

Closed-loop in Neuroscience: can a brain be controlled?

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Abstract—An experimental setup capable of stimulating, inhibiting, sensing, processing and analyzing a biological system is presented. The experimental setup is based on open-source, open hardware commercial devices. It can be applied to record and control neuronal activity, for example to establish causal links between neurons and behavior in a closed-loop fashion. Cells or group of cells can be triggered by acoustical, electrical or light impulses. This last case is based on a technique defined as *optogenetics*. The latter is used in an example that reads and controls neuronal activity of a population of neurons in laboratory rats forcing them to transition between neuronal states, illustrates the system developed in this work.

Index Terms—Optogenetics, closed-loop, automatic control, open-source, open-hardware

I. INTRODUCTION

Understanding the Neural Code requires the development of tools that allow causality testing of neuronal activity patterns and behaviors. As a general principle the logical pathway of causality testing would involve preventing and recreating patterns of neuronal activity and evaluating behavioral outcomes associated with those manipulations. Thus for causality testing

it is necessary to develop tools to monitor and control neuronal activity in a closed-loop fashion.

Neurons are connected by transforming electrical activity to metabolic signals, that code behavior, thoughts, motor expressions or emotions. To decipher how such a complex system works it is necessary to develop new tools to control their activity. Controlling a high number of neurons is a task that has not been resolved. In turn, this requires to understand and predict the dynamic behavior of the network. Optogenetics¹ on a closed-loop configuration would potentially allow real-time replay or inhibition of specific patterns of neuronal activity. This research could lead to better and scalable control of complex structures, like cortical areas, that will have basic and clinical applications, for example in invasive therapies to treat epilepsy or Parkinson disease.

Technologies to perform extracellular recordings have been present for decades, experiencing a sustained increase in the number of neurons that can be recorded simultaneously [2]. In the last decade the development of the optogenetics technology enabled millisecond scale optical control of neural

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¹The term *optogenetics* is a biological technique that involves the use of light to control cells in living tissue that have been genetically modified to express light-sensitive ion channels. They are tools designed to control cell's activity which share the common principle of being based in the recombinant expression of light sensitive protein (opsins) in selected cell types. This technique has enabled a millisecond-scale optical control of neural activity and has been selected as the **Method of the Year** by Nature in 2010 [1].

activity in defined cell types. The term optogenetics refers to a series of tools designed to control cell's activity which share the common principle of being based in the recombinant expression of light sensitive proteins (opsins) in selected cell types. In the neurosciences field this approach implies the expression of opsins which constitute ionic channels or pumps which increase or decrease neuron activity when activated.

Therefore, the current capabilities to record and manipulate neurons by means of optogenetic have created the conditions to establish causal links between neuronal activity and behavior. To achieve this goal, electrophysiological-optogenetics closed-loop systems to control the activity of large groups of neurons is required [3], [4].

In this context the term closed-loop refers to an approach by which excitatory or inhibitory optogenetic stimulation of a group of neurons is fed-back by firing patterns. Thus closing the loop implies computing the difference between the observed and the targeted pattern of activity, and updating the optogenetics stimulus to minimize this difference. Several works have proposed electrophysiology-optogenetics closed-loop systems in the last few years. With different approaches, they have achieved closed-loop control neuronal activity *in vitro* [5], [6], in single cells [5], in multiple cells [6], or *in vivo* [6]–[10]. The following references [11]–[13] consider different types of control loops and also the simulation of large optical stimulated networks [14].

Different states of brain information processing are associated with specific patterns of activity in population of neurons. These states can be detected by means of extracellular electrophysiological records such as Local Field Potentials (LFP). They are characterized by oscillatory activity in defined frequencies, ranging from 0.5 up to 500Hz [15]. Therefore LFP could be used as a readout of neuronal activity of a group of neurons.

This approach offers significant computing advantages over direct detection of cell's action potential, which are one order of magnitude faster (1 KHz) than LFP. Here, we developed an experimental setup to control neuronal activity based on brain status, that is, an LFP/optogenetic based closed-loop system. This setup is assembled using commercial devices and consists in execution-time acquisition, processing and data analysis system. It is capable of commanding input signals, either electrical or luminical or even trigger visual or sound excitations. It differentiates from the previous attempts in terms of its flexibility and low cost implementation, in the framework of open-source and open-hardware.

II. METHODS

The system acquires electrophysiological signals, compares the observed with the target ones and computes the error, in order to generate the output signal to optogenetics actuators (Fig. 1).

In this work, the acquisition of electrophysiological signals is performed by custom designed boards based on Intan Technologies microchips. Amplified signals are streamed *via*

a USB interface using Future Technologies Devices International (FTDI).

The opsin chosen was the excitatory fast variant oChIEF which reliably follows light pulses at high frequencies (> 20 Hz) [16]. Optogenetics excitation is performed by a blue laser commanded by the Field Programmable Gate Array (FPGA) output signal.

A. Electrophysiological experiment

The experiments which illustrate this setup (see section IV and Fig. 1) were performed in Wistar male rats (Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina) weighting 300-350 g. Rats were injected with a virus carrying the opsin gene fused to a fluorescent protein sequence in the Medial Septum/Diagonal Brocca Band area (MS/DBB). After three to five weeks; electrophysiological experiments were done. Briefly, animals were anesthetized with urethane (1.4g/kg) and local anesthesia was applied in all pressure points and scalp (durocaine 0.5%) and placed in an stereotaxic frame. LFP recordings were performed by means of four single wires (tungsten, 50 μ m diameter) from the CA1 region of the hippocampus (AP: -3.6 mm from bregma; ML: -2.6 mm from bregma; DV: -2.2 mm from brain surface). A 200 μ m thick fiber optic was placed with an angle of 20°, at (AP: +1.2 from bregma; ML: -2.8 from bregma; DV: -6.7 from brain surface) to illuminate the MS/DBB. At the end of the experiments, the whole brain was removed and fixed in paraformaldehyde 4% PBS for almost 24 hs. After that, the brain was cut into coronal slices of 100 μ m in thickness, in order to determine the location of the electrodes and the fiber optic, and to assess the viral expression (see Fig. 2).

The laser used in this experiment for optogenetic excitation, was triggered by an external acquisition board commanded by the PC software.

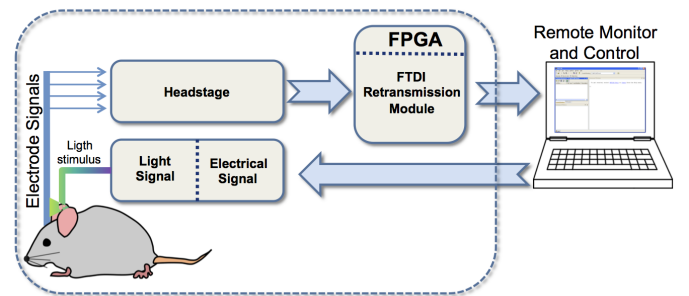


Fig. 1. *In-vivo* closed-loop optogenetic setup.

III. RESULTS

A real-time acquisition, processing and data analysis system is described. It can command input excitations: electrical, luminical, visual or sonic. To this end, the RHA2116 Evaluation board [2] low cost embedded platform is considered and combined with software programmed in Labview. The INTAN

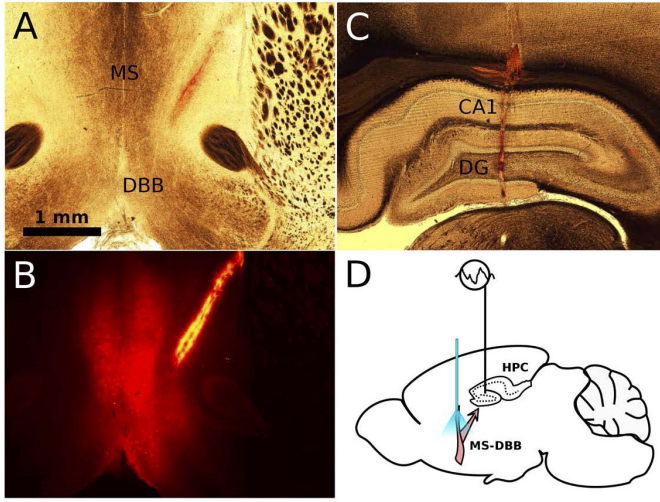


Fig. 2. Representative example of light stimulation and LFP recording areas. A, B. Transmitted light (A), and fluorescence (B) pictures of a coronal section of the medial septum/diagonal band of Broca (MS-DBB) complex, showing the localization of the viral infection (AAV2/8-hSyn-oChIEF-tdTomato) and the trace of the optic fiber (DiI). C. Coronal section of the hippocampus, showing the trace of the 4-electrodes array across cornu ammonis 1 (CA1) and dentate gyrus (DG) regions. D. Sagittal diagram depicting the approximate location of the stimulation and recording sites.

software and hardware used here have been developed within a free and open-source/hardware paradigm. Furthermore, a link for the Labview programs developed by the authors can be distributed freely. The combination of an embedded acquisition system with online processing software allows the automatization of *in-vivo* electro-physiological experiments, e.g. spectral analysis of LFP [6].

Platform RHA2116-Eval is composed of two clearly different elements. The first one is a head capable of acquiring the electrode signals (inputs). This headstage has a 16 channel multiplexed analog amplifier (integrated circuit RHA2116) with a constant voltage gain of 200. Each channel can be accessed sequentially (default) or randomly, depending on the user's choice selected through the configuration pins. These values are digitalized (16 bits AD7980 converter) and transmitted through a Serial Peripheral Interface (SPI) and latter captured by a FPGA (Xilinx-Spartan). The latter is defined as a retransmission module, which formats and streams the data to the PC by means of FTDI through a USB connection (virtual COM). This is also used for remote monitoring.

In addition, six auxiliary external digital signals can be added, for example, to include synchronic marks to a visual stimulus while studying vision nervous cells. All these data are configured in 3 byte packages for each electrode, with an additional auxiliary signal information package. Therefore, 48 bytes should be considered for a 16 electrode input transmission with 6 auxiliary channels. Fig. 3 shows the retransmission module, the headstage, the connectors and the fiber optic and recording electrode detail, used in the experiments.

Finally, the data received by the PC are formatted for their

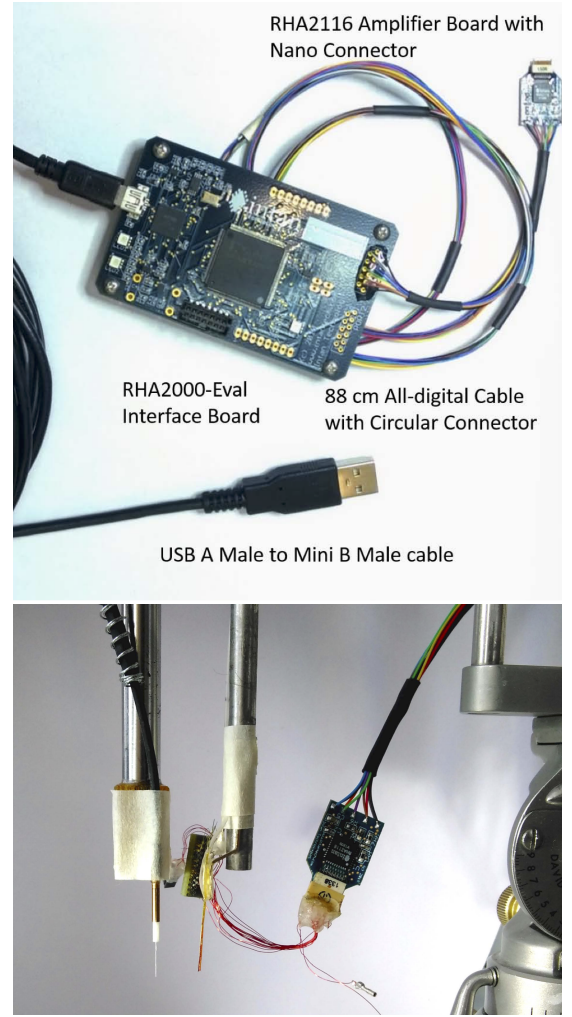


Fig. 3. (upper) Intan RHA2116-Eval platform (head and retransmission module) and (lower) close-up of the setup with the fiber optic and recording electrode.

visualization, processing and analysis.

IV. APPLICATION EXAMPLE

Here an application example illustrates the use of the soft/hardware setup for data analysis and control. It implements a closed-loop procedure to detect and control transitions between two different states of the hippocampus. Selected LFP states are δ and θ rhythms. The δ state is characterized by high amplitude oscillations of 0.5 to 2 Hz, and is observed during slow wave sleep and during repose. In turn, θ is a lower amplitude oscillation at 4-8 Hz that is thought to be relevant for memory acquisition and is associated with REM sleep [17], [18].

Both δ and θ states could be readily observed in urethane anesthetized animals [19], being δ the most prominent. In order to induce δ to θ transitions the system generates an output to a blue laser that activates oChIEF at the MS/DBB, a manipulation previously shown to induce transitions to θ state [20].

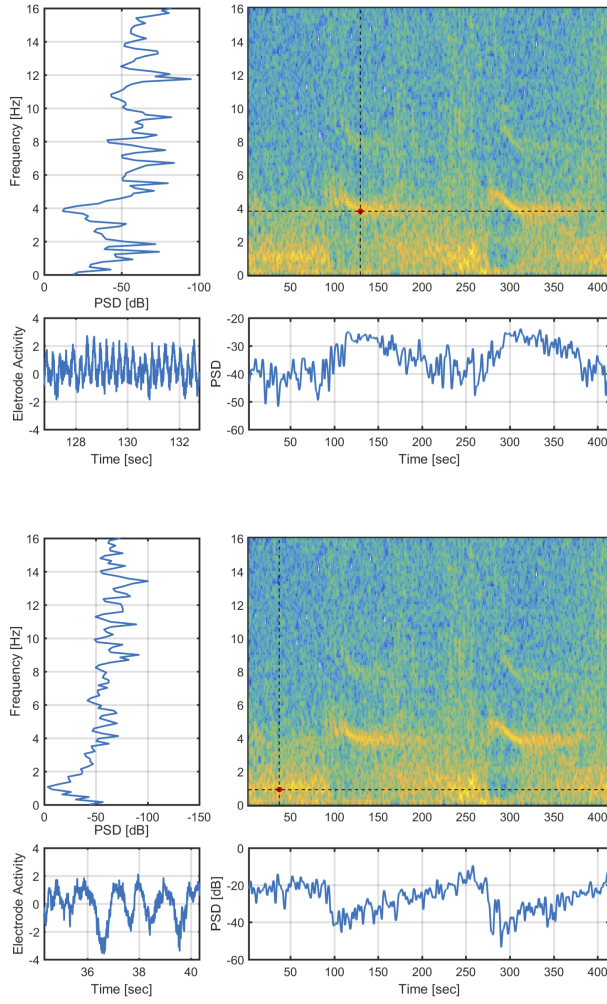


Fig. 4. Analysis of a acquired/registered signal in a θ -state (upper) and δ -state (lower) situations. In both, the specific analysis time is indicated with the red point in the spectrogram. In each plot, the upper-left diagrams represent the FFT of the signal interval centered at the analysis time. The time evolution of the analyzed point is indicated in the lower-left plot. The lower-right plot represents the temporal evolution of the red point frequency in the spectrogram. The upper-left and lower-right plots, indicate the horizontal and vertical cuts respectively, around the red point in the spectrogram.

In this example, an electrode channel is selected, acquired and finally decoded in order to analyze its Power Spectral Density (PSD) for selected frequency bands. In this way, δ and θ states are detected (Fig. 7). In this particular case, the PSD of the δ and θ rhythms are compared, each defined approximately in the 0.5–1.5 Hz and 2.5–7 Hz bands, respectively. A comparison among both PSD is made to decide the current state. The band limits can be tuned by the user in each particular experiment. Fig. 4 shows two different records of a registered signal. The upper one, at approximately 127 seconds, indicates that the rat is in the θ -state. The lower one, at approximately 37 seconds indicates that it is in the δ -state. Both figures show the time-frequency representation (spectrogram) around a particular analysis point.

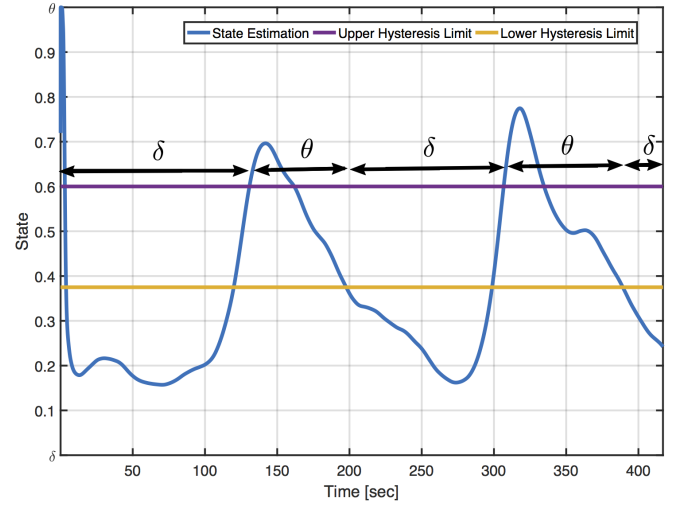


Fig. 5. Estimation procedure applied to the experimental signal. The hysteresis limits are also plotted. This figure corresponds to the same experiments illustrated in Fig. 4.

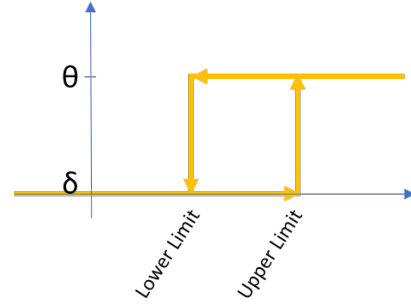


Fig. 6. Hysteresis cycle used in the state estimation procedure to avoid high frequency switching.

As a proof of concept we will keep the system in one of the detected states (θ), taking advantage of the possibility to induce the θ rhythm in the hippocampus by activation of the MS/DBB [21]. This maintains the animal in a particular brain state in a secure way, to prevent its prolonged exposure to the stimulus. This is important to evaluate the effects of different brain rhythms and its consequences in cognition.

The proposed procedure is detailed below:

- 1) Acquire and decode the signal.
- 2) Apply two different frequency band filters to the raw signal. One is a bandpass 6-th order Butterworth filter that recovers the θ -band, and generates a time signal $e_\theta(t)$. The second applies the same process to recover the δ -band, obtaining $e_\delta(t)$.
- 3) Each current RMS value is calculated from the filtered signal. The filtered signals in each band are e_δ and e_θ and the powers are $\delta_{PSD} = RMS\{e_\delta\}$ and $\theta_{PSD} = RMS\{e_\theta\}$, respectively.
- 4) An *estimation index* is computed as a relative PSD

measure between bands, as follows:

$$\tilde{s} = \frac{\theta_{PSD}}{\theta_{PSD} + \delta_{PSD}}$$

Fig. 5 shows the result of the estimation procedure and the index from the experimental signals used also in Fig. 4.

- 5) The RMS relationship obtained is smoothed using a low pass second order Butterworth filter. In this way, the transition dynamics can be tuned during the experiment.
- 6) For the state detection and switching between states, an hysteresis cycle is implemented to prevent high frequency commutations. The $\delta \rightarrow \theta$ transition is achieved once the high level limit is crossed. The $\theta \rightarrow \delta$ transition is achieved once the lower level is crossed, as indicated in Fig. 6.
- 7) Once the state detected is δ , the stimulus is activated to modulate the transition $\delta \rightarrow \theta$. To this end, the light is applied to the MS/DBB. As a stimulation pattern, a rectangular signal of 15 Hz and 10 msec pulses is commanded through a USB board (NI USB-6212). The signal is generated in an auxiliary loop, and by the use of local variables, it is activated or de-activated depending on the analysis criterion, in this case, the detected state. The use of multiple loops running in parallel threads, is an advantage of using Labview.

In addition, other tools can be implemented in this Labview-based acquisition system. For example, a simultaneous command which inserts a stimulation signal or a user-tunable notch filter that attenuates the noise interference. The system has also the option of eliminating constant values which are added when acquiring the information due to intrinsic characteristics of the amplifier. Overall the previous assembly composes a closed-loop system as indicated in Fig. 1. Fig. 7 shows the transition time between both states, i.e. $\delta \rightarrow \theta$ in different time scales.

V. CONCLUSIONS AND FUTURE RESEARCH

Here we present an acquisition system based on a low cost commercial platform built within an open source and open hardware paradigm. The proposal, has the possibility to analyze signals at run-time, which can trigger stimuli actions in closed-loop experiments. This is illustrated by means of an application example in the area of Neuroscience.

The versatile hardware support of this work is an interesting feature to highlight. For example, cameras, serial communications, sensors and other acquisition platforms could be added. This allows to acquire and analyze electrophysiological signals combined with images from a camera simultaneously with stimuli triggered by optical signals. In this case, a trade-off should be established between this flexibility and the computation time.

Future research in this area will focus on the signal processing stage, either for model identification or control [22]–[24]. Both could be achieved by using a FPGA that provides the necessary features for large and complex dynamical system

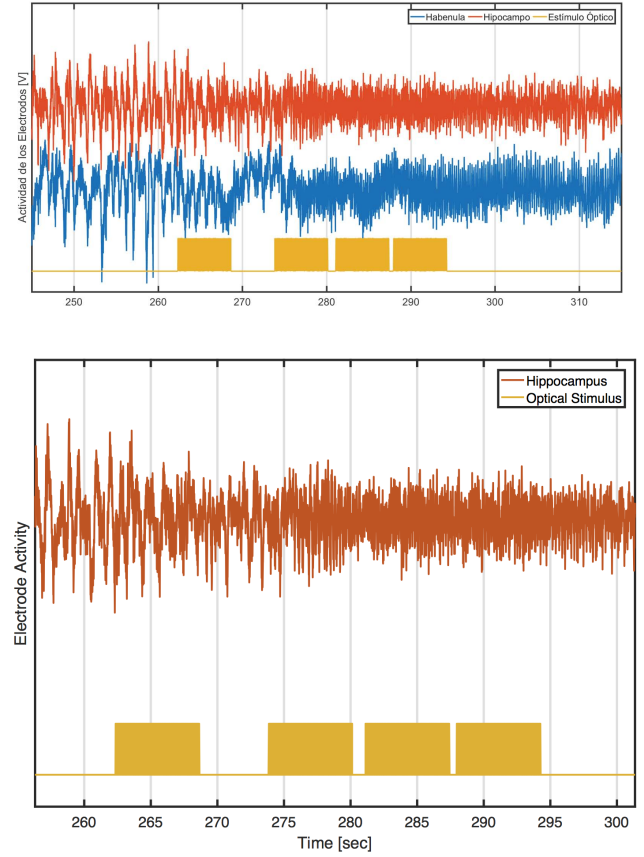


Fig. 7. (Upper) application example on an anesthetized rat. (Lower) a detail between 260 and 300 seconds of the previous plot. A safety protocol was applied to avoid long exposures to excitation signals.

treatment, due to its high processing speed and computing performance. Hardware suited for these type of applications based on FPGAs, are provided by National Instruments (NI), in particular, the Compact-RIO family and the FlexRIO FPGA modules for PXI. Both, based on FPGA technologies, take advantage of Labview Programming.

In addition, identification techniques based on subspace methods [25], [26] will be applied to large number of neurons, via LFP. By using Hankel norm model reduction [22], [23], control-oriented low order models could be used for design purposes. Due to the fact that the problem at hand has a substantial model uncertainty and nonlinearity, \mathcal{H}_∞ , Linear parameter varying (LPV) or switched-LPV controller design methods could be instrumental to achieve good performance [22], [23], [27].

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