incubator. The network electrical activity is non-invasively recorded by a set of 60 amplifiers (Multi-Channel Systems, Germany) with a sampling rate of 12 kHz and transferred to a computer and saved to disk using Alpha Map data acquisition software (Alpha Omega Engineering, Israel). Spike detecting and sorting of the recorded action potentials were performed by our wavelet packets decomposition method as described in Hulata, Segev, and Ben-Jacob (2002).

Table 1
Similarity values of SBE motifs from 5 cultures

Culture	Session	Similarity, mean (stdev)		Cooling/Control (%)
		Control	After cooling	
1	1	0.76 (0.08)	0.97 (0.04)	128
	2	0.65 (0.12)	0.68 (0.09)	105
	3	0.57 (0.17)	0.51 (0.11)	88
2	1	0.72 (0.10)	0.31 (0.14)	43
3	1	0.20 (0.10)	0.88 (0.19)	437[a]
4	1	0.98 (0.02)	0.71 (0.14)	73
5	1	0.37 (0.18)	0.39 (0.13)	105
	2	0.55 (0.08)	0.83 (0.15)	151
	3	0.90 (0.12)	0.99 (0.01)	111
	All:	0.64 (0.24)	0.70 (0.25)	100 (33)

For each cooling session, the similarity was computed between SBEs of the most prominent motif, taken from 1 h before and 5 h after application of cold. For control, we computed the similarity of the same motif between SBEs from 6 h before the application of cold and 1 h before. In two cultures (1 and 5) we applied multiple sessions of cold.

[a] This value was excluded from the calculation of the mean of all sessions.

2.3. Profound hypothermia application

Before application of profound hypothermia, the neuronal activity was recorded for 10 h under normal physiological growth conditions in order to establish a base line for normal neuronal activity. The physiological atmosphere was maintained and continuously supplied to the external recording chamber throughout the entire experiment. The recording was continued while profound hypothermia was applied by pumping chilled water stored in a container submerged in a Neslab RTE-221 cooling bath (Neslab RTE-221, Thermo Electron Corporation, USA) through channels in the walls of the recording chamber. This effectively reduced the temperature to 12 ± 1 °C. Temperature was verified using a thermocouple-measuring device (Digi-Sense Thermometer, Cole Parmer, USA). The thermocouple measurements indicated that cooling of the chamber began immediately and the designated temperature was achieved within 5 min. The cultures were maintained in this hypothermic state for 1 h.

Following 1 h of profound hypothermia the cooling system was shut down and the temperature was returned to 37 °C by circulating warm water through the walls of the recording chamber. The culture activity was continuously recorded afterwards for a minimum of 10 h. Thermocouple measurements showed that the physiological temperature was achieved within 5 min.

We applied the above procedures on a total of 6 cultures. Of these, 2 of the networks were subjugated to repeated exposures of profound hypothermia with a 1–2 day time period between sessions. This was done to assess the cumulative effects of repeated hypothermia application on neuronal networks.

2.4. Synchronized bursting activity of in-vitro cultures

After 10 days *in-vitro*, the culture forms a dense interconnected network that exhibits rich spontaneous dynamical behavior characterized by the formation of synchronized bursting events (SBEs). SBEs are short time windows (200–1000 ms) during which most of the recorded neurons participate in relatively rapid firing of action potentials (Fig. 1A) (Segev et al., 2002). The SBEs are separated by long intervals (above seconds) of quiescence with few sporadic neuronal firings. A closer inspection reveals that each SBE has a distinctive spatio-temporal activity pattern (a motif) between the neurons (Fig. 1B). It is noticed that in a single culture a small number of different SBE spatio-temporal patterns may appear, and these patterns repeat themselves overtime (Segev et al., 2002, 2004).

Download English Version:

https://daneshyari.com/en/article/10326515

Download Persian Version:

https://daneshyari.com/article/10326515

Daneshyari.com