Electrophysiological imaging of epileptic brain slices reveals pharmacologically confined functional changes

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Abstract
Microelectrode arrays (MEAs) are employed to study extracellular electrical activity in neuronal tissues. Nevertheless, commercially available MEAs provide a limited number of recording sites and do not allow a precise identification of the spatio-temporal characterization of the recorded signal. To overcome this limitation, high density MEAs, based on CMOS technology, were recently developed and validated on dissociated preparations (Berdondini et al. 2009). We show the platform capability to record extracellular electrophysiological signal from 4096 electrodes arranged in a squared area of 2.7 mm x 2.7 mm with inter-electrode distance of 21 µm at a sampling rate of 7.7 kHz/electrode. Here, we demonstrate the performances of these platforms for the acquisition chemically evoked epileptiform activity from brain slices. Moreover the high spatial resolutions allow us to estimate the effect of drugs in spatially modulating Inter-Ictal ((I-IC) activity.

1 Introduction

Electrophysiological recording of neuronal activity in slices is a common experimental method for investigating complex brain processing circuits, brain plasticity or neuropharmacology. To this end, it is crucial to enable recordings of electrophysiological activity from large neuronal populations at sufficient spatial and temporal resolution, thus making possible to localize and track electrically or chemically evoked neuronal activity and to identify induced functional changes. Conventional electrophysiological include the use of electrodes and light-imaging methods to record action potentials from multiple single neurons as well as local field potentials generated by neuronal ensembles. Patch-clamp and field electrodes, approaches based on micro-positioned single glass pipettes, enable intracellular or extracellular recordings respectively, from single neurons or from neuronal populations surrounding the electrode respectively. Optical imaging based on fluorescent Ca2+ indicators [1] or voltage sensitive dyes (VSDs) [2, 3] are the current choice for spatially resolving electrical activity in the brain, but this approach suffers a modest temporal resolution due to sampling frequencies in the range of 1-3 kHz. Moreover, this approach does not offer a high signal to noise ratio and is not suited to record continuously for long periods of time (usually it can records for few seconds after a triggered electrical stimulus). Consequently, these recording performances are not sufficient to effectively track the electrical activity propagations over large areas of the brain tissue in real time, either due to insufficient spatial or temporal resolutions.

2 Materials and Methods

Horizontal cortico-hippocampal slices (400 µm of thickness) were placed on the active area of the chip (Fig.2). A platinum anchor of the same dimension was used to keep the slice in position for the whole recording. Epileptic-like discharges were induced in slices by treatment with the convulsant agents 4-aminopyridine (4AP) at a concentration of 100µM [4] and/or bicuculline (BIC) at a concentration of 30µM. Epileptiform activity was stable during recordings for at least two hours. Epileptiform activity in the form of I-IC was detected with a previously described Precision Timing Spike Detection algorithm (PTSD) [5] and represented in raster plots (see fig.1B). This algorithm, originally developed to detect spiking activity, has been re-adapted to detect Inter-Ictal (I-IC) events. To this aim, the threshold was set to 5 times the standard deviation of the noise while the refractory period and the peak lifetime period were set to 50 msec and 40 msec, respectively. Movie sequence frames as reported in Fig.1 were generated in *Matlab* after low pass filtering of single electrode traces.

3 Results

The large-scale high-density electrode array allow us to visually describe the involvement of cortical and hippocampal circuitries during propagation of epileptiform activity (Fig. 2). In this experiment the I-

IC was observed to originate in CA3 and propagating to CA2 (10 ms) and CA1 (30 ms). By litterally imaging the extracellular activity it is possible to study the spatial extention of the epileptic event which might be reflect different mechanisms of propagation through the network (e.g. synaptic versus non-synaptic propagation).

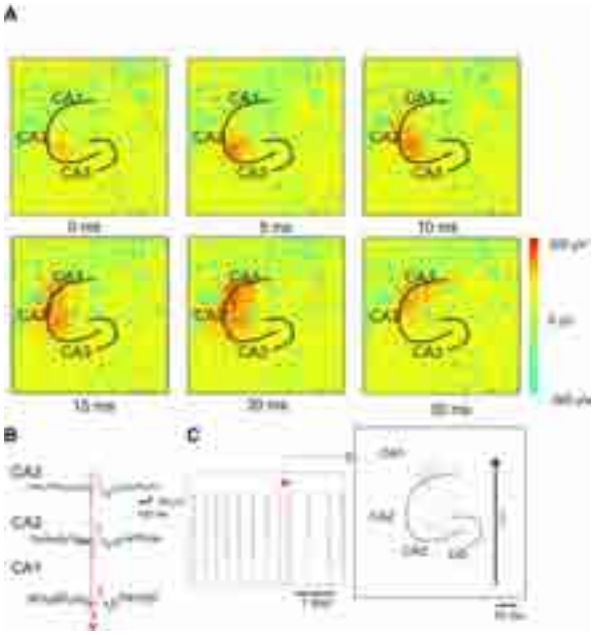

Fig. 1. Inter-Ictal like propagation. (A) Color-map coding voltages of extracellular activity. The map sequence is showing the consecutive activation of the distinct areas: CA3 first, then CA2 and finally CA1. (B) Inter-Ictal (I-IC) activity recorded in three representative electrodes in the hippocampus (CA3, CA2, CA1). Red vector is indicating the direction of propagation of the signal (C) Raster plot representation of detected I-IC events. (Left) On the large scale raster plot (10 minutes) the distribution of I-IC events seems to be synchronous on all the electrodes whereas on the close up (right) it is possible to note that different hippocampal regions are recruited at different times.

Moreover, our method enable to spatially depict the firing rate of different cortico-hippocampal regions over the active area of the chip (Fig. 2). In this experiment by adding BIC to the extracellular solution containing 4AP, the mean firing rate of I-IC events was found to spatially increase differently in different cortico-hippocampal regions (cfr. Fig. 2 middle and left)

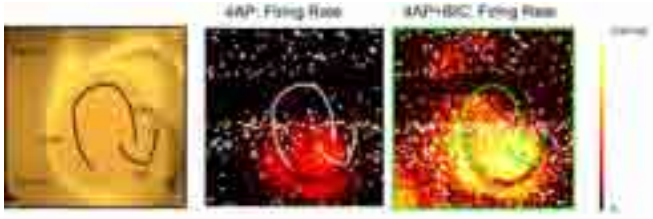

Fig. 2. (left) Cortico hippocampal slice over the active area. The mean firing rate has been calculated for every pixel in the slice under 4AP treatment (middle) and under 4AP+BIC treatment (right)

4 Discussion

Our system enables us to literally imaging the extracellular activity and to describe signal propagations over large areas. The spatial extention and propagation latencies of I-IC can be finely appreciated with the system. These are important aspects to evaluate the different types of synchronization mechanism which could play a role in the propagation of epileptic events (for review see[6]).

A part from propagation studies our system appears also to be a useful tool to assess the effect of chemical compounds in different regions in brain slices.

References

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