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Research Report

Characterization of the *in vitro* propagation of epileptiform electrophysiological activity in organotypic hippocampal slice cultures coupled to 3D microelectrode arrays

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ABSTRACT

Dynamic aspects of the propagation of epileptiform activity have so far received little attention. With the aim of providing new insights about the spatial features of the propagation of epileptic seizures in the nervous system, we studied in vitro the initiation and propagation of traveling epileptiform waves of electrophysiological activity in the hippocampus by means of substrate three-dimensional microelectrode arrays (MEAs) for extracellular measurements. Pharmacologically disinhibited hippocampal slices spontaneously generate epileptiform bursts mostly originating in CA3 and propagating to CA1. Our study specifically addressed the activity-dependent changes of the propagation of traveling electrophysiological waves in organotypic hippocampal slices during epileptiform discharge and in particular our question is: what happens to the epileptic signals during their propagation through the slice? Multichannel data analysis enabled us to quantify an activity-dependent increase in the propagation velocity of spontaneous bursts. Moreover, through the evaluation of the coherence of the signals, it was possible to point out that only the lower-frequency components (<95 Hz) of the electrical activity are completely coherent with respect to the activity originating in the CA3, while components at higher frequencies lose the coherence, possibly suggesting that the cellular mechanism mediating propagation of electrophysiological activity becomes ineffective for those firing rates exceeding an upper bound or that some noise of neuronal origin was added to the signal during propagation.

1. Introduction

Paroxysmal population discharges and epileptiform activity characterize the in vitro electrophysiological activity of many pharmacologically disinhibited neurobiological systems such as neocortical and hippocampal slices which largely retain the anatomical and physiological features of the intact in vivo circuitries (Gutnick et al., 1982; Knowles et al., 1987; Menendez de la Prida and Pozo, 2002; Poulsen et al., 2002; Salazar et al., 2003, Miles et al., 1988). Such reduced neurobiological preparations are therefore often used as a simplified experimental model of in vivo neuronal networks synchronization, under

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physiological as well as pathological conditions. In particular, hippocampal slices received great attention in the last two decades, and they have been extensively studied because the in vivo pathological increase in the synchronization of epileptiform activity through the hippocampus is thought to contribute to temporal lobe epilepsy, in which reverberating activity between entorhinal cortex and hippocampus is a central event (Pare et al., 1992). Actually, it is widely accepted that hippocampal networks can be rapidly recruited due to the large positive feedback provided by recurrent axon collaterals, and they are believed to play a crucial role as an in vivo generator of epileptic activity, or even as an amplifier of epileptic activity (Lothman et al., 1991; Pallud et al., 2008).

The study of the propagation of electrical synchronous activity in such neuronal networks is of a paramount importance for the understanding of the cellular basis of some form of central nervous system pathologies.

In addition, the propagation of synchronous discharges in many different neurobiological system may be used as a probe for the investigation of the network circuitry and synaptic physiology (Gutnick and Wadman, 1986; Traub et al., 1993, Hu et al., 2005; Orman et al., 2008) and it might provide important information at the network level.

In the last few years, substrate arrays of microelectrodes have proved to constitute an excellent tool for the investigation of the spatiotemporal evolution of in vitro electrophysiological activity, as they can be effectively employed to simultaneously record and stimulate the collective activity of networks of dissociated neurons in culture (Chiappalone et al., 2006; Vajda et al., 2008; Berdondini et al., 2009) as well as of brain tissue slices (Köhling et al., 2005; Heuschkel et al., 2003; Thiébaud et al., 1999), emphasizing the electrophysiological dynamics at the network level. In the case of the propagation of travelling waves of excitation through a brain slice, these arrays are useful tools for the assessment of the spreading direction and velocity of the population spikes (PSs).

In this work, we demonstrate what happens to the epileptic signal during propagation through the hippocampal slice. More precisely, we have studied the problem through the use of spectral coherence analysis that provides complementary information to the classical analysis of temporal signals. We measured the propagation of epileptiform bursts of activity in a standard experimental model of in vitro epilepsy by means of 3D substrate microelectrode arrays, supporting experimental data with nonconventional signal processing tools. From the physiological point of view, many different biophysical mechanisms are known to determine and dynamically modulate the properties of the propagation of travelling pulses of excitation through an in vitro brain tissue slice (Holsheimer and Lopes da Silva, 1989).

2. Results

Experiments were carried out on nine organotypic hippocampal slices coupled to the MEAs. During each experiment, epileptic activity was recorded from all the array microelectrodes to spatially monitor the electrophysiological activity across the whole slice. Within a few minutes after the application of picrotoxin 100 μM, spontaneous episodes of epileptiform activity could be detected extracellularly by the MEA microelectrodes located in CA1–CA3 areas (Fig. 1A), but not in dentate area (data not shown) (Traub et al., 1993). Such electrophysiological bursts could be also elicited by a weak monopolar electrical stimulus, delivered via one microelectrode of the array, located in the CA1–CA3 areas.

With the aim of characterizing the propagation velocity of epileptiform activity bursts through the hippocampal tissue,

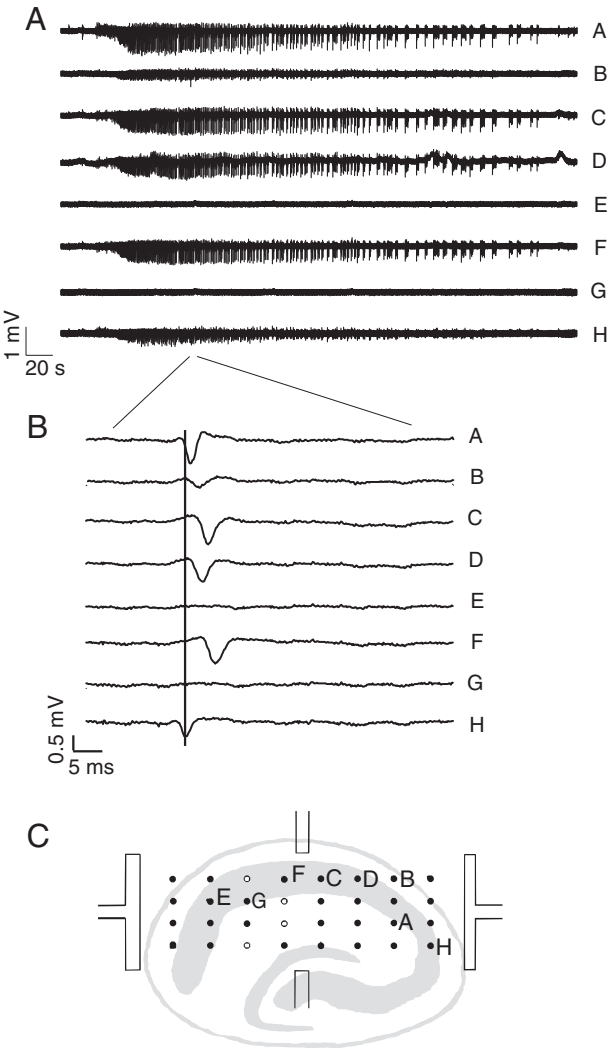

Fig. 1 – Epileptic activity induced by 100 μM picrotoxin in an organotypic brain slice. A) Burst activity recorded by means of eight different electrodes of the array. B) Population spikes (PS) that constitute the bursts in A. The time delay was calculated between the time corresponding to the minimum value of the PS recorded by H electrode and all the other time values recorded by the different electrodes. C) Sketch of the actual alignment of an organotypic hippocampal slice coupled to the microelectrode array, during a typical experiment. Letters indicate the location of 8 active array microelectrodes, selected out of the 28 available. Light gray-coloured areas identify the granule cell body layer of the dentate gyrus (DG) and the pyramidal cell body layer of the cornu ammonis (CA).