040.04

High Density Multi Electrode Array: a new tool to monitor seizure-like activity evoked by different convulsant drugs

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Introduction

Epilepsy is a neurological disorder characterized by recurrent and sustained neuronal discharges. Different approaches have been used to evoke seizure-like activity but chemoconvulsants are the most used. In this work, we challenged mouse hippo-cortical slices with different compounds. Activity evoked by acute application of these compounds was monitored through high-density multi electrode array (HD-MEA) in order to characterize in time and space seizure like activity. In particular we challenged slices with kainic acid (KA, 10 µM) and the high-selective agonist (RS)-2-Amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl) propanoic acid (ATPA, 10 µM) in order to acivate kainate receptors. Furthermore, we compare the effect of these compounds with the convulsant 4-aminopyridine (4-AP, 100 µM), the voltage-dependent potassium channels blocker.

The aim of this work is to characterize different patterns of seizure like activity evoked by different compounds with HD-MEA.

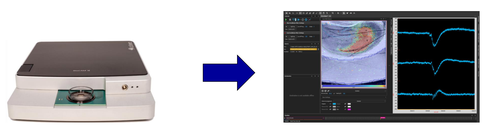


Figure 1. High Density MEA platform and BrainWave v.4.0 software recording screen

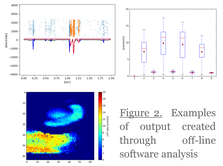


Figure 2. Examples of output created through off-line software analysis

Methods

High density micro electrode arrays recordings

Spatio-temporal signal in adult mouse slices have been acquired by using High Density Multi Electrode Arrays (HD-MEAs) from 3Brain (www.3brain.com). These devices, providing 4096 recording electrodes (64x64) covering a total active area of 2.7 by 2.7 mm, were able to record simultaneously local field potential (LFP) from hundreds of neurons. LFPs were monitored for up to 90 minutes and then analysed off-line with a 3Brain software. For LFP detection, traces were filtered with a low-pass Butterworth filter; events were selected as field potentials when parameter of amplitude (> +110 or < -90 µV), duration (< 1 s) and refractory period (> 10 ms) were satisfied. Custom-made denoise filtering was applied in order to reduce the number of false positive events. Cluster were defined manually according to mouse brain atlas. All the analysis presented were performed with a 3Brain analysis software.

Seizure-like activity evoked by acute application of kainic acid (10 µM) in mice hippocampus and cortex

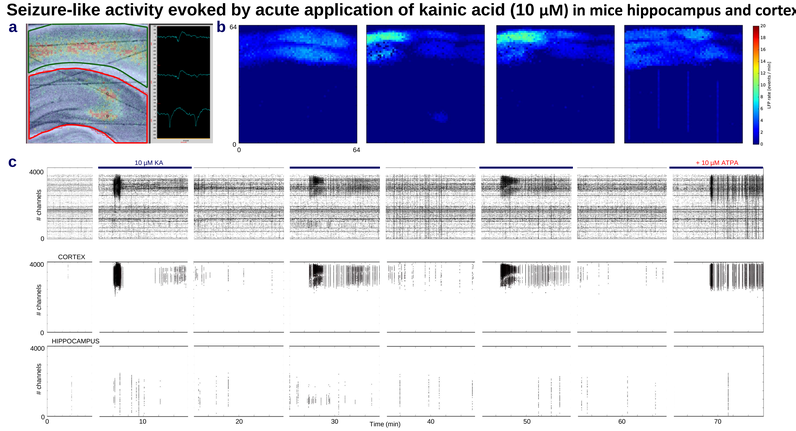


Figure 3. Kainate-dependent activity in mouse hippocampus and cortex.
a. Representative image of a cortico-hippocampal slice superimposed to the pseudocolor activity map of the MEA array. Clusters analysed are highlighted in red (hippocampus) and dark green (cortex). On the right: representative detected events obtained from 3 different channels located in hippocampal (CA3 and CA1) and cortical area. **b.** Pseudocolor activity map resuming average event rate during consecutive compound application (see c). **c.** Raster plot of compound application and wash-out of the representative experiment in a. Upper plot represents all events found after LFP detection. Lower plots represent LFP found in cortex (upper plot) and in hippocampus (lower plot) after denoising filter.

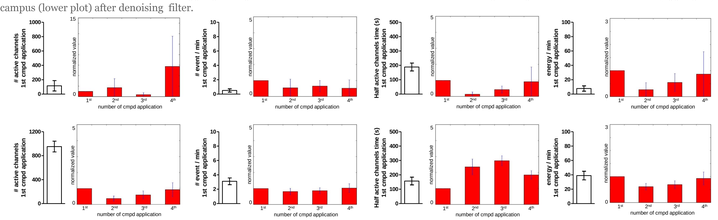


Figure 4. Average kainate-dependent activity in mouse hippocampus and cortex.
Normalized average of event rate, number of active channel, 50% active channel time and energy histogram obtained from the analysis of 5 slices (5 mice). Upper histograms: cortex, lower histograms: hippocampus.

Seizure-like activity evoked by acute application of ATPA (10 µM) in mice hippocampus and cortex

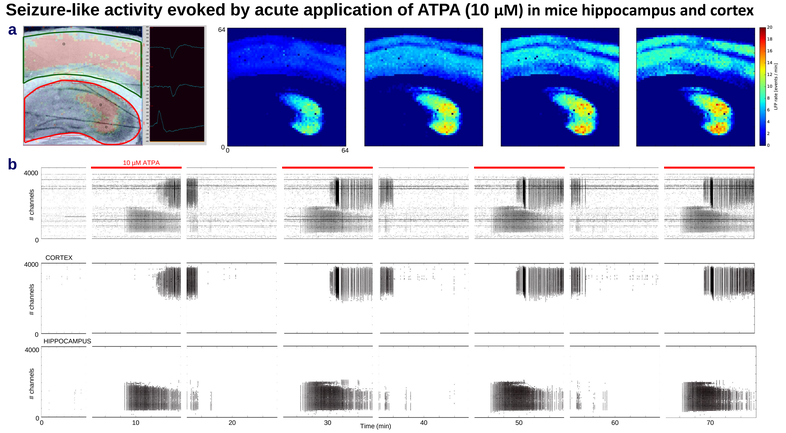


Figure 5. ATPA-dependent activity in mouse hippocampus and cortex.
a. Representative image of a cortico-hippocampal slice superimposed to the pseudocolor activity map of the MEA array. Clusters analysed are highlighted in red (hippocampus) and dark green (cortex). On the right: representative detected events obtained from 3 different channels located in hippocampal (CA3 and CA1) and cortical area. **b.** Pseudocolor activity map resuming average event rate during consecutive compound application (see c). **c.** Raster plot of compound application and wash-out of the representative experiment in a. Upper plot represents all events found after LFP detection. Lower plots represent LFP found in cortex (upper plot) and in hippocampus (lower plot) after denoising filter.

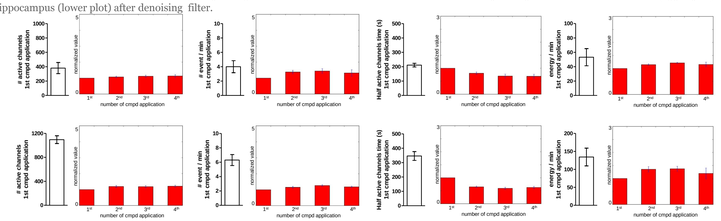


Figure 6. Average ATPA-dependent activity in mouse hippocampus and cortex.
Normalized average of event rate, number of active channel, 50% active channel time and energy histogram obtained from the analysis of 5 slices (5 mice). Upper histograms: cortex, lower histograms: hippocampus.

Seizure-like activity evoked by acute application of 4AP (100 µM) in mice hippocampus and cortex

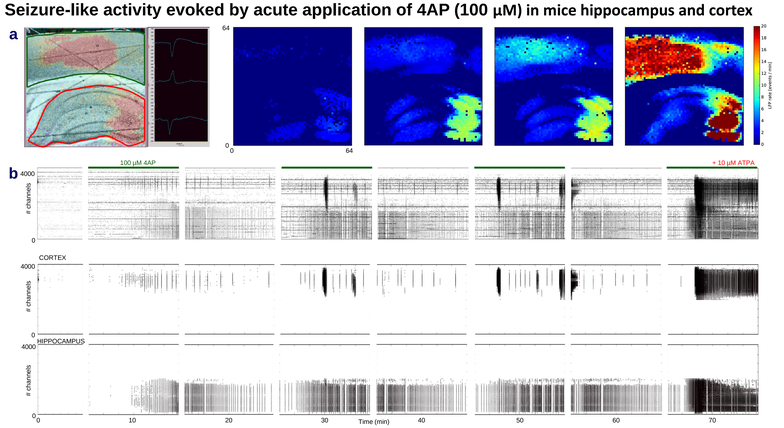


Figure 7. 4AP-dependent activity in mouse hippocampus and cortex.
a. Representative image of a cortico-hippocampal slice superimposed to the pseudocolor activity map of the MEA array. Clusters analysed are highlighted in red (hippocampus) and dark green (cortex). On the right: representative detected events obtained from 3 different channels located in hippocampal (CA3 and CA1) and cortical area. **b.** Pseudocolor activity map resuming average event rate during consecutive compound application (see c). **c.** Raster plot of compound application and wash-out of the representative experiment in a. Upper plot represents all events found after LFP detection. Lower plots represent LFP found in cortex (upper plot) and in hippocampus (lower plot) after denoising filter.

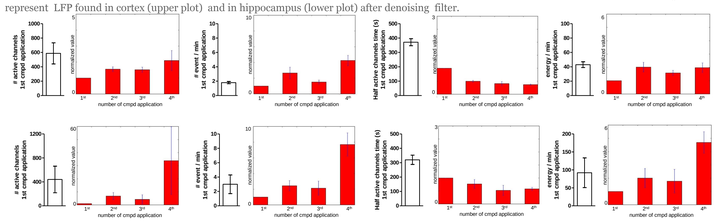


Figure 8. Average 4AP-dependent activity in mouse hippocampus and cortex.
Normalized average of event rate, number of active channel, 50% active channel time and energy histogram obtained from the analysis of 5 slices (5 mice). Upper histograms: cortex, lower histograms: hippocampus.

Hippocampal event propagation evoked by acute application of ATPA (10 µM) or 4-AP (100 µM)

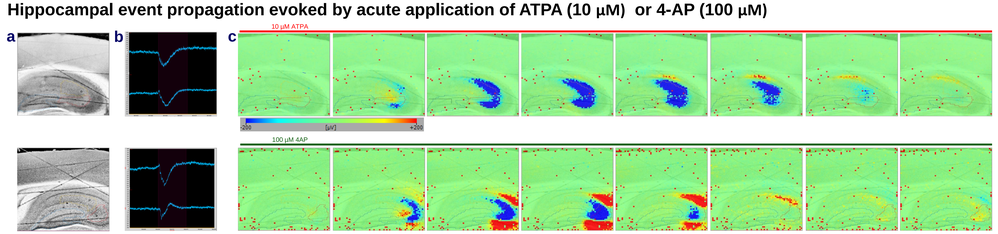
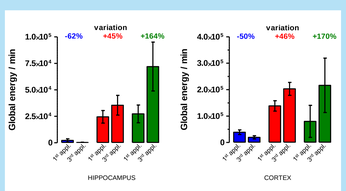


Figure 9. Different event propagation in cortico-hippocampal slice challenged with 10 µM ATPA or 100 µM 4-AP.
a. Schematic of a cortico-hippocampal slice on MEA with 3 cluster highlighted (dentate gyrus: blu, CA3 dendrite region: red, CA1 dendrite region: orange). **b.** Representative events recorded in slices during ATPA (up trace) and 4-AP (lower trace). **c.** Pseudocolor activity map of 80 ms event (time bins 10 ms).

Take home message



- All three compounds tested can activate cortico-hippocampal slices but with different outcomes.
- Although active in cortex, consecutive applications of 10 µM kainic acid evoke a poor seizure-like activity in hippocampus.
- 100 µM 4-AP effect is characterized by high variability in both cortex and hippocampus.
- Consecutive 10 µM ATPA applications evoke reproducible seizure-like activity in both hippocampus and cortex.
- ATPA represents a useful tool to investigate the effect of new molecule in acute seizure-like activity.

Experimental procedures

Briefly, brains from 6 weeks old CD1 mice were dissected in a high sucrose ice-cold solution. After brain dissection on the coronal plane, 300-µm-thick slices were cut and transferred at room temperature in a low magnesium oxygenated modified ACSF. Recordings were performed at 30 ± 1°C on cortico-hippocampal slices continuously perfused at a rate of 4-5 ml/min with modified ACSF. Compounds were applied in bath perfusion after at least 15 min of activity stabilization.

References

- Fritsch B *et al.*, J Neurosci. 2014 Apr 23;34(17):5765-75. doi: 10.1523/JNEUROSCI.5307-13.2014
- Ferrea E *et al.*, Front Neural Circuits. 2012 Nov 14;6:80. doi: 10.3389/fncir.2012.00080. eCollection 2012