Development of a Stand-alone Integrated MEA Biochip System for Chronic Recordings

Paolo De Pasquale[1,2]; Antonin Sandoz [1]; Igor Charvet [1]; Philippe Passeraub [1]; Michela Chiappalone [2]; Luc Stoppini [1]

1 Hepia, HES-SO Geneva, Switzerland;
2 Istituto Italiano di Tecnologia (IIT), Genova, Italy
* Corresponding author. E-mail address: michela.chiappalone@iit.it; luc.stoppini@hesge.ch

Abstract
Stand-alone integrated MEA system were developed and tested to investigate the effect of toxic compounds on network neural activities during chronic recordings. The system, consisting of specifically designed MEA aim to receive microfluidic organic substances and to communicate using wireless technology with the computer device. The system has been tested with several types of 2D and 3D neuronal cultures. The electrophysiological data are processed by NeuroSpy, a modify version of SpyCode. The software developed is able to analyze the basal activity, drug and stimulation evoked response.

1 Background

"Cell biochips" containing engineered tissue interconnected by a microfluidic network allows the control of microfluidic flows for dynamic cultures by continuously feeding of nutrients and waste removal. Thus, these types of systems can enhance functionality of cells by mimicking the tissue architecture complexities when compared to in vitro analysis, but at the same time present a more rapid and simple process when compared to in vivo testing procedures. To date, one of the most promising tools for neuropharmacological functional tests is the Micro-Electrode Array (MEA). MEAs have been applied to study multiple aspects of electrically excitable cells and to explore the pharmacological and toxicological effects of numerous compounds on spontaneous activity of electrogenic cells (A. Novellino, T. J. Shafer et al. 2011). As a complementary tool to standard MEA platforms, we are developing a novel stand-alone system able to acquire, stimulate and feed the cultures. This new approach will keep the cultures inside the incubator providing a useful tool for chronic long-term recordings, with the possibility to continuously submit exposure to drugs or chemicals.

2 Methods and Statistics

We have developed a small-volume in vitro system in which neural cells/tissues can be cultivated in four separate porous membrane microchambers. These are connected by microchannels with the presence of a MEA in each well (Fig. 1). Each MEA, in this configuration, is composed by 8 recording electrodes plus two additional electrodes which can be used for electrically stimulating the cultures through the STG4002 stimulator (MCS). Electrophysiological activities recorded from the 4 integrated MEAs are transmitted through the Wireless System W32 (developed for in vivo applications by Multi Channel Systems, Reutlingen, Germany) and adapted to this particular in vitro application.
The wireless headstage is directly linked to the chip and integrated into the main body of the system. A specific micro-peristaltic pump, developed in order to feed the culture during the incubating period, is controlled by a dedicated software to operate it via Bluetooth or wireless mode.
We developed a modified version of the Spycode software (Bologna et al. 2010), called NeuroSpy (Fig. 2), to perform the analysis of electrophysiological data of neuronal cultures, acquired by MEAs. NeuroSpy allows performing basic and high precision offline analyses . Moreover, the software is able to perform a specific type of analysis aim to study the behaviour of the 2D or 3D neural cultures, as a result of both chemical and electrical stimulation.

Fig. 1. Complete system: MEA chip, wifi system, perfusion pump.

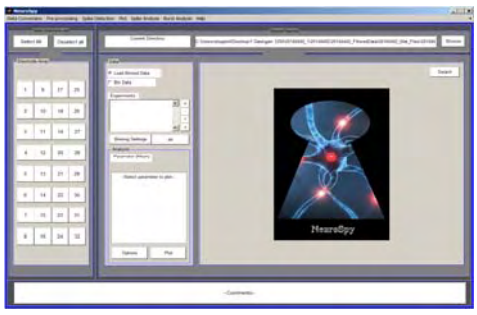

Fig. 2. Analysis software: Neurospy interface.

3 Results

In the first series of experiments, we could record the electrophysiological signals from several types of cells, with the aim at testing the system performance.

Basically, the behaviour of the system has been studied with 2D and 3D cells structure, derived from human sphere stem cells, rat brain cortex, rat hippocampus slices and dissociated cells.

Fig.3 shows a *network burst activity*, recorded from a 3D neural network (derived from H9 or iPs stem cells), through the *raw data* and a *raster plot*. Fig.4 Shows the *Mean Firing Rate* and the *Mean Bursting Rate* from dissociating cells from rat brain cortex. Fig.5 shows the *ISI histogram* relative to the same experiment of Fig.4 and Fig.5.

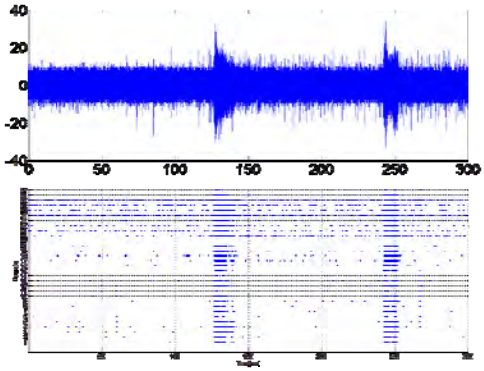

Fig. 3. Electrophysiological activity from a representative experiment of a neural network derived from H9 cells. *Top panel*, raw data recorded from one electrode. *Bottom panel*, raster plot depicting the activity from the 32 channels of the array.

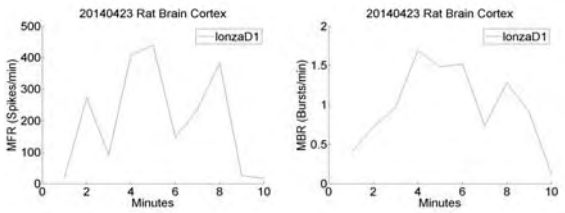

Fig. 4. MFR and MBR from a representative experiment of a neural network derived from rat brain cortex. *Left panel*, Mean Firing Rate (spikes/sec). *Right panel*, Mean Bursting Rate (Bursts/Min).

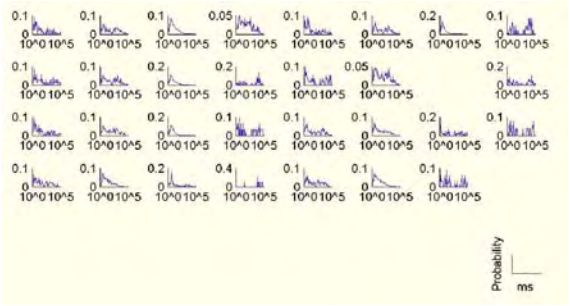

Fig. 5. ISI histogram from a representative experiment of a neural network derived from rat brain cortex

4 Conclusion

These human surrogate integrated Biochips will enable the determination of toxicological profiles of new drug candidates. In addition, the developed system will provide scientists with alternative ways to test more thoroughly new drugs or chemicals. It will also help to understand the process that causes an organism to react and adapt when exposed to drugs over a long timescales.

Moreover, the system will provide scientists with a new independent device able to reduce contaminations factors, due to interactions between the organic components and external agents, thanks to the feeding, recording and stimulating remote wireless control. Scientists will be able to monitor the culture since the first day in vitro, without the risk of expose them to an excessive stress due to the behaviours changing.

Statement of financial support: Supported by the HES-SO, CTI/KTI and the SCAHT.

References

[1] Tieng V, Stoppini L, Villy S, Fathi M, Dubois-Dauphin M, Krause KH. Engineering of Midbrain Organoids Containing Long-Lived Dopaminergic Neurons. Stem Cells Dev. 2014 Apr 3.

[2] Kern I, Xu R, Julien S, Suter DM, Preynat-Seauve O, Baquié M, Poncet A, Combescure C, Stoppini L, Thriel CV, Krause KH. Embryonic stem cell-based screen for small molecules: cluster analysis reveals four response patterns in developing neural cells. Curr Med Chem. 2013;20(5):710-23.

[3] Krug AK, et al. A. Human embryonic stemcell-derived test systems for developmental neurotoxicity: a transcriptomics approach. Arch Toxicol. 2013 Jan; 87(1):123-43. doi: 10.1007/s00204-012-0967-3. Epub 2012 Nov 21.

[4] Sundstrom L, Biggs T, Laskowski A, Stoppini L. OrganDots--an organotypic 3D tissue culture platform for drug development. Expert Opin Drug Discov. 2012 Jun;7(6):525-34. doi: 10.1517/17460441.2012.686488. Epub 2012 May 19.

[5] Bologna, L. L.; Pasquale, V.; Garofalo, M.; Gandolfo, M.; Baljon, P. L.; Maccione, A.; Martinoia, S. and Chiappalone, M.Investigating neuronal activity by SPYCODE multichannel data analayze.Neural Networks, 23 (6): 685-697, 2010.

[6] Chiappalone, M., Novellino, A., Vajda, I., Vato, A., Martinoia, S. & van Pelt, J. (2005) Burst detection algorithms for the analysis of spatio-temporal patterns in cortical networks of neurons. Neuro-computing, 65-66, 653-662.

[7] A. Novellino, Bibiana Scelfo, T. Palosaari, A. Price, Tomasz Sobanski, T. J. Shafer, A. F. M. Johnstone, G. W. Gross, A. Gramowski, O. Schroeder, K. Jügelt, M. Chiappalone, F. Benfenati, S. Martinoia, M. T. Tedesco, E. Defranchi, P. D'Angelo and M. Whelan. (2011) Development of micro-electrode array based tests for neurotoxicity: assessment of interlaboratory reproducibility with neuroactive chemicals. Front. Neuroeng., 27 April 2011