B083

Purkinje cells firing recorded by a high density multi-electrode array: a new tool for compounds validation

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Introduction

Restoring altered firing pattern of cerebellar Purkinje cells has been proposed as a promising approach for the treatment of movement disorders such as ataxia (Kasumu *et al.,* 2012; Alviña and Khodakhah, 2010). However new therapeutic molecules would require a full characterization of their effect on Purkinje cells firing in normal and pathological conditions. Here, high-density multi electrode array (HD-MEA) was used to monitor and characterize spiking activity of hundreds Purkinje cells simultaneously in 90 minute long recordings. Since Ca^{2+}-activated K^+ channels have been demonstrated to modulate Purkinje cells firing, we used different positive and negative modulators in order to assess their effect on this neuronal population.

The aim of this work is to demonstrate that HD-MEA is a suitable tool for a deep characterization and validation of molecules that can modulate Purkinje cells spike activity.

Methods

High density micro electrode arrays recordings

Spatio-temporal signal in adult cerebellar mouse slices have been acquired by using High Density Micro Electrode Arrays (HD-MEAs) from 3Brain (www.3brain.com). These devices, providing 4096 recording electrodes (64x64) covering an area of ~7 mm^2, were able to record simultaneously spontaneous firing from hundreds of putative Purkinje cells (PCs). Firing was monitored for up to 90 minutes and then analysed off-line with a 3Brain software. For spike detection, events characterized by larger amplitude than -100 µV separated at least by 0.5 ms (refractory period) were selected as action potentials. For tonic firing PCs selection, only units showing a tonic firing with interspike interval lower than 300 ms during 10 minutes control recording were subsequently analysed and presented in this poster. No sorting analysis was applied. All the analysis presented were performed with a 3Brain custom made analysis software.

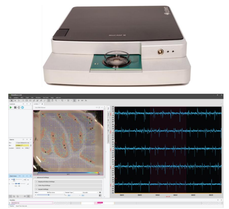


Figure 1. High Density MEA platform
The platform consists of disposable recording Biochips, an acquisition hardware unit (Biocam) and BrainWave, a software tool for real-time/off-line visualization and analysis of the recorded data. Each square electrode has a 42 µm side pitch (21 µm x 21 µm the recording area) covering a total active area of 2.7 by 2.7 mm^2. The BioCam enables simultaneous recording from the whole array at a frame rate of 18 kHz per electrode (Ferrea *et al.,* 2012).

Characterization PCs firing activity in 90 minute long control experiments

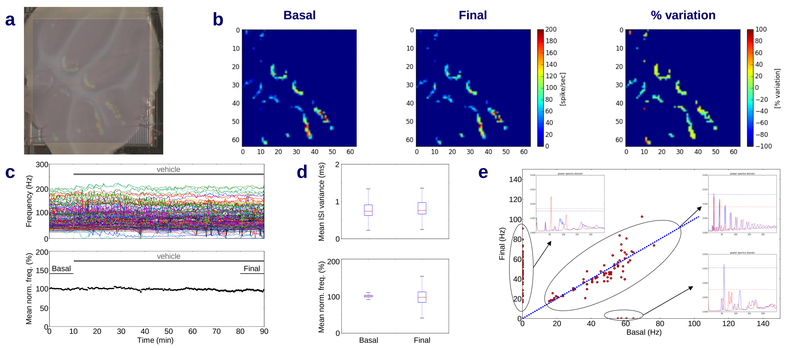


Figure 2. Representative PCs firing activity in a control slice
a. Representative image of a cerebellar slice on a chip with pseudocolor map of the MEA array superimposed in order to highlight PCs position. **b.** Pseudocolor map of firing rate activity in the first (basal) and last (final) ten minutes of recording. On the right the normalized percentage of firing variation between basal and final color map. **c.** Absolute and mean normalized firing frequency time course of 166 units presented in b (bin size 30 s). **d.** Box plots representing the mean variance of inter spike interval (ISI) and mean normalized frequency in basal and final ten minute recording. **e.** Most represented frequency peaks obtained from power spectra analysis of all PCs from slice in b. Inset: representative power spectra of basal (blue spectrum) and final (red spectrum) from the 3 major groups highlighted in the distribution.

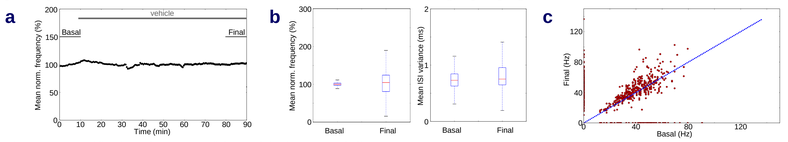


Figure 3. Quantification of mean PCs firing activity in 6 control slices
a, b and **c**. Same analysis presented for the single slice obtained from 997 units (6 slices, 4 mice, bin size 30 s).

Different effects of Ca^{2+}-activated K^+-channel positive modulator of SK/IK channels on PC firing activity

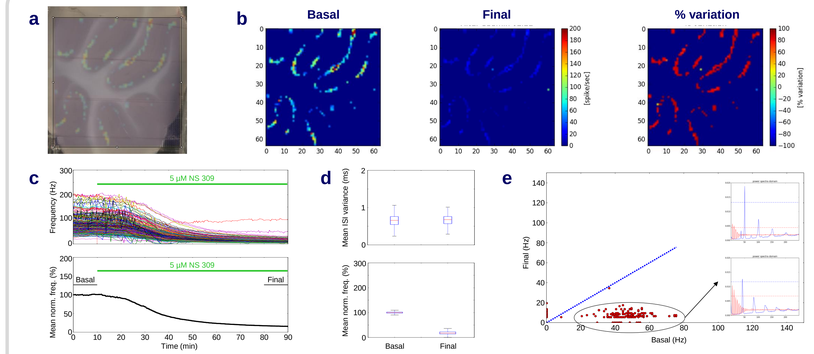


Figure 4. PCs firing modulation by NS 309, a $K_{Ca}2$ and $K_{Ca}3.1$ positive modulator, in a treated slice
a. Representative image of a cerebellar slice on a chip with pseudocolor map of the MEA array superimposed in order to highlight PCs position. **b.** Pseudocolor map of firing rate activity in the first (basal) and last (final) ten minutes of recording. On the right the normalized percentage of firing variation between basal and final color map. **c.** Absolute and mean normalized firing frequency time course of 261 units presented in b (bin size 30 s). **d.** Box plots representing the mean variance of inter spike interval (ISI) and mean normalized frequency in basal and final ten minute recording. **e.** Most represented frequency peaks obtained from power spectra analysis of all PCs from slice in b. Inset: representative power spectrum of basal (blue spectrum) and final (red spectrum) from the major group highlighted in the distribution.

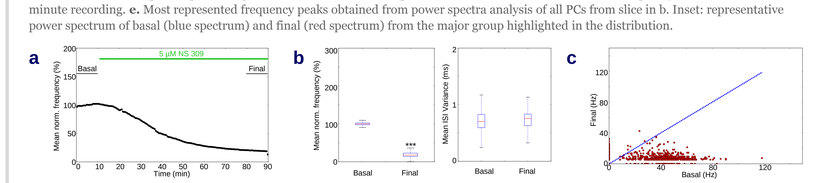


Figure 5. PCs firing modulation by NS 309, a $K_{Ca}2$ and $K_{Ca}3.1$ positive modulator, in treated slices
a, b and **c**. Same analysis presented for the single slice obtained from 1362 units (7 slices, 7 mice, bin size 30 s).

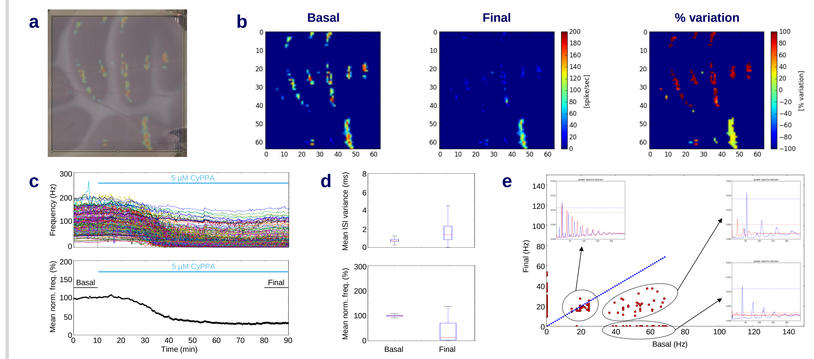


Figure 6. PCs firing modulation by CyPPA, a $K_{Ca}2.2$ and $K_{Ca}2.3$ positive modulator, in a treated slice
a. Representative image of a cerebellar slice on a chip with pseudocolor map of the MEA array superimposed in order to highlight PCs position. **b.** Pseudocolor map of firing rate activity in the first (basal) and last (final) ten minutes of recording. On the right the normalized percentage of firing variation between basal and final color map. **c.** Absolute and mean normalized firing frequency time course of 195 units presented in b (bin size 30 s). **d.** Box plots representing the mean variance of inter spike interval (ISI) and mean normalized frequency in basal and final ten minute recording. **e.** Most represented frequency peaks obtained from power spectra analysis of all PCs from slice in b. Inset: representative power spectra of basal (blue spectrum) and final (red spectrum) from the 3 major groups highlighted in the distribution

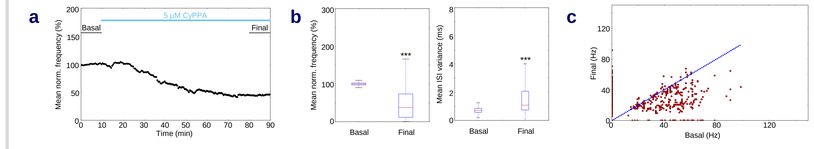


Figure 7. PCs firing modulation by CyPPA, a $K_{Ca}2.2$ and $K_{Ca}2.3$ positive modulator, in treated slices
a, b and **c**. Same analysis presented for the single slice obtained from 676 units (4 slices, 4 mice, bin size 30 s).

Ca^{2+}-activated K^+-channel blocker apamin alters PCs firing pattern without affecting mean firing frequency

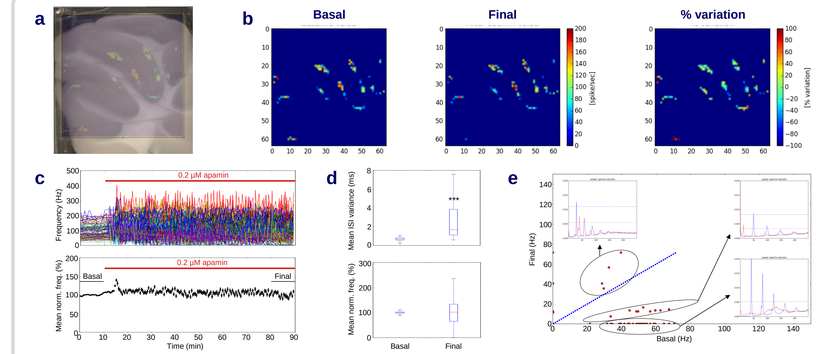


Figure 8. PCs firing modulation by apamin, a selective inhibitor of $K_{Ca}2$ channels, in a treated slice
a. Representative image of a cerebellar slice on a chip with pseudocolor map of the MEA array superimposed in order to highlight PCs position. **b.** Pseudocolor map of firing rate activity in the first (basal) and last (final) ten minutes of recording. On the right the normalized percentage of firing variation between basal and final color map. **c.** Absolute and mean normalized firing frequency time course of 99 units presented in b (bin size 30 s). **d.** Box plots representing the mean variance of inter spike interval (ISI) and mean normalized frequency in basal and final ten minute recording. **e.** Most represented frequency peaks obtained from power spectra analysis of all PCs from slice in b. Inset: representative power spectra of basal (blue spectrum) and final (red spectrum) from the 3 major groups highlighted in the distribution.

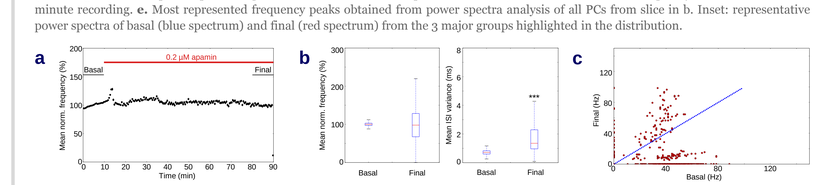
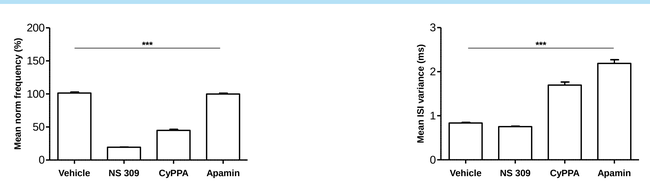


Figure 9. PCs firing modulation by apamin, a selective inhibitor of $K_{Ca}2$ channels in treated slices
a, b and **c**. Same analysis presented for the single slice obtained from 827 units (5 slices, 5 mice, bin size 30 s).

Take home message



- PCs activity recorded with high-density multi electrode array is stable for up to 90 minutes
- Ca^{2+}-activated K^+-channels have a strong impact on PCs firing with different outcomes that can be evaluated through different analyses
- Although the average PCs firing frequency is unaltered, Ca^{2+}-activated K^+-channel blocker apamin strongly affect PCs firing pattern
- HD-MEA is a suitable tool to deeply characterize compound effects on PCs in cerebellum

Experimental procedures

Briefly, brains from 6 weeks old C57BL6/J mice were dissected in a high sucrose ice-cold solution. After brain dissection on the sagittal plane, 250-µm-thick vermis slices were cut and transferred at room temperature in an oxygenated standard ACSF. Recordings were performed at 35 ± 1°C on cerebellar slices continuously perfused at a rate of 4-5 ml/min with standard ACSF with picrotoxin (100 µM) and kynurenic acid (5 mM) in order to isolate PCs firing activity.
For statistical analysis, extreme outliers were removed from initial data through Tukey's method for all data sets.
One sample Wilcoxon signed-rank test were applied to statistically compare the normalized frequencies of vehicle sample to the value of 100%. Then, each treatment sample were compared to vehicle group using Welch's ANOVA ($p<.0001$) followed by Fisher's least significant difference (LSD) test. Since values of ISI variances at baseline were not homogeneous then change from baseline has been used to compare treatments *versus* vehicle using Welch's ANOVA ($p<.0001$) followed by Fisher's LSD test..

References

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