Investigation of the spatio-temporal propagation pattern of epileptic events on cortico-hippocampal slices by means of high-density CMOS micro electrode arrays

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ABSTRACT

Micro electrode arrays (MEAs) are employed to study extracellular electrical activity in neuronal tissues (Morin et al., 2005). Nevertheless, commercially available MEAs provide a limited number of recording sites and do not allow a precise identification of spatio-temporal oscillations. To overcome this limitation, high density MEAs were recently developed and validated on dissociated preparations (Berdondini et al., 2009). The platform enables extracellular electrophysiological recordings from 4096 electrodes arranged in a squared area of 2.7 mm x 2.7 mm with inter-electrode distance of 21 µm (Imfeld et al., 2008), at a sampling rate of 7,7 kHz/electrode (fig.1).

Here, we demonstrate the performances of this high-resolution platform for the acquisition of electrophysiological activity from acute brain slices.

Furthermore, we show that the high density MEA platform can be mounted on a microscope stage and can be coupled with conventional extracellular electrodes or intracellular patch clamp electrodes for local stimulation or recording (fig.2,5,6,7).

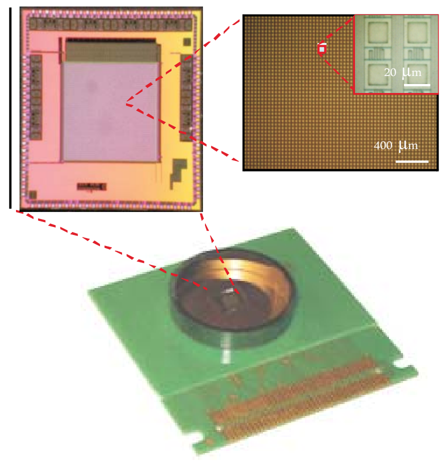

Fig 1. **Biocam 4096 high density MEA device.** The packaged chip and close-up of the active area of 2.7 mm x 2.7 mm with 4096 integrated electrodes. (www.3brain.com).

Materials and Methods

Epileptic-like discharges were induced on horizontal hippocampal slices by applying:

- 4-aminopyridine (4AP, 200 µM);
- bicuculline (BIC, 30 µM).

Spontaneous activity was recorded over the CMOS-MEA up to one hour under constant perfusion and at controlled temperature conditions.

An extracellular stimulator as well as an intracellular patch clamp electrode was coupled to the system for stimulation and intracellular recordings respectively (fig.2).

Fig 2. High density MEA system (Biocam 4096) coupled with an upright microscope for simultaneous extracellular and patch clamp recordings.

In vitro epilepsy induced by 4-AP (200 µM) and Bicuculline (30 µM). Interictal as well as Ictal discarges are observed during recording

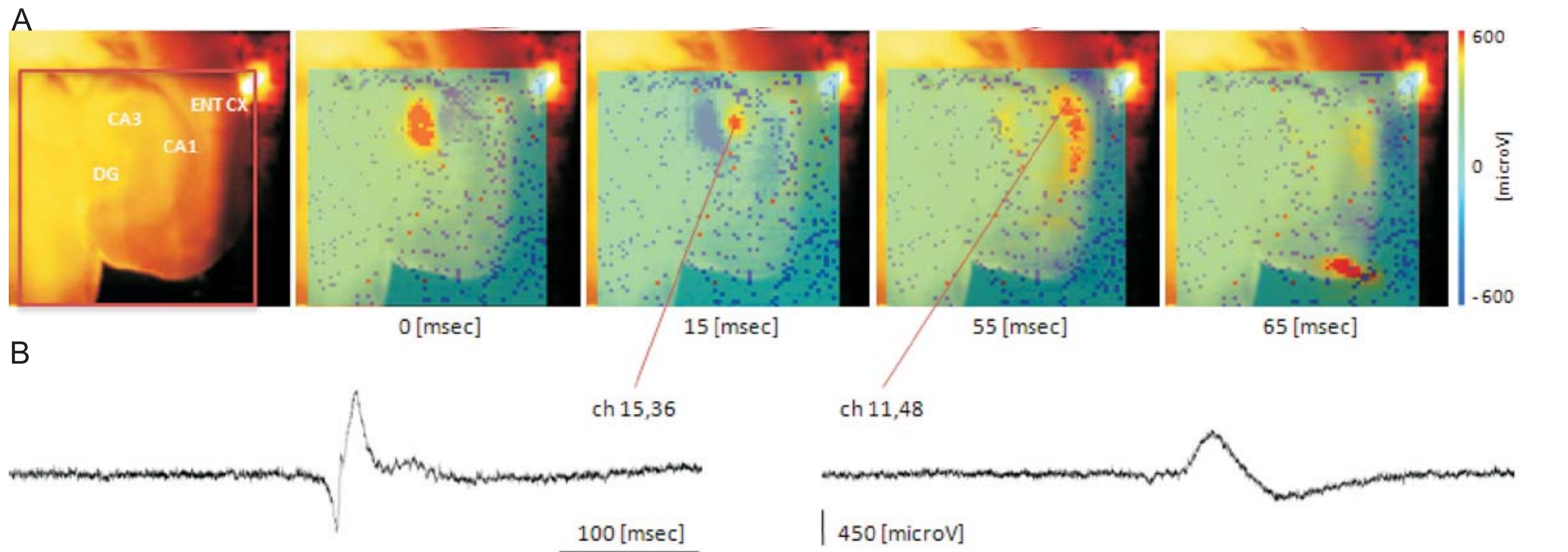

Fig 3 **Spatio-temporal propagation of epileptic activity.** (A) Superimposition on the slice image of a red square representing the recording area of the device; the following images show a false color-map of the voltage values recorded at different instances. Signal propagation from CA3 to CA1 and to Entorhinal Cortex can be appreciated in detail. (B) Raw data of two representative channels showing alternate phases of de- and hyper-polarization.

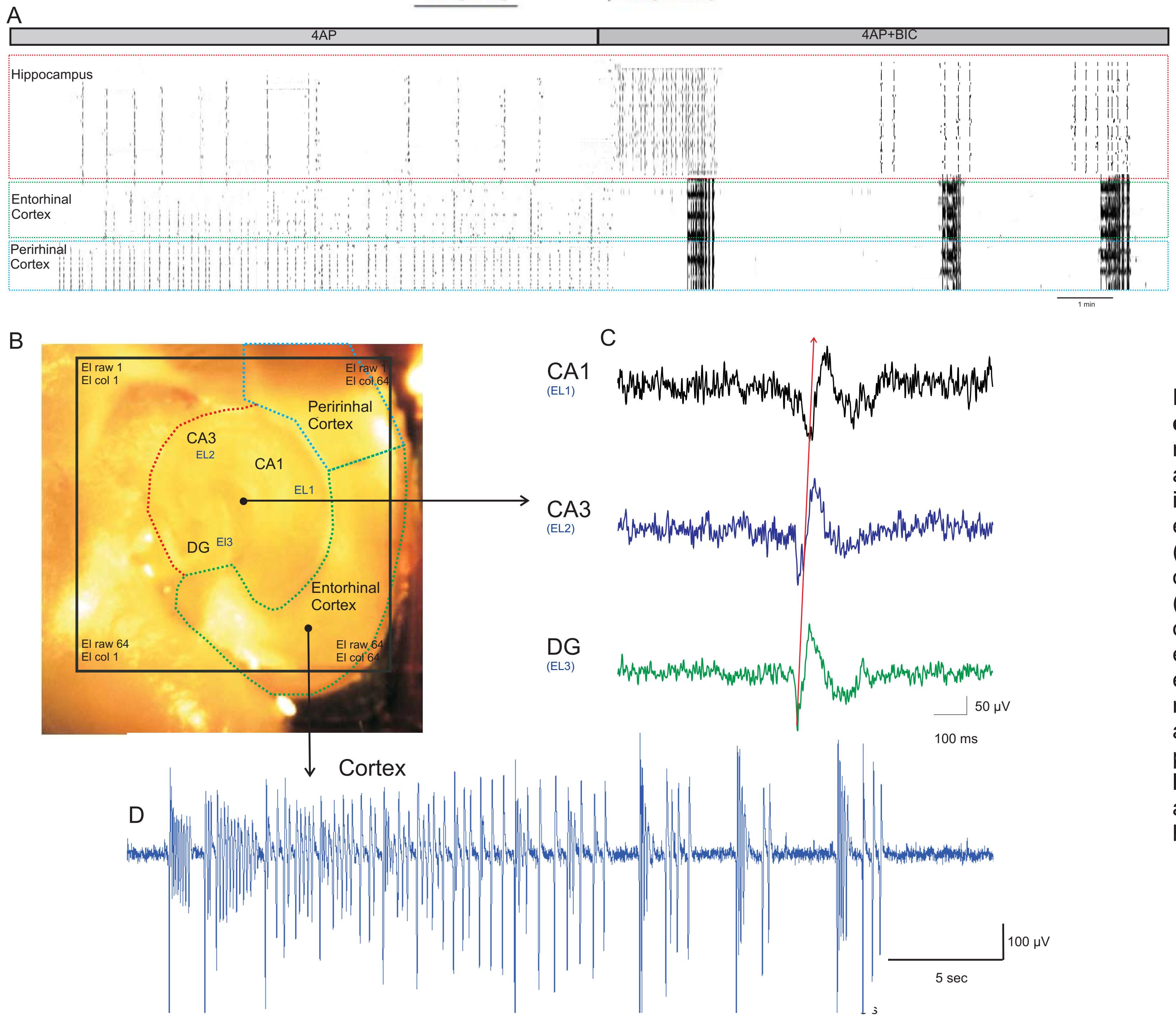

Fig 4 **Single electrode traces of epileptic events.** (A) Raster plot representation of spontaneous activity characterized by trains of fast interictal events recorded by ~900 electrodes under perfusion of 4AP (20') and 4AP+BIC (20') (B) Picture of the brain slice over the active area (the black square indicates the edges of the active area containing the 4096 electrodes). (C) Single Interictal event recorded in three distinct regions of the hippocampus, the red arrow indicating the direction of propagation. (D) Ictal discharge, lasting up to 30 s, was recorded in PC and EC, and never observed in the hippocampus.

The high density CMOS micro electrode arrays recordings can be coupled with conventional electrophysiological techniques

Combined with patch clamp recordings:

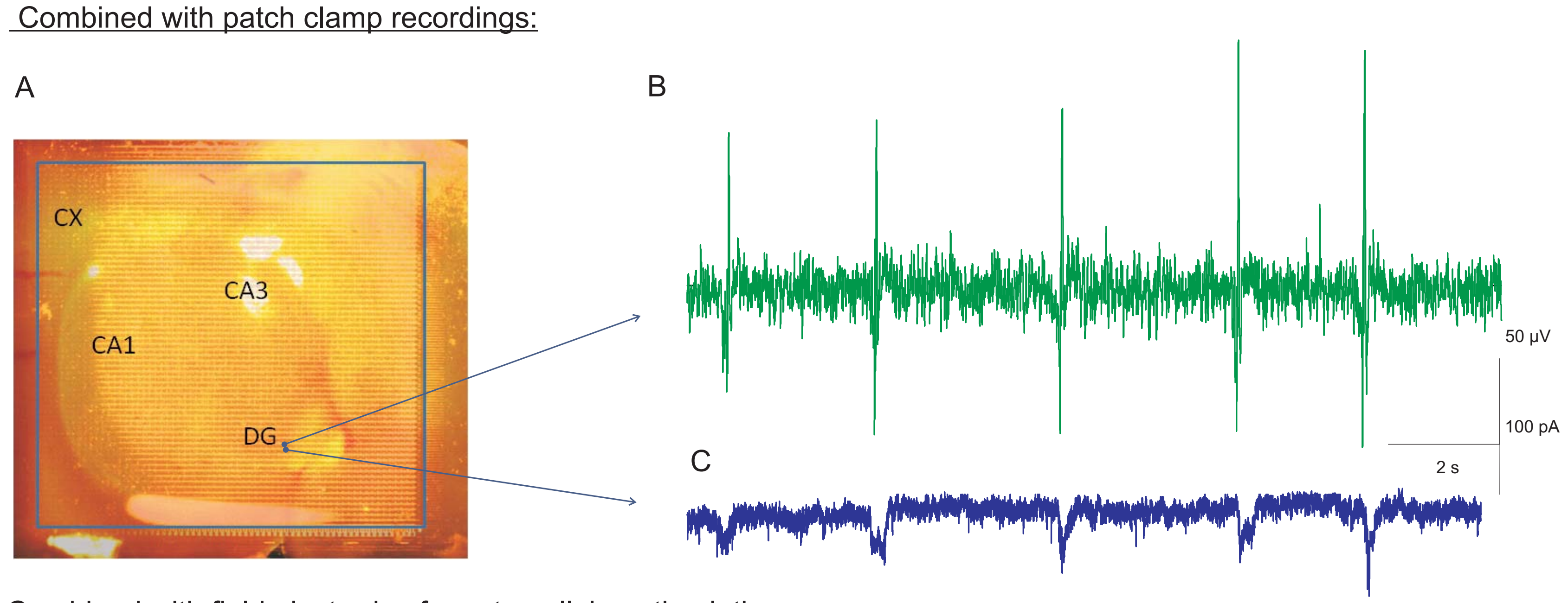

Fig 5 **Combined patch clamp and multi site elctrode recordings.** (A) A hippocampal slice placed onto the chip area and perfused with 4AP. Extracellular recordings (green trace, B) coupled with intracellular recordings (dark blue trace, C) from the granular cell layer of the dentate gyrus (see figure 4A). It is important to note the similar time course of the two traces which are recorded not only with different techniques but also from opposite sides of the slice.

Combined with field electrodes for extracellular stimulation:

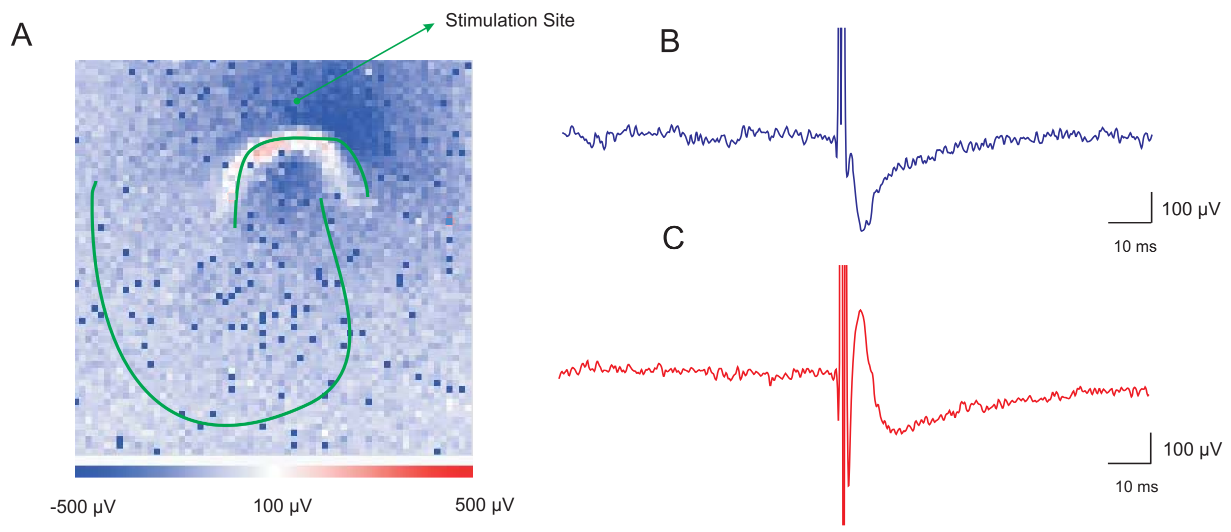

Fig. 6 **Electrically evoked responses.** (A) Color-map of electrically evoked extracellular field potential in the dentate gyrus. Horizontal bar shows color-scale code used to generate the spatial maps. (B) Single electrode response of the system in the dendritic region after stimulation of the medial perforant path. (C) Single electrode response in the granular cell layer after stimulation of the medial perforant path.

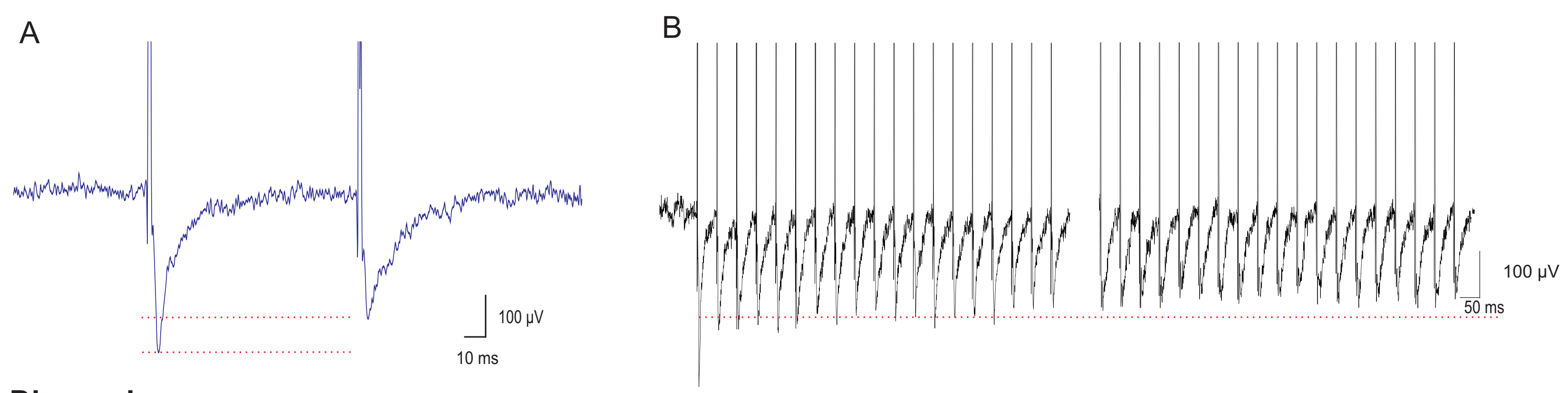

Fig. 7 **Short term plasticity protocols can be evaluated with the system.** (A) Paired-pulse stimulation (inter pulse interval, 100ms) applied to the medial perforant path induces a clear synaptic depression. (B) A stimulation train at 20 Hz, lasting 10 sec, induces a short term depression. On the left panel are shown the synaptic responses during the first second of stimulation, while the responses of the last second are depicted on the right panel.

Discussions

High density MEA offer a unique opportunity to perform long-lasting recordings on a large brain area with high spatial and temporal resolution. In particular we demonstrated the effectiveness of these devices in recording epileptic like events. Moreover, thanks to the versatility of the system it can be coupled to standard electrophisiological techniques. Such a complementary methodological combination supports the platform validation and paves the way to detailed electrophisiological studies in acute brain tissue.

References:

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