

Implementación de un Modelo Funcional del Registro Extracelular Neuronal en Ratas Wistar en
el Laboratorio de Neurociencias de la Facultad de Salud

Henry Andres Mancipe Arias, Luisa Fernanda Torres Acelas

Trabajo de Grado para optar al título de Ingeniero Electrónico

Director

Julián Gustavo Rodríguez Ferreira

PhD en Astrofísica

Co-Directores

Sergio Andrés Conde Ocazonez

PhD en Neurociencia

María Carolina Velásquez Martínez

PhD en Fisiología Humana

David Eugenio Forero Martínez

M.Sc. en Ciencias de la Información y las Comunicaciones

Universidad Industrial de Santander

Facultad de Ingenierías Fisicomecánicas

Escuela de Ingenierías Eléctrica, Electrónica y de Telecomunicaciones

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Resumen

Título: Implementación de un Modelo Funcional del Registro Extracelular Neuronal en Ratas Wistar en el Laboratorio de Neurociencias de la Facultad de Salud *

Autor: Henry Andres Mancipe Arias, Luisa Fernanda Torres Acelas **

Palabras Clave: Spike, potencial de campo, potencial de acción, EEG, Ratas Wistar, adquisición, RNI, laboratorio, registro.

Descripción: Aquí describimos el proceso de diseño, implementación y validación de un algoritmo automatizado y eficiente de detección de picos para el registro extracelular de múltiples sitios localizados y el análisis de la actividad neuronal en ratas Wistar para el laboratorio de neurociencias y comportamiento UIS-UPB en la Universidad Industrial de Santander. Este sistema extracelular multisitio existente está compuesto por una placa de evaluación RHA2000-EVAL con un amplificador RHA2116 y una matriz de electrodos. Los primeros registros mostraron que las señales estaban dominadas por el ruido, por lo que se propuso llevar a cabo una campaña de validación en el entorno de las instalaciones de laboratorio para determinar las posibles fuentes de ruido. Se implementaron mediciones de resistencia de puesta a tierra, radiación no ionizante y espectro electromagnético. Para procesar las señales adquiridas, se diseñó una interfaz gráfica de usuario desarrollada en Matlab, manteniendo los principios de diseño como ser amigable para el usuario y tener una variedad de herramientas de análisis de datos para finalmente obtener un producto confiable y de bajo costo. Esta interfaz se validó con 3 tipos diferentes señales proporcionadas por el grupo de investigación de neurociencia. Se realizó una prueba de comparación entre los ritmos cerebrales de Theta, validando nuestra interfaz. Los resultados de las pruebas propuestas arrojaron un valor elevado en el sistema de puesta a tierra el cual podría ser la

* Trabajo de grado

** Facultad de Ingenierías Físico-Mecánicas. Escuela de Ingenierías Eléctrica, Electrónica y telecomunicaciones. Director: Julián Gustavo Rodríguez Ferreira, PhD en Astrofísica.

causa del mal funcionamiento de la jaula de Faraday encargada del blindaje del sistema. Por otra parte, las mediciones de radiación no ionizante y espectro electromagnético demostraron que no son una posible fuente de ruido. Se logró determinar que existían problemas en la conexión entre el amplificador y la matriz de electrodos, causando la aparición de artefactos en la toma de los registros.

Abstract

Title: Implementation of functional model of the neuronal extracellular record in wistar rats in the neurosciences laboratory of the health faculty *

Author: Henry Andres Mancipe Arias, Luisa Fernanda Torres Acelas **

Keywords: Spike, field potentials, action potentials, EEG, Wistar rats, acquisition, RNI, laboratory, recording.

Description: Here we describe the process of design, implementation and validation of an automated and efficient spike-detection algorithm for localized, multi-site extracellular recording and analysis of neuronal activity in Wistar Rats for the neurosciences laboratory at Universidad Industrial de Santander. This system is based on an existing multisite extracellular recording system consisting of an RHA2000-EVAL evaluation board with a RHA2116 amplifier amplifier with nano connector from the company Intan technologies. First records showed that signals were dominated by noise, whereby it was first proposed to carry out a validation campaign to laboratory facilities environment in order of determine possible noise sources candidates. Measurements of grounding resistance, non-ionizing radiation and electromagnetic spectrum were implemented. In order to process acquired signals, a graphical user interface developed in Matlab was designed. So a GUI was implemented, keeping design principles like to be user friendly, even to non-specialist users, and to have a variety of data analysis tools to finally obtain a low cost and reliable product. This interface was validated with 3 different kind of signals provided by the neuroscience research group. A comparison test was performed between the Theta brain rhythms, validating our interface. The results of the proposed tests showed also high values on ground current measurement, that it is supposed to be zero. These results led us to suppose an issue on the grounding system which could be the origin of the malfunction of a Faraday cage, used for

* Bachelor Thesis

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the electrical shielding of the system. On the other hand, we will see that measurements of non-ionizing radiation and electromagnetic spectrum showed that they are not a possible noise source. It was determined that there were problems in the connection between the amplifier and the electrode array, causing the appearance of artifacts on acquired data.

Introduction

Due to the importance of getting deep in the understanding of the brain and the way it works, new technologies on the field of bio-engineering and bio-medical engineering has been recently implemented. They try to mitigate the effects of diseases such as epilepsy, alzheimer among others diseases Villalon et al. (2018), Adeli et al. (2003). The research group Grupo de Investigación en Control, Electrónica, Modelado y Simulación (CEMOS) from the Engineering School in Electronics at Universidad Industrial de Santander (UIS) its currently working on the strengthening on this research field, collaborating alongside with the neurosciences and behavior laboratory UIS-UPB (NYC) of the medical school at UIS.

Some research projects carried by the CEMOS group in bioengineering have been focused on the design, construction and implementation of systems for the measurement of physiological variables Conde et al. (2005), Silva et al. (2018) as well as in the monitoring and control of glucose in patients with diabetes Greyeb et al. (2016) among others. The work presented here has been developed as a final career research project, led by last year students, under supervision of their advisors, booth, in electronics and medical schools at UIS. The goal is to contribute to the strengthening on bioengineering research by providing to the scientific community tools that allow solutions to real life problems, and also to advance research in these areas.

One of the main research subjects at NYC research group is to perform experiments in ani-

mal models based on the analysis of electroencephalographic (EEG) signals. This duty seeks to understand the functioning of the brain. An EEG signal, usually in Volts, is a measure of the electrical currents flowing during the synaptic excitations of the dendrites of many pyramidal neurons in the cerebral cortex Sanei and Chambers (2007). The oscillations recording in the EEG could be local field potentials (LFP) which reflect the result of the sum of the postsynaptic potentials generated in the region near the recording electrode, or action potentials (spikes) that are the response of the neurons located around the active tip of the electrode Artieda et al. (2009). In addition, this research group has a neuroscience laboratory, and it is equipped with an electrophysiological signal recordings instrument (RHA2000-EVAL board) IntanTechnologies (2012). The first records showed that acquired signals were dominated by noise (see Figure. 1) since the information (field and action potentials) could not be distinguished easily at first sight.

The company Intan Technologies distributes their acquisition boards with their own acquisition software Intan Technologies (2018) however, it is kindly limited, because users are not allowed to perform signal processing at any level, the software is limited to acquire and plot signals. Another more efficient solutions are found in the market, but at a high cost. Some toolbox for processing neural signals such as EEGLAB Delorme and Makeig (2004) and Signal Processing MathWorks (2019), are open source but it is mandatory for their operation, to have licensed Matlab. A prior knowledge of the programming language Matlab is also a requirement. Using alternative software such as Open Ephys Buschman et al. (2017), it is also limited because there is not possible the use of files with the extension (.int) generated by the registration software. These situations represent

an issue for the NYC group; solving them is one of the main motivations for this work.

We have started this work by conducting a series of studies that seeked to explore the possible noise sources on the acquired signal at the NYC laboratory. A validation campaign of the quality of the laboratory environment was carried out. First, we have measured the quality of the ground resistance, defined as the relationship between the potential of the laying system to ground to measure, with respect to a remote ground and the current that flows between these points. A good grounding, guarantees people's safety, but also instruments and tools protection, likewise the laboratory facilities RETIE (2013).

An RFI study was also carried out (electromagnetic field intensity measurement (non-ionizing radiation) and electromagnetic spectrum measurement). Electromagnetic radiation consists of electrical and magnetic energy moving together through space. The low energy radiations are known as non-ionizing, which are not able to break the bonds of the molecules Martínez et al. (1999) an electrophysiology pilot test was performed on three groups of anesthetized rats to corroborate the correct operation of the signal acquisition system. The analysis of these signals were done through a graphical user interface designed in MATLAB® software with a variety of basic tools and methods in time and frequency, in addition to the creation of a user manual, and finally, the operation of The interface was verified using real electrophysiology data arranged in the NYC laboratory where a Theta brain rhythm analysis was performed.

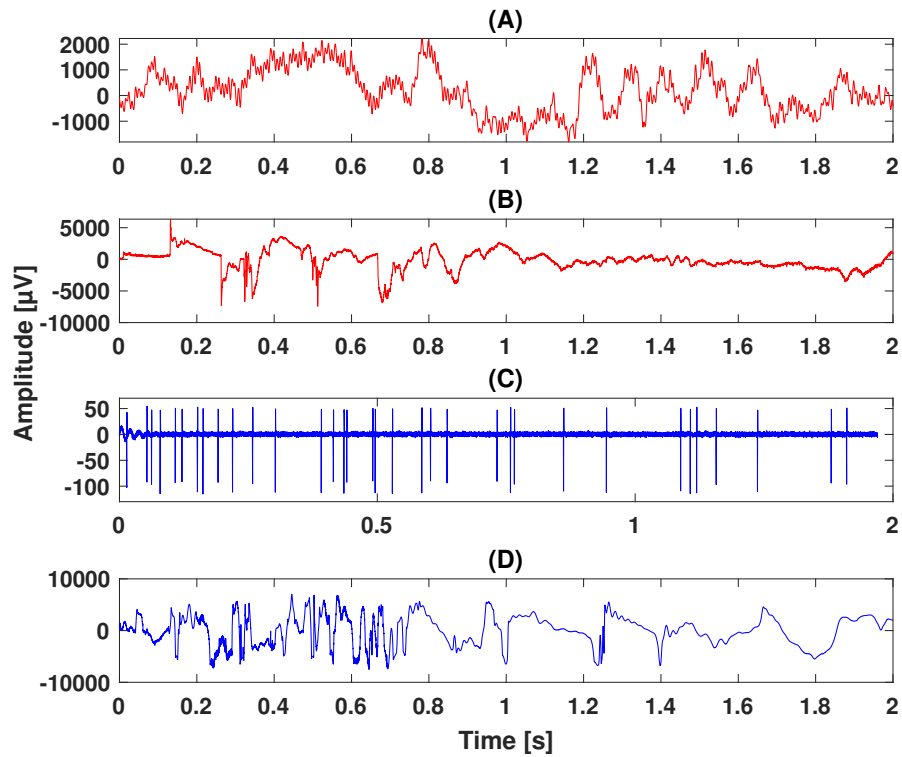


Figure 1. Comparison of noise between field potential and action. (A) and (B) are field potential signals. (C) and (D) are action potential signals. (A) and (C) present the typical expected form; but (B) and (D) are affected by noise. The information can not be visually distinguished from the noise. This situation is an issue for the NYC group, solving it is one of the main motivations of this research.

1. Facilites

1.1. NYC laboratory

To perform experiments in animal models based in the EEG signal analysis, the research group NYC has a laboratory located in the health faculty at Universidad industrial de Santander in Bucaramanga, Colombia. Laboratory facilities are described in Figure 2.

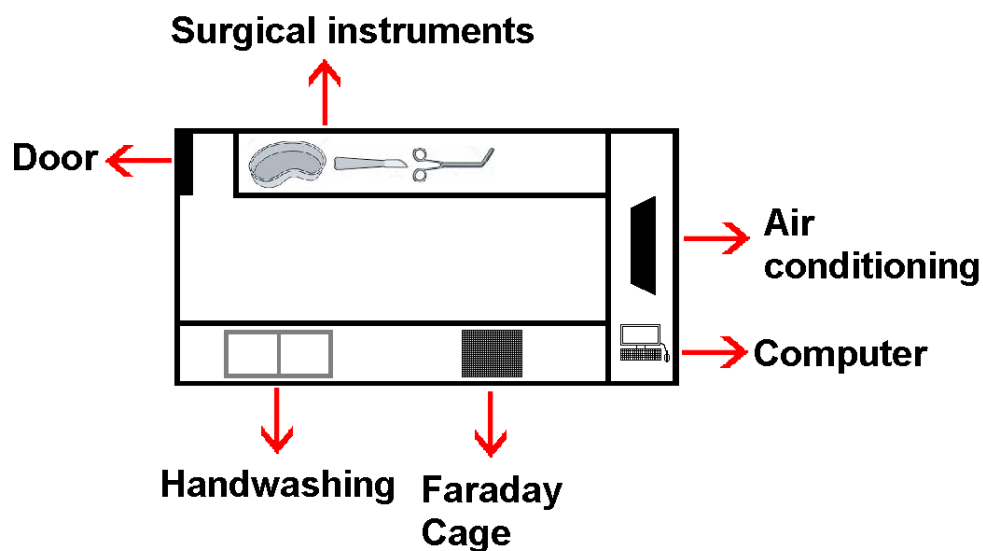


Figure 2. Distribution of the laboratory of neuroscience and behavior

This laboratory has a INTAN electronic board to acquire the EEG signals, the facilities are equipped with all the surgical implements needed to implant the electrodes arrays on Wistar rats. These rats are also provided by their own bioterium. A home-made Faraday cage is used to perform experiments with animals in movement or anesthetized depending the type of study that is desired to do, on one side it serves as an electromagnetic shield to reduce noise on the acquiring signal system; on the other side it serves also as a cage to keep rats. For signal processing, display and

register the system uses a Dell computer with 16GB RAM and a processor core i7 (see Figure 3).

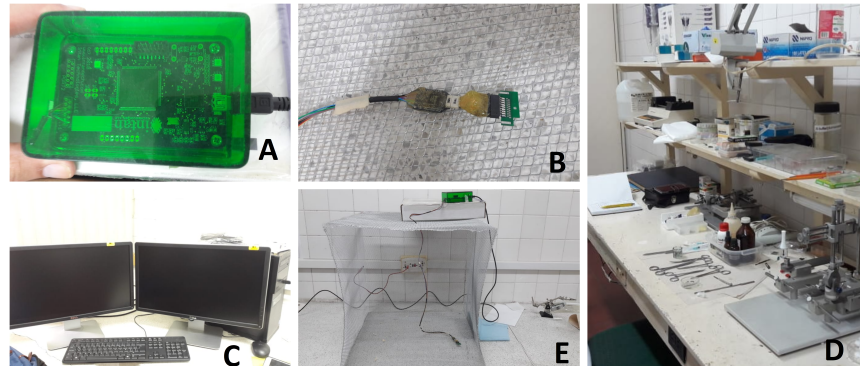


Figure 3. Implements of the NYC laboratory. (A) RHA2000-EVAL board. From the company intan technologies. (B) Connection between the Amplifier RHA2116 with Nano connector and the electrodes matrix through an Omnetics A7944 connector. (C) Computer equipment arranged with the real time signal recorder software. (D) Surgical implements to the implantation of the electrodes on the wistar rats. (E) Faraday's cage to perform experiments in animals.

2. Measuring equipment

2.1. Equipment for the acquisition of electrophysiological signals

The recordings of electrophysiological signals in the neuroscience and behavior laboratory are made by a board distributed by Intan Technologies, the RHA2000-EVAL board with an Amplifier Board (Type 1): RHA2116 with Nano connector of double row of 18 pins (Omnetics A79041), provides access to the 16 amplifiers inputs (in0 - in15), as well as a reference for the channels (ref_elec) and a ground connection (GND). Through an Omnetics A79044 connector, connections are provided by a cable for each channel, which it turns allows the connection with the electrodes matrix. The board includes real-time signal recorder software Intan Technologies (2018) installed in a computer ready to record data.

2.1.1. RHA2000-Eval board and RHA2116 amplifier with Nano connector. The RHA 2000-Eval board (see Figure. 4) with which is performed the electrophysiological signals acquisition have the following characteristics IntanTechnologies (2012):

- Monitor the real time signals of 16 channels in a RHA2116 amplifier chip.
- Each channel is digitalized with an accuracy of 16 bits with a sampling rate of 26 KHz.
- The maximum bandwidth of the amplifiers can be programmed to any value between 10 Hz and 20 KHz, and the minimum bandwidth between 0.02Hz to 1.0 Hz.

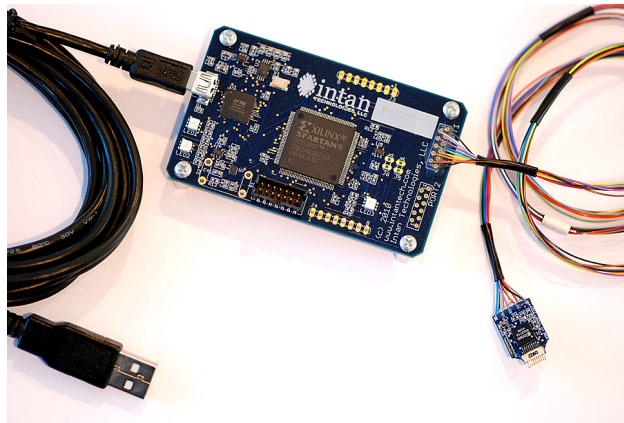


Figure 4. RHA2000-Eval and RHA2116 amplifier with Nano connector. Taken from IntanTechnologies (2012)

2.1.2. Electrodes matrix. The electrodes matrix used consists of 16 channels, a common ground (GND) and a reference (ref_elec) (see Figure 5). The electrodes are made of 50 [μm] tungsten filaments.

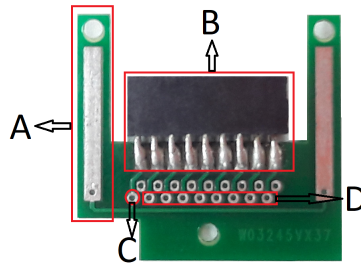


Figure 5. Electrodes matrix. (A) is the ground of the system (GND) (B) 2.54mm dual female pin header socket connectors strip (C) reference for channels (ref_elec) (D) pin header connectors to the first eight channels, arranged for the tungsten electrodes.

2.2. Description of the system used to characterize the electromagnetic environment at the NYC lab facilities

2.2.1. Tellurometer METREL MI2088. The tellurometer(see Figure. 6) was used to perform ground resistance measurements and it has the following characteristics METREL (2002):

- Ground resistance (4 threads method, one tweezer method, two tweezers method).
- Isolation scale: large scale of test voltages to the isolation, from 50 V to 1000, with a measurement scale of the resistance up to 30 G Ω
- Change of polarity: automatic reversal of the polarity in the continuity test.



Figure 6. Tellurometer METREL MI 2088. Taken from METREL (2002).

2.2.2. Spectrum analyzer HMS-X ROHDE Y SCHWARZ. The spectrum analyzer (see Figure. 7) used to perform spectral power measurements of environmental signals to know the levels of noise by spectral band capable to produce interference with the acquisition system. Some equipment characteristics are Rohde and Schwarz (2013):

- Frequency range: 100 kHz to 1.6 GHz/3 GHz
- Spectral purity greater than -100 dB/Hz (at 100 kHz)



Figure 7. Spectrum analyzer HMS-X ROHDE Y SCHWARZ. Taken from Rohde and Schwarz (2013).

2.2.2.1. Vertical antenna tri-band VERT400.

- Maximum power: 10 watts
- Frequencies: 144-400-1200 MHz.
- Connector: SMA.



Figure 8. Vertical antenna tri-band VERT400.

2.2.2.2. Logarithmic antenna LP850.

- Frequency range: 800 a 6500MHz.
- Gain: 5-6dBi.
- Connector: SMA.



Figure 9. Logarithmic antenna LP850.

2.2.3. Narda NBM 550 broadband field meter. The Narda (see Figure. 10) with which is performed non-ionizing electromagnetic radiation measurements have the following characteristics Narda (2013) :

- Automatic zeroing.

- Memory to store up to 5,000 results of measurement.
- Measurement and monitoring of the field strengths around the broadcasting and radar hardware.
- Measurement of the field strength of the transmitters of phones, cellphones and satellite communication systems to demonstrate compliance of the limit values of human security.
- Measures to protect the users of diathermy equipment and other medical devices that generates high frequency radiation.
- Measurement of the field strength in TEM cells and absorption chambers to show the electromagnetic compatibility.



Figure 10. Narda NBM 550 broadband field meter whit BM E-Field-Probe 0391 Antenna.Taken from Narda (2013).

2.2.3.1. NBM E-Field-Probe 0391 Antenna .

- Frequency range: 100 kHz to 3 GHz.

- Measuring range: 0.2 to 320 V / m.
- Gamma dynamic: 64 dB.
- Absolute error: $\pm 1,0dB$.

3. Description graphical userinterface

The interface was designed and programmed with the GUI development environment tool-box of Matlab which provides design tools of user interface for custom Apps, using the student license provided by Universidad Industrial de Santander. Figure 11 shows a screen copy of the main window of the graphical interface. For more information and details, please refer to the user manual in Torres et al. (2019).

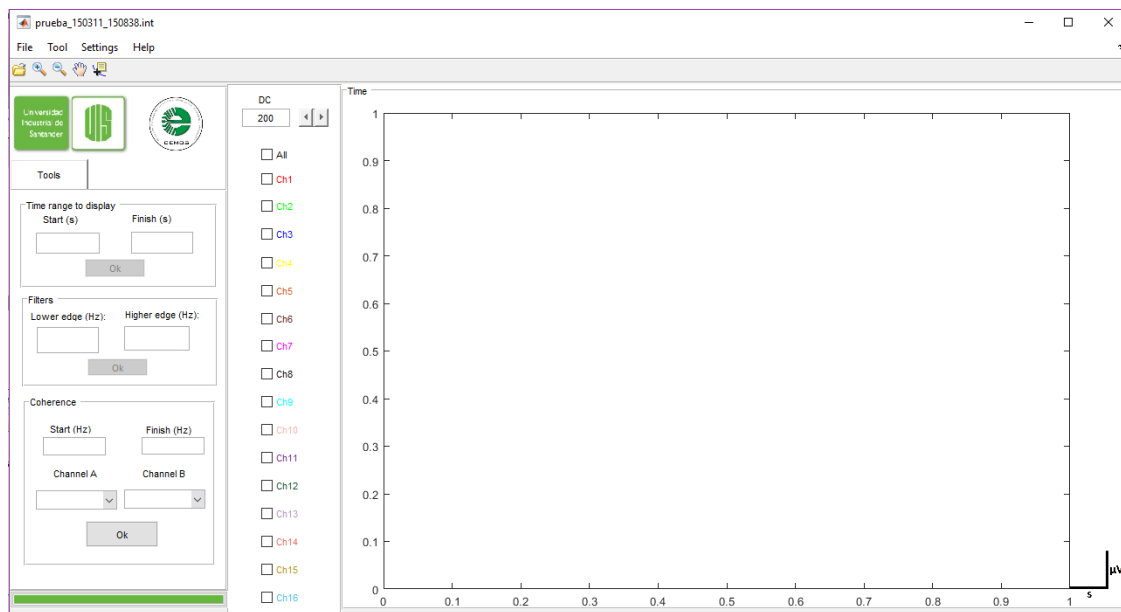


Figure 11. Screen copy of the developed graphic interface.

The designed software was designed not also to acquire and plot signals, but moreover to

be an electrophysiological signal processing tool that lead to non-expert users to perform a data analysis in time or frequency of field and action potential, described as:

3.1. Brain Rhythms

Brain rhythms are periodically fluctuating waves of neuronal activity that are readily observed using local field potential (LFP) recordings Andrés et al. (2016). These rhythms are the following:

3.1.1. Alpha. It is located at frequencies between 8 to 12 Hz. Reflects a state of relaxation, tranquility, start of full activity of the left hemisphere and disconnection of the right hemisphere, this cerebral rhythm is not found in rodents Andrés et al. (2016), Buzsáki (2006).

3.1.2. Gamma. The spectral band covers from 30 to 80 Hz. It is thought that these signals reflects the underlying mechanism of the consciousness and it has been demonstrated that their appear during a variety of memory task in rats, monkeys and humans Buzsáki (2006), Zheng et al. (2016).

3.1.3. Beta. These signals covers the spectral band of frequencies from 10 to 30 Hz. Corresponds to a maximum alert state, watchful or scared. They are generated also on normal situations, for example when we are awake in alertness and anxiety Andrés et al. (2016),Buzsáki (2006).

3.1.4. Theta. This rhythm is observed in frequencies from 4 to 10 Hz. It represents a watchful state, balanced between the left and right hemispheres and anxiety Andrés et al. (2016),Buzsáki (2006).

3.1.5. Delta. A frequency from 1.5 to 4 Hz. Corresponds to a hypnotic state, deep sleep and meditation Andrés et al. (2016),Buzsáki (2006).

3.1.6. Fast Gamma. It is located at frequencies between 80 to 200 Hz. It is studied in rats to investigate how is related with the memories codification to new object-place associations Buzsáki (2006),Zheng et al. (2016).

The implementation of this analysis method requires the use of digital filters (matlab **filtfilt()** function), the order of the filter is variable and depends purely on the number of samples of the signal. To find the average power of these rhythms, it is used a Matlab function **bandpower()**.

3.2. Spectrogram

Represents how the spectral power concentration changes over time.

Spectrogram is defined as:

$$S_x(n, \omega) = |X(n, \omega)|^2 \quad (1)$$

Being the short-time Fourier transform (STFT) is defined as the discrete-time Fourier transform evaluated over a sliding window.

$$X(n, \omega) = \sum_{\tau=-\infty}^{\infty} x(\tau)w(n - \tau)e^{-j\omega\tau} \quad (2)$$

Where the discrete-time index n refers to the position of the window $w(n)$ Sanei and Chambers (2007).

This method uses the function **spectrogram()** of Matlab.

3.3. Power Spectral Density (PSD)

The power spectral density shows how the power changes as a function of the frequency described by the following equation:

$$PSD = \frac{X_{xx}^*(f)X_{xx}(f)}{fs} = \frac{|X_{xx}(f)|^2}{fs} \quad (3)$$

Where $X_{xx}^*(f)$ is the conjugate complex of the Fourier transform of the autocorrelation function $X_{xx}(f)$ and fs is the sampling frequency.

$$X_{xx}(f) = \sum_{k=0}^M corr_{xx}(k)e^{-i2\pi fk/fs} \quad (4)$$

The Turkey's method uses the complex Fourier transform as a function of the autocorrelation function $corr_{xx}(k)$, where M is the maximum delay and fs is the sampling frequency Trauth (2010), Unde and Shriram (2014).

This method uses the **periodogram()** of Matlab.

3.4. Visualization, identification and counting of spikes

The action potential or spike is the functional unit of the brain. The spikes are transient signals of non-stationary short-term broadband with high instant energy and clearly distinguishable Kermani et al. (2012).

Usually in the spikes detection it is used an amplitude threshold (limit) that in most of the systems is manually set. The crossing of events by this limit determines the amount of detected spikes. To select the threshold we had realized an algorithm that determines the average value of the previously filtered signal, at the same time it determines the standard deviation and establish a threshold value equal to n times (value entered by the user) the standard deviations below the average. With the established values, the spikes detection function was performed, which detects and stores the peaks in a vector of temporal components and amplitude, allowing the visualization of the waveforms detected. Thanks to this detection tool, users can study the operation of the cerebral process as memories, emotions, consciousness or sensorial processing.

3.5. Coherence

The coherence is a type of study based on the spectral analysis that can describe the synchronization of the electrical activities in two different areas of the brain at different frequencies and is described by the equation:

$$\gamma_{xy}^2(\omega) = \frac{|S_{xy}(\omega)|^2}{S_{xx}(\omega)S_{yy}(\omega)} \quad (5)$$

Where $S_{xy}(\omega)$ =Cross-spectral density between x and y, $S_{xx}(\omega)$ =Auto spectral density of x and $S_{yy}(\omega)$ =Auto spectral density of y.

The Amplitude of $\gamma_{xy}^2(\omega)$ will have a oscillating value between 0 (without coherence) and 1 (with perfect coherence) Unde and Shriram (2014), Wolpaw and Wolpaw (2012).

This method uses the Matlab function **mscohere()**

3.6. Peri-stimulus time histogram (PSTH)

The PSTH is related with some stimulus of event, dividing the period of time in a series of intervals and counting the amount of spike that falls in every division in all the tests, this information is presented in a histogram or in a bar chart Wallisch et al. (2014).

For this method, we have developed a counting spikes algorithm with time intervals defined by users. To plot the graphic chart we have used the **bar()** function of Matlab.

3.7. Identification of latency from the PSTH

The latency is the time that takes to present the higher concentration of spikes in the PSTH.

4. Description of the validation of the electromagnetic environment at the NYC lab facilities

4.1. Pilot test on Wistar rats.

To the detection of possible error sources in the record of electroencephalographic signals (EEG) a pilot test was performed just like the system seen in Figure 12. Previously, the NYC perform the preparation of the electrodes matrix (see Figure 13) for their subsequence implant in three groups of rats: reactive (R), anxious animals, no reactive (NR) and control (G) can be R or NR.

The process to acquire signals from the rats requires a previous preparation with the animal inside the Faraday cage and the electrodes matrix implanted on his head. Electrodes are connected to the signal amplifier and at the same time to the data acquisition board. A PC is used to visualize and record signals using the software of the RHA2000-EVAL board itself, at the end of this research project this software was replaced by own version developped as a main result of our work.

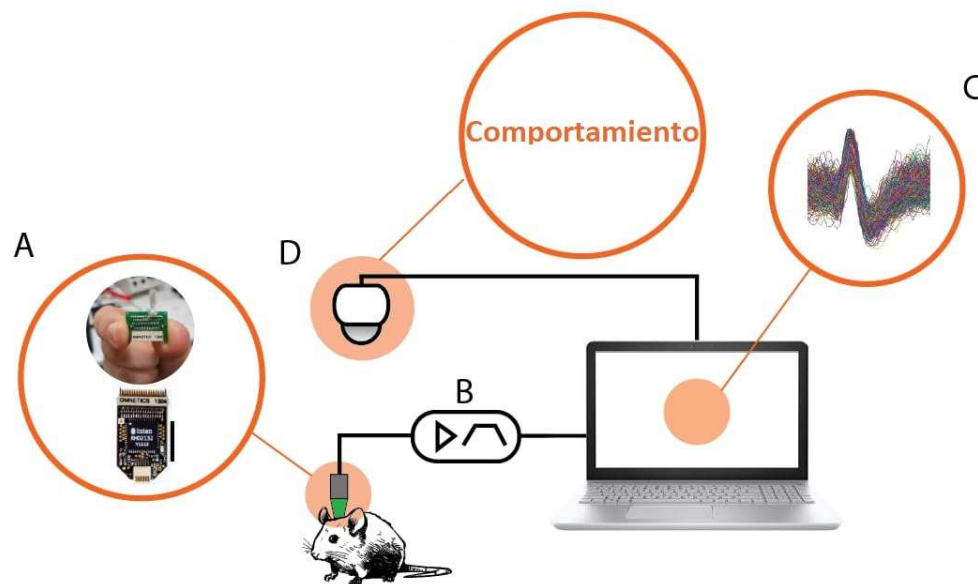


Figure 12. Schematic of a registration system of EEG. (A) The signal is captured by the electrodes of tungsten implanted in the cerebral cortex of the rat that is inside the Faraday cage. This signal is pre-amplified before being transmitted to the amplifier central module. (B) amplification and filtering module of the signal. (C) the neural activity is recording and stored to further analysis. (D) camera that records the physical activity of the animal in video synchronized with the EEG signal record.



Figure 13. Electrodes matrix. Matrix with 8 50mm tungsten electrodes ready for recording, a common ground (GND), a reference for the channels (ref_elec) and a resin coating to fix all the components to their respective place.

4.2. Measurement campaign of non-ionizing electromagnetic radiation (RNI).

In this campaign far field incision measurements were made (electric field strength E), taking into account the measurement protocol established in the ITU-T K.83 recommendation ITU-T K.83 (2011). It was used a broadband field meter Narda NBM 550, along with a NBM E-Field-Probe 0391 antenna isotropic in a frequency range from 100 KHz to 3 GHz. These measurements were made with the goal of determine if the electromagnetic field intensity were into the limits to the human exposure of the recommendation ITU-T K.52 ITU-T K.52 (2004), and if this value could affect the signal recordings system.

Close field incision measurements were not performed, because it was not necessary to measure the radiation level of the magnetic field (H), since both are related by the power vector

(S).

$$S = \frac{E^2}{Z_0} = H^2 * Z_0 \quad (6)$$

Where Z_0 is the impedance of free space ($Z_0 = 377\Omega$) and E and H are expressed in units of V/m and A/m , respectively, and S in units of W/m^2 . ITU-T K.52 (2004).

The Figure 14 shows a scheme of the NYC laboratory, where the marked points (1, 2 and 3) represents the location in which measurements were made with a spatial average.

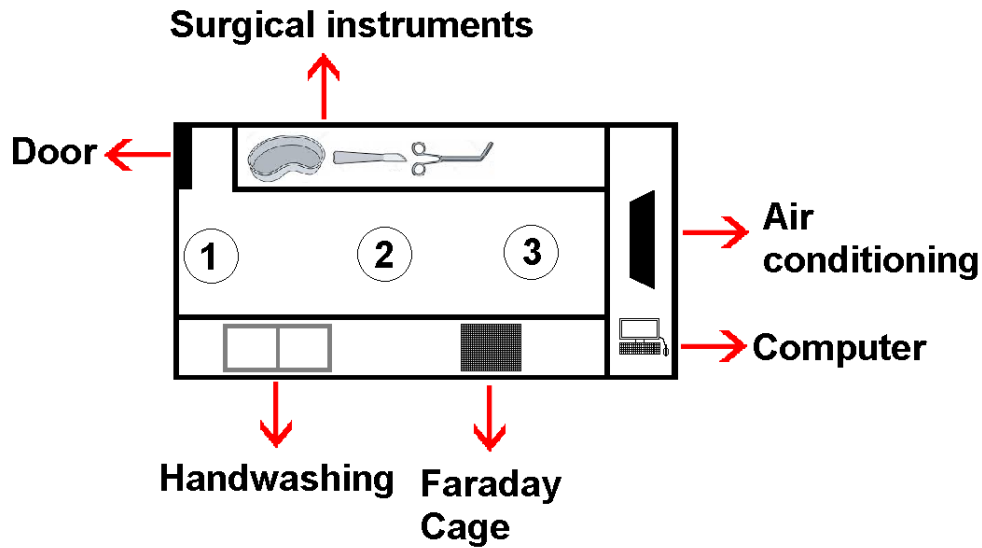


Figure 14. Distribution scheme of the NYC laboratory neuroscience room. The marked dots 1, 2 and 3 show the zones where were made the RNI measurements.

Also, following the recommendations of the ITU-T K.83 ITU-T K.83 (2011), emission measurements were made to know the levels of signals present in each frequency band. It was used a spectrum analyzer HMS-X ROHDE Y SCHWARZ (narrowband instrument), connected to a computer equipment and to an antenna as showed in Figure 15, doing a sweep of 80 MHz to

3GHz. Cover this interval of frequencies required using antennas of different range described in section 2.2.

With the goal of validate the Faraday's cage, a non-ionizing radiation test was performed inside and near the cage, taking into account the aforementioned measurement protocol.

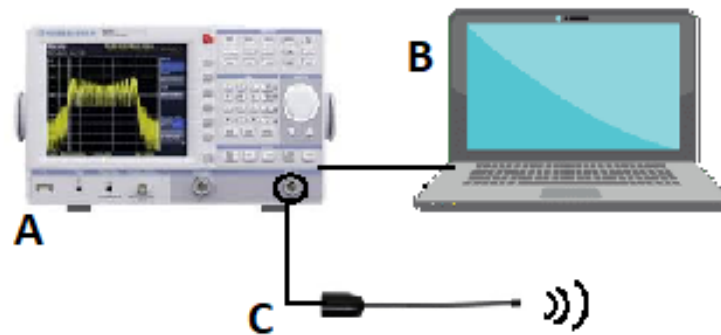


Figure 15. Scheme of measurement of electromagnetic spectrum. (A) HMS-X ROHDE Y SCHWARZ Spectrum analyzer. (B) Computer equipment with acquisition software installed. (C) receiving antenna.

4.3. Grounding measurement

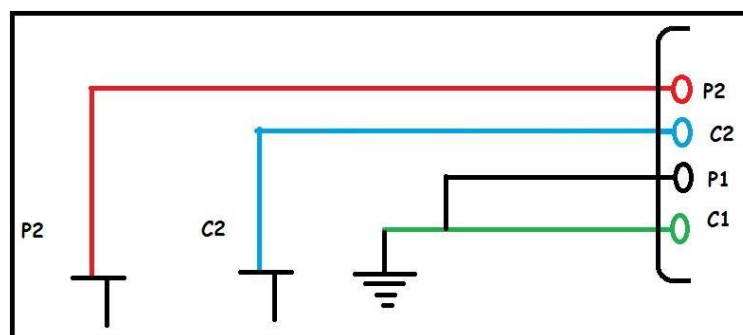


Figure 16. 4 pole method with additional connection between the field to measure (P1) and the measurement instrument (C2). Taken from Chauvin Arnoux (2015).

The soil resistance measurements were made with a tellurometer METREL MI2088, using the 4 poles method, this is based in the same principle of the 3 poles method Chauvin Arnoux

(2015), but with an additional connection between the ground to measure P1 and the measurement instrument (see Figure 16). Thus, this method allows to obtain a better resolution and get rid of the resistance of the cables. The ground measurement is based in the 62 % method, this requires the use of 2 auxiliaries (voltage and current) electrodes (of "stakes") as shown in Figure 17.

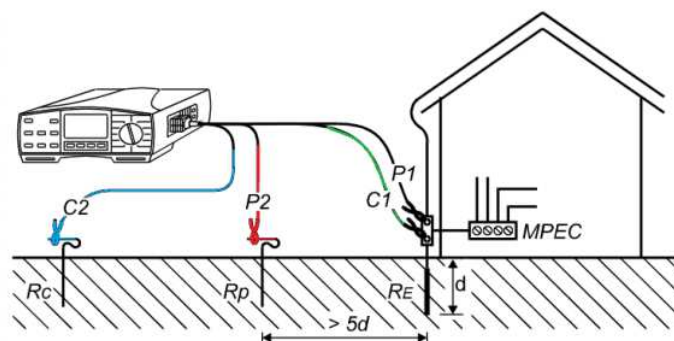


Figure 17. 4-pole method for the measurement of grounding resistance with a METREL MI2088 tellurometer, with P1 and P2 (voltage electrode) and C1 and C2 (current electrode). Taken from METREL (2002).

To calculate the distance of the stakes it was taken the proposed method in RETIE (2013), obtaining the following distances:

Counting with the measure of the electrode or the ground rod of the structure (usually is 2,4 m).

- Distance from the ground to the current stakes C2 = depth (stake or electrode) $\times 5 = 12,4m$
- Distance to the voltage pike P2 (62 %) = distance C2 $\times 0.62 = 7,75m$.

It must be indicating the upper limit value of ground resistance, for them is taken to count the reference values adopted from the electric installations technical regulation (RETIE) (see Table 1).

Table 1

Maximum values of grounding resistance

APLICACION	MAXIMUM VALUES OF GROUNDING RESISTANCE
Structures and metal turretsof lines or networks with guard cables	20 Ω
Substations of high and extra high voltage.	1 Ω
Medium voltage substations.	10 Ω
Lightning protection.	10 Ω
Neutral connection point in low voltage.	25 Ω
Networks for electronic or sensitive equipment.	10 Ω

Note: Adapted from RETIE (2013).

At the time of making the upper limit it was adjusted, since the output value was higher than the previous table.

4.4. Working test of the graphic interface with real data.

To perform the validation test of the developed interface, the research group NYC provided us real data of electrophysiological signals (EEG) of three groups of rats: Reactive (R) anxious animals, non-reactive (NR) not anxious animals and control (G) can be R or NR. We took an specimen of each group and 2 minutes of its own record (one minute at the beginning and one minute at the end of the test) in eight channels to which is made a calculation of the Theta rhythm (4 to 10 Hz), since this is the oscillatory activity more studied in the hippocampus of rodents, in which during the Theta locomotors behaviors shows how a high power peak between 4 and 10 Hz Colgin (2016).

In this validation test, the following parameters were analyzed: instant power, average power and frequency in the interval of 2 seconds, choosing the channel with the strongest average power. Also, the PSTH (width of 0.5 seconds) was performed, and spikes were detected and visualized with a threshold of three standard deviation below the average. To assure the spike detection, filtering of the signals between 700 and 2000Hz was necessary to delete the low frequencies components.

5. Measurements results

5.1. Results of the measurement campaign of non-ionizing electromagnetic radiation (RNI).

To the broadband non-ionizing measurements, it was made according to the measurement protocol established in the ITU-T K.83 rule. These measures were made in 3 different zones of the laboratory, obtaining values of the spatial average of the electric field, from these measurements in the magnetic field and the power spectral density in equation (6), in Table 2 the appropriate result is shown.

Table 2
Spatial average of RNI in the NYC laboratory

Zone	Electric Field [V/m]	Magnetic field [A/m]	Power density [W/m²]
1	0.69	1.83e-03	1.26e-03
2	0.93	2.46e-03	2.29e-03
3	0.87	2.31e-03	2.01e-03

This results are compared to the maximum value allowed in the ITU-T K.52 by the general public ($61V/m$, $0.16A/m$ and $10W/m^2$), it is not found in higher values.

The broadband tests made inside and in the surrounds of the Faraday cage, this test gave the

following results: in the inside 0.42 [V/m] and in the vicinity 0.92 [V/m] with attenuations of 54.36 %. The narrow band measurements made with the spectrum analyzer for a sweep of 80MHz to 3GHz, it throws the following most important results: Broadcasting frequencies by modulated frequency FM (88MHz to 108MHz) shows signals of maximum levels of -58.33 dBm and a standard deviation of -64.06 dBm in the vicinity of the Faraday cage (see Figure 18) at a frequency of 95.7MHz.

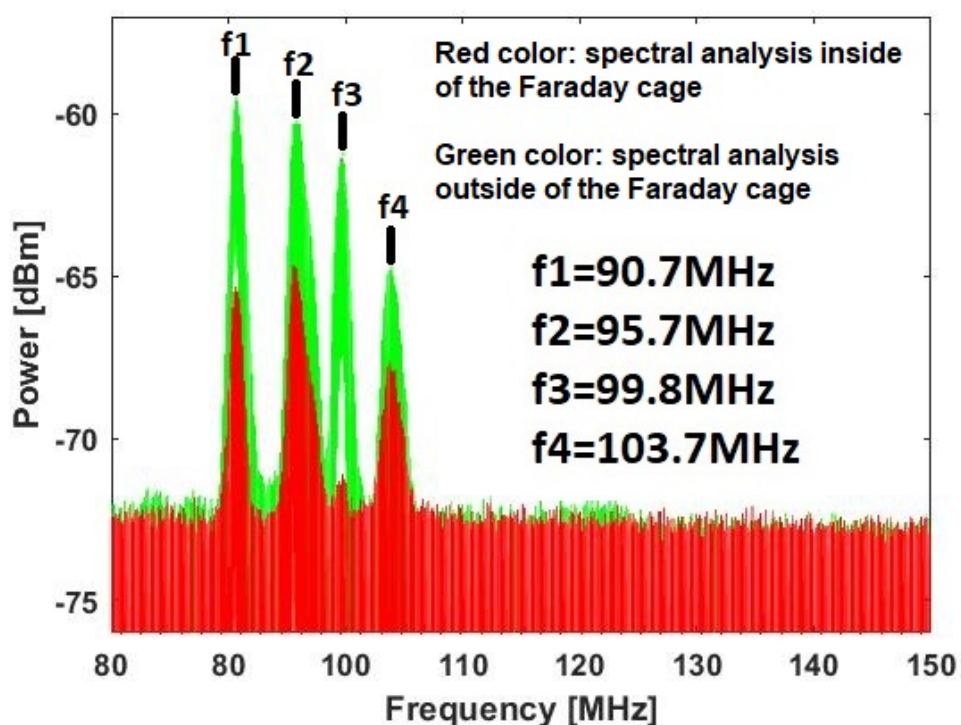


Figure 18. Spectral analysis between 80MHz and 150 MHz inside (red) and outside (green) of the Faraday cage, for an average of 5 measurements per spectral band. The signals f1, f2, f3, and f4 correspond to the spectrum of some local stations in FM.

At the same frequency inside of the Faraday cage it was obtained a value of 64.61 dBm with a standard deviation of -65.27 dBm having a attenuation of 25.54 %.

5.2. Pilot test results.

The execution of the pilot test allowed evaluating failures in the connections, errors in the data collection and noise presence in the acquired signals.

The electrode connection implanted in the rats was not fixedly attached, since any sudden movement of the animal caused a loss of connection with the amplifier, generating in the record the appearance of noise (see Figure 19).

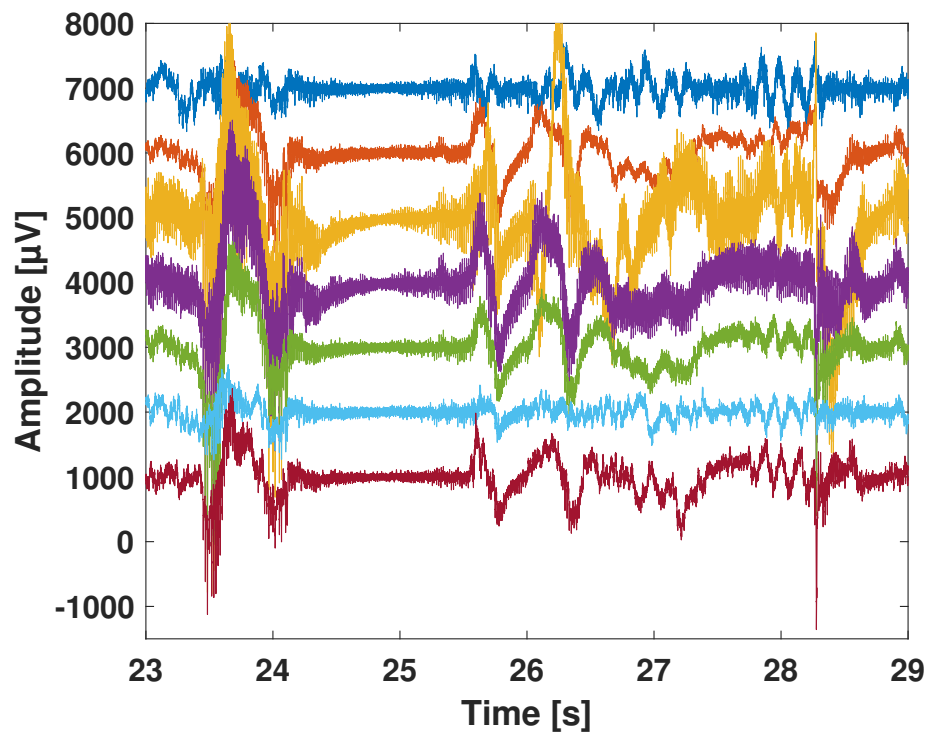


Figure 19. Appearance of artifacts in the signal by sudden movements of the animal.

Furthermore, it can be observed that other possible error source is founded in the electrodes matrix, since the pins without connection can't be found grounded (GND), also the common ground of the system could be working as an antenna. We suggested the NYC group checking the

design and manufacturing process of the matrix with the purpose of amend the error sources and be able to record signal that have the desired shape.

5.3. Grounding measurement results

The measurements of soil resistance (described in section 4.3) they were made 9 data collection giving the results seen in Table 3. With an average value of 80.3Ω and a standard deviation of 0.3798Ω .

Table 3
Grounding resistance

Events	R_ Tierra Ω
1	80.1
2	81.0
3	80.09
4	80.8
5	79.9
6	80.4
7	79.9
8	80.5
9	80.7

If we take the average value obtained in the measurements and we related with the value allowed (see table 1) we see that this value is 7.3 times higher than expected.

5.4. Graphical user interface

In the validation tests of the interface (average power, instant power and frequency) with data provided by the research group NYC it was obtained the following results:

5.4.1. Average power of the cerebral rhythm Theta. In Table 4 are consigned the results obtained of average power of the cerebral rhythm in the first and last minute of recording.

Table 4
Average power of the cerebral rhyth

Rate/min	Average power	Average power
	$[\mu v^2/Hz]$	$[\mu v^2/Hz]$
	first minute	last minute
Reactive(R)	5.3005e+04	4.8300e+04
Not reactive(NR)	3.4470e+04	2.1656e+04
Control(G)	7.1359e+04	3.8346e+04

Making a comparison between the three groups of animals, it can be observed that the R group used to have higher average power than the NR, indicating that the animal of the R group has a state of anxiety much higher.

5.4.2. Power in the highest peak of the cerebral rhythm theta. In Table 5 you have the peak power of the cerebral rhythm theta of each group of animals, having the X axis the frequency and the Y axis the power as seen in Figure 20 .

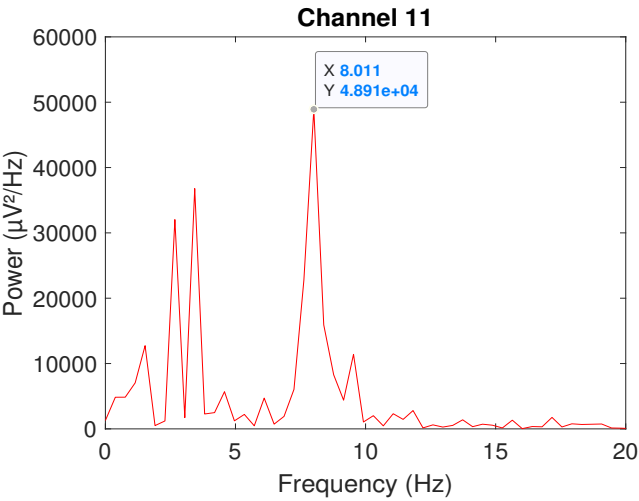
Table 5
Maximum peak power in the first and last minute.

Rate/min	First minute		Last minute	
	x[Hz]	y $[\mu v^2/Hz]$	x[Hz]	y $[\mu v^2/Hz]$
Reactive(R)	8.11	2.893e+04	7.63	1.442e+04
Not reactive(NR)	7.24	4.377e+04	8.11	1.151e+04
Control(G)	8.11	4.891e+04	7.63	4.391e+04

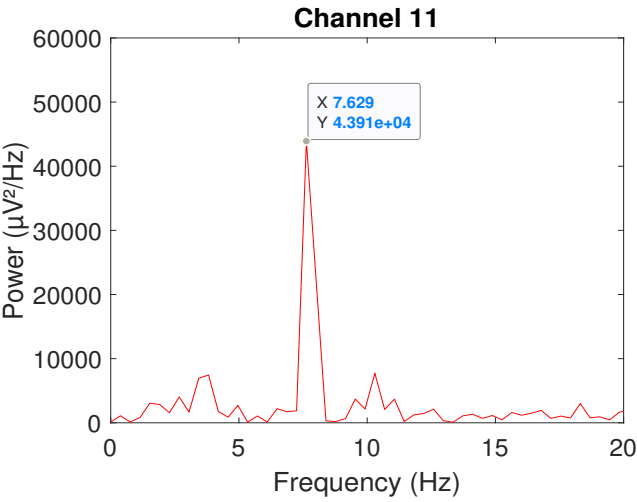
The Figure 20 shows that the frequency of the highest peak it is found in the range of 4 and 10 Hz. This corresponds to the cerebral rhythm Theta Colgin (2016).

In the development of other validation test of the interface, it was used methods as: peri-

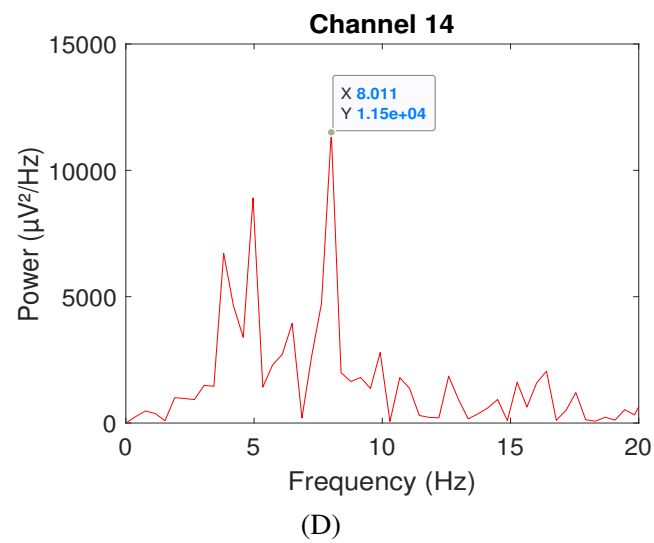
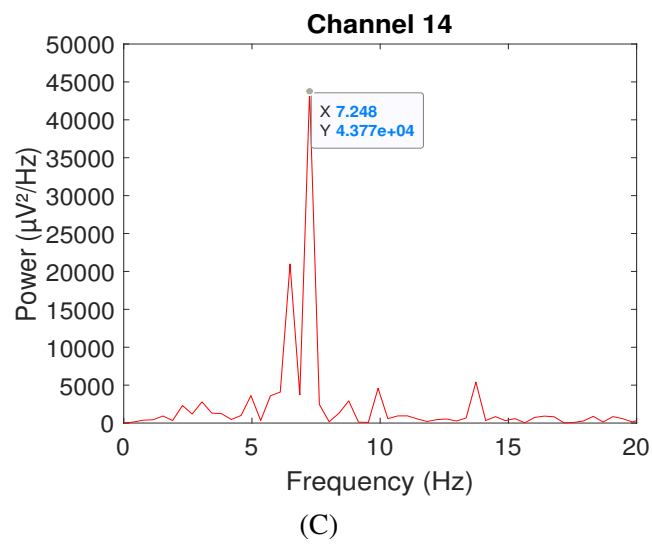
stimulus time histogram (PSTH), detection and visualization of spikes (see Figure 21).



(A)



(B)



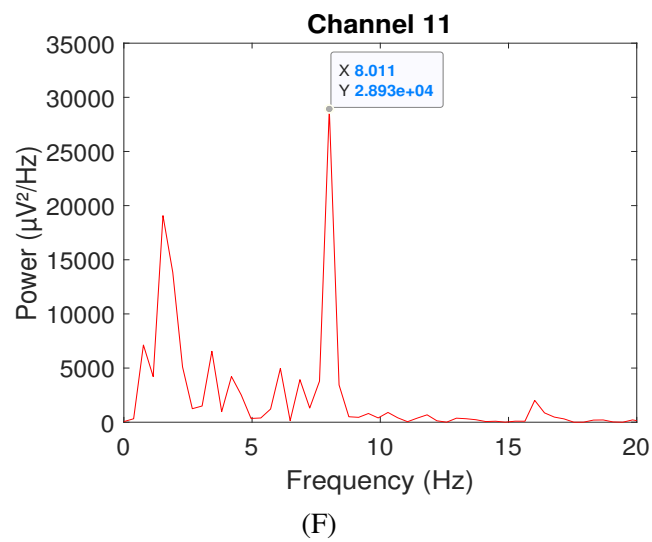
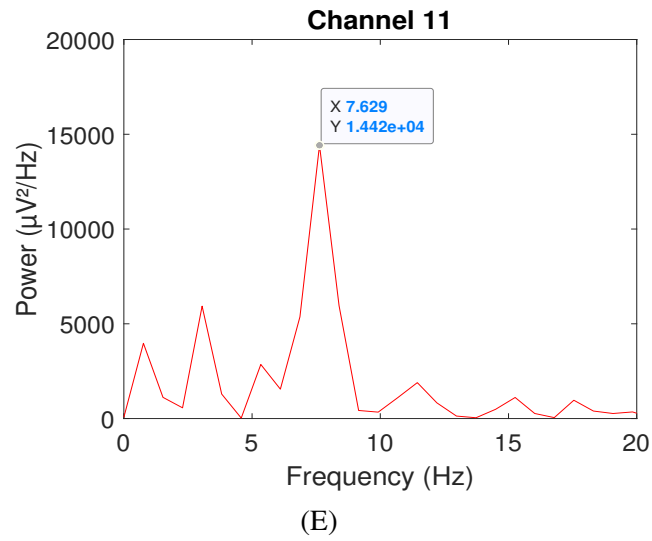
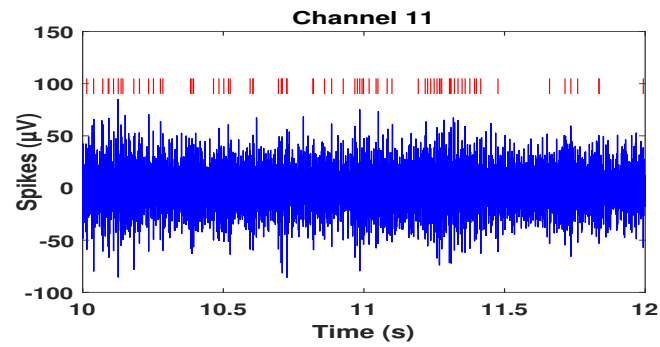
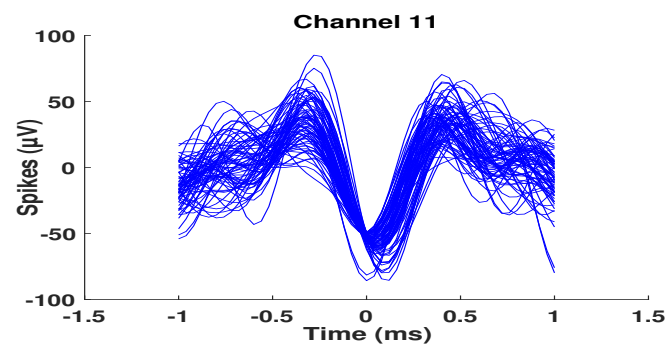


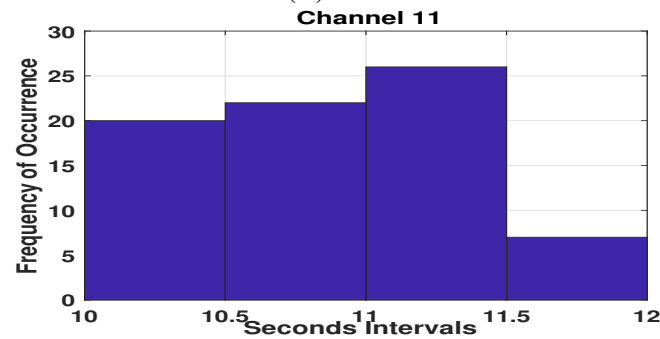
Figure 20. Validation tests of the interface. (A)(B) Instant power of the channel 11 of the control rats (G) in the first and last minute respectively. (C) (D) instant power of the channel 14 of non-reactive (NR) rats in the first and last minute respectively, (E) (F) instant power of the channel 11 and 15 in reactive (R) rats in the first and last minute.



(A)



(B)



(C)

Figure 21. Spikes method detection. (A) is the EEG filtered signal between 700 to 2000Hz, it is shown on the top of the graphic some red bars, which indicate the detected events of action potentials using a threshold of 3 standard deviations below the average. (B) It is shown the waveform of the spikes, which indicates that the detected events have the desired characteristic shape and it can be classified Buzsáki et al. (2012). (C) Peri-stimulus time histogram (PSTH), histogram that shows the frequency of occurrence of the detected spikes for a range of 10 to 12 seconds with a bar width of 0.5 seconds.

6. Recommendations for future projects

The results presented in section 5 allow performing the following recommendations:

- The grounding of the NYC laboratory must be checked since it may be related with the poor attenuation of the Faraday's cage, as the measured value fails with the TECHNICAL REGULATION OF ELECTRICAL INSTALATIONS (RETIE) RETIE (2013).
- Some improvements of the connectors that goes between the amplifier and the electrodes matrix in order to avoid that the movements of the animal may disconnect this and may generate disturbances in the acquired signal.

As future Works it is proposed to perform a characterization of the electrodes matrix with the purpose of stablish a elaboration protocol. Also it is recommended to extend the analysis methods of the interface to offer a more advanced tool of neural electrophysiological signal processing. Finally, the design of a wireless acquisition system, that allows the NYC group to carry out experiments without losing the connection between the amplifier and the electrode matrix.

7. Conclusions

Starting from the maximum values obtained in the non-ionizing radiation tests, a comparison was made with the maximum non-occupational exposure levels allowed in the ITU-T K.52 rule ITU-T K.52 (2004) in which you can establish that these levels are below the limit that could come to produce adverse effects in the health of the people and in the signal recordings system. The high value of the ground resistance, which is approximately seven times greater than the value set in the TECHNICAL REGULATION OF ELECTRICAL INSTALLATIONS (RETI) RETIE (2013), may be related to the low attenuation of the Faraday cage, which it doesn't allows a shielding of the recordings system to external noise sources.

The execution of the pilot test determined the presence of artifacts (noise) in the signals acquired due to the bad connection between the amplifier and the electrode array, an improvement in the design of this connection would allow obtaining records without the appearance of devices.

The NYC research group followed the proposed recommendations, making improvements in the electrode matrix, thus achieving signal recordings with the expected shape (see Figure 22).

With the acquired signals in the NYC laboratory (real data), it was possible to verify the correct functioning of the designed graphical interface, delivering a data analysis tool that facilitates students and researchers from the faculty of health and the E3T community, to perform works in the study of neuronal electrophysiological activity of the brain.

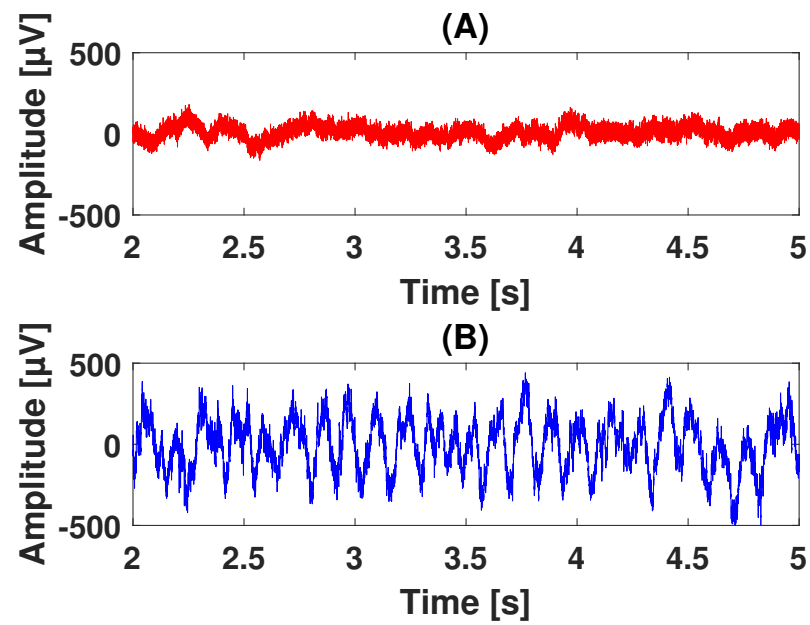


Figure 22. (A) First acquisitions registered in the NYC laboratory, does not have the expected shape. (B) Signal acquired after modifications of the electrode matrix, has the expected shape.

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