PUBLISHABLE SUMMARY

Project context and objectives

Brain-Computer Interface (BCI) research has seen an explosive growth in the past decade given the major application potential of these technologies. Despite the many advances, there are still a significant number of problems to be resolved before achieving the complete 'brain-computer symbiosis'. Effective communication between brains and computers can only be achieved through the implementation of highly parallel communication channels to and from the brain and through a better understanding of the brain's actual information coding scheme, the adaptive dynamics of its behavior, and the predictive nature of the its sensorimotor internal models.
The ENLIGHTENMENT project consortium aims to develop a combination of advanced multisite, single unit neural activity monitoring, closed-loop patterned and cell specific activations, and computational techniques that would identify cell ensemble activation in real time. We will create a technological platform for directly **interacting with cell assemblies** in a **two-way dialogue**.

The **technological objectives** addressed in this project are:

- Realization of an ***experimental platform combining cell type specific light-induced neuronal activation with multisite extracellular recordings of ensemble activity*** with a probe combining tetrode electrodes for recording and optic fibers for stimulation in the same site. The platform will be configured as a ***closed-loop approach*** thus being capable of reading instantaneous ensemble activity, detecting "important" activity configurations, and consequently driving an array of independent optical stimulators with specific patterns. This should be obtained within an implant of small size, compatible with freely moving experiments in rats and mice. Selective expression of the ***optogenetic channels*** (Channelrhodopsins (ChR2) and archaerhodopsin-3 (Arch)) in specific cell populations is obtained by viral vector transduction.
- Development of ***methods allowing to infer functional interactions between neurons from the recording of the concerted activity of the neural population, in response to a given stimulation***. We will develop robust and efficient learning algorithms for Boltzmann machines, which deduce interactions from the correlations in the activity, and inverse Integrate-and-Fire (IF) approaches, which infer the interactions of a network of IF neurons from their spiking times. This will allow us to characterize how interactions are modified in response to specific stimulation patterns. The algorithms must be fast enough that the outcome can be used to periodically update the stimulation protocol during the experimental session.

Main results

We summarize here the results obtained during the 2 project years. In the 1st project year, consortium partners have developed a first **prototype allowing light activation to be combined with multi-site tetrode-based recordings** of neural activity and demonstrated

a system capable of delivering light stimulation based on online spike detection. This included a **32-channel acquisition system** with **online spike-sorting** capabilities as well as a **control system** running **custom developed software** on Labview allowing closed recording/stimulation loops. The signal analysis is performed online through the implementation of a template-matching algorithm. Specifically, the neural activities are simultaneously recorded, detected, classified online (through spike sorting) from 32 channels, and used to trigger a light emitting diodes light source using generated TTL signals. The system performance was evaluated/validate in vivo in awake, freely moving rodents. A total processing time of **8 ms** is achieved, suitable for optogenetic studies of brain mechanisms online. The details on the realized architecture, signal processing and in vivo evaluations are given in T. Nguyen, Z. Navratilova, H. Cabral, L. Wang, G. Gielen, F. P. Battaglia, C. Bartic, *'Closed-loop optical neural stimulation based on a 32-channel low-noise recording system with online spike sorting'*, *Journal of Neural Engineering* (doi:10.1088/1741-2560/11/4/046005)

The main components of the closed-loop system are (see figure below): a head-mounting board that amplifies, multiplexes and digitally transmits the neural activity data to the digital I/O board, (2) a controllable optic set-up, (3) a data acquisition card providing the digital I/O and analog output pulses, and (4) a custom software that controls the acquisition, visualization, and analysis of the recorded signals. With the current version we can perform spike detection on 32 channels and control the optical stimulation (based on analyzed data) within 8 msec.

In the 2nd year, we have further expanded the recording capacity to **96 channels** (24 tetrodes) and a microdrive allowing precise positioning of both tetrodes and optic fibers has been realized and successfully tested in *vivo*.

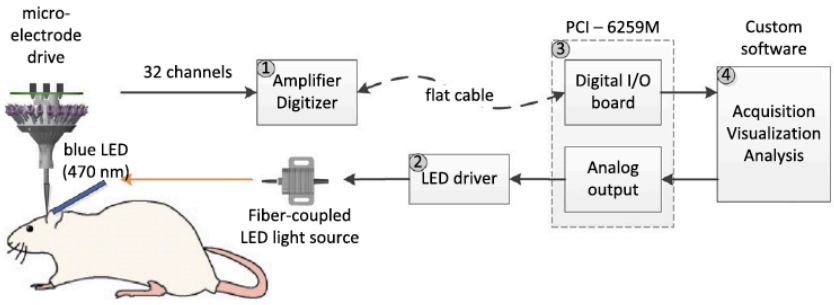

Figure 1. The implemented closed-loop system architecture. (1) A headstage acquires neural signals from 32 electrodes, amplifies, multiplexes, digitizes and sends them to the digital I/O board. (2) A LED driver controls the fiber-coupled LED light source. (3) A data acquisition card (National Instruments PCI-6259M) interfaces the digital and analogue inputs/outputs. (4) A custom software controls the acquisition, visualization and analysis of the recorded signals.

For **online activity detection**, we have implemented spike-sorting algorithms on both CPU and graphics processing unit (GPU) that contains hundreds of processing cores. The GPU can provide highly parallel computational capability, thus accelerating numerical signal processing in a real-time system. In the multi-channel signal recording system, individual channels (or tetrodes) can be treated independently, which leads to a well parallelizable problem. The GPU-based spike-sorting program has been successfully integrated into the current 32-channel recordings system. In order to evaluate its

computational speed, the system has been evaluated off-line with data recorded in the animal experiments. The performance is certainly sufficient for the real time operation of a closed-loop system with more than 128 channels and 25 kHz sampling rate. The system architecture and performance evaluation results have been presented at the 3rd International Symposium Frontiers in Neurophotonics, October 1-4 2013, Bordeaux France and at the SPIE Photonics Europe, April 15-17, Brussels, Belgium (L. Wang, T. Nguyen, H. Cabral, B. Gysbrechts, F. Battaglia, C. Bartic ,'*Closed-loop optical stimulation & recording system with GPU-based real-time spike sorting'*, in *Proc. SPIE* 9129, Biophotonics: Photonic Solutions for Better Health Care IV, 91293U (May 8, 2014); doi:10.1117/12.2052098).

In collaboration with Scientifica UK, partners at the University of Antwerp have constructed a system for localized laser photo-activation, based of galvo-mirrors, that can be efficiently combined with planar multi-electrode arrays (MEAs) for closed-loop in vitro neuronal activation based on activity data recordings.

In order to investigate the network activity in cultured neuronal networks and awake rodents, we have developed **viral vectors** allowing efficient and cell-type specific expression of light-activated proteins (in excitatory and inhibitory neurons). *ChannelRhodopsin 2* (*ChR2) and Archaerhodopsin (ARCH)* were shown to be well-expressed in different brain regions in vivo and also efficiently transduced in in vitro cultured primary neurons, using the adeno-associated viral vectors.

In parallel, we have developed methods for the inference of the neural connectivity maps along different lines. First we have improved the Boltzmann-machine inference procedure previously developed by the CNRS from both practical and algorithmic points of view. From a practical point of view we have rewritten the selective cluster algorithm into a versatile and user-friendly C++ code (J. Barton and S. Cocco, *Ising models for neural activity inferred via selective cluster expansion: structural and coding properties*, J. Stat. Mech. P03002 (2013)). This algorithm was made also faster by as series of improvements (U. Ferrari, G. Tavoni, F.P. Battaglia, S. Cocco, R. Monasson, *'Inferred Ising model unveils potentiation of pairwise neural interactions and replay of rule-learning related neural activity'*, BMC Neuroscience. 2013; 14 (Suppl 1) P276). We have then applied this inference algorithm to the analysis of the activity in the prefrontal cortex of a behaving rat. We have compared the connectivity maps found for three different phases: the awake phase, where the rat is learning a task, and two sleep phases, before and after learning. We have found that the discrepancies between the sleep connectivity maps can be related to the awake map in several experimental sessions: for some pairs of cells effective connections that were weak in the sleep phase prior to learning are potentiated in the awake and in the post-learning phases. Potentiated couplings connect a group of cells, which are found to co-activate much more frequently than expected if they were independent. An article reporting those results and our methods to identify cell-assemblies based on the connectivity map approach is now submitted for publication

Cellular and network responses to different light induced stimulation patters have been investigated using the previously described experimental platform in both in vitro and in vivo preparation.

The high-volume recording system and the optical stimulation setup have been used to characterize how neural systems, in particular the cerebral cortex and the hippocampus, respond to optogenetic stimulation. We recorded ensembles of tens of neurons during stimulation and we saw two patterns. First, 470 nm light stimulation of channelrhodopsin transfected neurons causes an increase in firing rate. At the network level, we observe increased oscillations in the gamma (from about 30 to 100 Hz) frequency range. The amplitude and frequency of these oscillations varied with the stimulation strength. While only excitatory neurons where transfected with the opsin, both excitatory and inhibitory effects were observed, highlighting effects at the network level. Interestingly, the effects of the stimulation were state-dependent, with stronger effects observed when the stimulation was applied during sleep, than during active behavior. These data highlight that the effect of optogenetic stimulation should be seen as a perturbation on the ongoing network activity and that the final outcome depends critically on the current network state. The closed-loop setup that we have developed is the ideal tool to study these intricate dependencies.

Expected final results and their potential impact and use

In this project we aim to demonstrate improved spatio-temporal resolution stimulation/recording systems in closed loop operation, develop algorithmic approaches for reliable, online, computer-aided neuronal data analysis as well as create computational models allowing to infer connectivity and to predict dynamic processes in neuronal systems.

Using the developed technological platforms and cellular preparation we will further attempt to control cell assembly activities and in this way reveal mechanisms responsible for dynamic processes in neuronal systems.

Neuroscience will benefit from a system that is capable of perform complex data analyses online, and change the system behavior based on the outcome of such computations. Such systems are of interest for experimental neuroscientists and theoreticians alike, as ideas about dynamical systems, neural networks, and neural computations may be easily translated into a closed-loop stimulation scheme and tested on the biological brain. Our initial results show that state dependencies are crucial to understand the outcome of stimulation, and these dependencies (as well as the effect on behavior) will be explored in the next year of the project.

Additional information on our consortium and publications can be found at:
www.enlightenment-fp7.eu