Universidade do Minho
Escola de Engenharia

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Miniaturized Wireless Electronic Device for Application on Rodents Thermal Neuromodulation

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Dissertation in Biomedical Engineering
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Professor Doutor Paulo Mateus Mendes

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DIREITOS DE AUTOR E CONDIÇÕES DE UTILIZAÇÃO DO TRABALHO POR TERCEIROS

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STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

RESUMO

A epilepsia é uma doença neurológica que afeta muitas pessoas em todo o mundo, e cerca de 30% dos pacientes epiléticos são resistentes aos medicamentos. Normalmente, estes são obrigados a ficar em casa, uma vez que a opção é uma cirurgia de ressecção focal, o que nem sempre é possível ou aceite. Portanto, e como em muitas patologias onde é possível o desenvolvimento de dispositivos médicos capazes de melhorar a qualidade de vida dos pacientes, a tecnologia pode-se associar à medicina e fornecer uma solução alternativa para tais pacientes. Dos vários estudos já realizados neste campo, a neuromodulação térmica é uma técnica promissora, uma vez que reduz ou possivelmente interrompe as crises epiléticas. Assim, a fim de potencializar uma solução futura para estes pacientes, é necessário primeiro realizar vários testes experimentais com modelos animais.

Com o intuito de melhor compreender o funcionamento dos testes *in vivo*, foram realizados testes em GAERS no *Grenoble Institute of Neuroscience* com um dispositivo previamente desenvolvido, a fim de recolher dados sobre o efeito de arrefecimento nos neurónios. Isso permitiu alargar o conhecimento sobre o arrefecimento focal, mas também permitiu encontrar limitações no dispositivo utilizado, dado que era necessário que este estivesse conectado por fios para ser possível aplicar frio e gravar o EEG. Assim, o objetivo desta dissertação foi melhorar a comunicação sem fios de um dispositivo eletrónico capaz de controlar um Peltier e registar a atividade elétrica cerebral de um roedor.

O sistema possui dois módulos, um que maioritariamente transmite dados e outro que os recebe. Nesse sentido, o transmissor possui toda a eletrónica associada à comunicação sem fios, à aquisição de sinais eletrofisiológicos e ao controlo do Peltier. Já o recetor possui a eletrónica para a comunicação sem fios e um conector USB para se conectar ao computador. Com o intuito de aumentar o débito binário da transação de dados, os dois módulos foram programados com o protocolo proprietário da *Nordic Semiconductor*, denominado *Enhanced ShockBurst* que opera na banda 2.4 GHz. Após a implementação do software, obteve-se um débito binário de 368640 bps, o que é 17 vezes superior ao protocolo usado anteriormente. Assim, é possível adquirir biossinais com frequências mais elevadas, ou então, com frequências mais baixas, mas com mais resolução e, ainda a visualização de mais do que um canal.

De forma a validar todo o sistema, realizaram-se vários testes. Um dos testes foi colocar nos elétrodos uma onda sinusoidal e observar no computador se a onda recebida era igual à onda de entrada. O outro foi determinar a taxa de erros associada à comunicação e o tempo de vida da bateria.

Palavras-Chave: Epilepsia, neuromodulação térmica, dispositivo eletrónico, comunicação sem fios e débito binário.

ABSTRACT

Epilepsy is a neurological disorder that affects numerous people worldwide, and around 30% of epileptic patients are drug resistant. Usually, they are restrained at home, since the option is a focal resection surgery, which is not always possible or accepted. Therefore, and as in many pathologies, where it allows the development of medical devices capable of improving patients' quality of life, technology may be used here to help medicine providing an alternative solution for such patients. Of the many studies already conducted in this field, thermal neuromodulation is a promising technique as it reduces or possibly interrupts epileptic seizures. Thus, in order to leverage a future solution for these patients, it's first necessary to run several experimental tests with animal models.

To better understand how *in vivo* tests work, tests were initially performed on GAERS at the Grenoble Institute of Neuroscience with a previously developed device in order to collect data on the cooling effect on neurons. This allowed to extend the knowledge about focal cooling, but also allowed to find limitations in the device used, as it was required to be wired in order to be able to apply cold and record the EEG. Therefore, the aim of this dissertation was to improve the wireless communication of an electronic device capable of controlling a Peltier module and recording the electrical brain activity of a rodent.

The system has two modules, one that mostly transmits data and another that receives them. In this sense, the transmitter has all the electronics associated with wireless communication, electrophysiological signal acquisition, and Peltier control. The receiver, on the other hand, has the electronics for wireless communication and a USB connector to connect to the computer. In order to increase the binary throughput of the data transaction, the two modules were programmed with the 2.4 GHz proprietary protocol from Nordic Semiconductor, called Enhanced ShockBurst. After implementation of the software, a binary throughput of 368640 bps was obtained, which is 17 times higher than the previously used protocol. Thus, it is possible to acquire biosignals at higher frequencies, or at lower frequencies but with more resolution, and to visualize more than one channel.

In order to validate the whole system, several tests were performed. One of the tests was to place a sine wave on the electrodes and observe in the computer if the signal received was equal to the input wave. The other, on the other hand, was to determine the error rate associated with communication and battery life.

Keywords: Epilepsy, thermal neuromodulation, electronic device, wireless communication and binary throughput.

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List of Abbreviations and Acronyms

A

ACK	Acknowledgment
ADC	Analog-to-digital Converter

B

BLE	Bluetooth Low Energy

C

CLK	Clock
CS	Chip Select
CTS	Clear To Send

D

DBS	Deep Brain Stimulation
DSI	Data Sciences International

E

EEG	Electroencephalography
ESB	Enhanced ShockBurst

F

FDA	Food and Drug Administration
FIFO	First-In-First-Out

G

GAERS	Genetic Absence Epilepsy Rat from Strasbourg
GIN	Grenoble Institute of Neuroscience
GUI	Graphical User Interface

M

MISO	Master In, Slave Out
MOSI	Master Out, Slave In

P

PCB	Printed Circuit Board

PRX Primary Receiver

PTX Primary Transmitter

PWM Pulse Width Modulation

R

RF Radiofrequency

RNS Responsive Neurostimulation

RTS Request To Send

S

SoC System-on-a-Chip

SPI Serial Peripheral Interface

SUDEP Sudden Unexpected Death in Epilepsy

U

UART Universal Asynchronous Receiver/Transmitter

UM University of Minho

USA United States of America

V

VNS Vagus Nerve Stimulation

CHAPTER 1 INTRODUCTION

Epilepsy is considered the third most diagnosed neurological disorder, so it affects many people worldwide in all age groups [1]. This disease is characterised by recurrent and unpredictable disruptions of the normal brain functioning, causing the epileptic seizures [2], [3]. These seizures occur when neurons are intermittently activated in an abnormally excessive and highly way, which can be easily detected by EEG (Electroencephalography). For most patients there are solutions to control epileptic seizures, namely antiepileptic drugs. However, around 30% of patients are drug resistant, being the options for them a focal resection surgery, which is not always possible because the epileptogenic foci can be located in critical areas such as motor and speech cortices, or staying restrained at home [4], [5]. Either way, and despite some advances, they lack an alternative treatment. In this sense, several studies have been carried out in this area for attenuating and/or interrupting epileptic seizures for these patients [6].

Until now, the various therapies studied to help these patients have been electric neuromodulation, optogenetics and thermal neuromodulation [5], [7]. The latter has great potential for the desired effect, so more emphasis will be placed on this method.

Therefore, this chapter will cover the different therapies in more detail, as well as explain the entire process required to obtain an approved medical device suitable for patients.

1.1 Several Approaches for Patients Suffering from Epilepsy

Epilepsy is a neurological disease that has a very negative impact on patients' daily lives since epileptic seizures are unpredictable. Therefore, the treatment of this disease, meaning the control of seizures, is extremely relevant in research, since the aim is for these patients to have a better quality of life.

One of the main treatments for epilepsy are antiepileptic drugs [3]. There are a large number of medications available for seizure control, however only a few are considered first line. Note that medication has different mechanisms of action, so the choice of medication to be used should always be made for the specific case of the patient. In other words, age, sex, potential for pregnancy, comorbidities and tolerability problems must be considered, as well as the type of seizure and epilepsy syndrome.
Moreover, it is necessary that these patients are constantly monitored, since medication should be withdrawn if there is no effect on seizure control, if there are suspected tolerability problems and if there are no longer any long-term seizures despite taking medication. However, there are major obstacles in

this type of treatment, as there are associated cognitive, psychological and behavioral side effects, as well as a high percentage of patients who are resistant [7], [8].

1.1.1 Clinical Intervention

Patients who have epilepsy are considered drug-resistant when there is failure in properly performed trials of two antiseizure drugs chosen and used according to the patient's epilepsy to achieve seizure freedom [8]. Drug resistance remains a major challenge as there is still no treatment for every patient, so this issue has been the focus of many studies in order to find a solution for these patients [7]. An important help to better understand why patients are resistant to pharmacological treatments and thus to develop more efficient treatments are animal models of drug refractory epilepsy [9].

Currently, there is only one alternative for these patients to be free of seizures, and that is resection surgery. This consists of removing brain tissue from the area affected by the epilepsy, so it is essential to know the exact location of the focus. A major obstacle to this treatment is the fact that it is not possible to perform the surgery on all patients, since the epilepsy can be multifocal, bilateral, generalized, or even arise from the eloquent cortex, a cortex that has crucial functions such as movement, speech, and vision. In this sense, there is a need to investigate other techniques [7], [8], [10].

1.1.2 Electrical Neuromodulation

Given the limitations of pharmacological therapy, as well as resection surgery, the need has arisen to study alternative therapeutic modalities in order to prevent or interrupt the process by which the brain develops epilepsy, the epileptogenesis. Neuromodulation is a growing area, which has experienced a considerable increase in applicability in recent years. This technique consists in stimulating or blocking neuronal activity, which can be done through electrical, electromagnetic, chemical, optogenetic, or thermal methodologies.

Electrical neuromodulation is a technique that has been in vogue for the palliative treatment of these cases. As the name implies, it can modulate neuronal activity through electrical pulses applied to peripheral nerves or to specific areas of the brain. Currently, there are three types of devices already approved for the treatment of refractory epilepsy, such as VNS (Vagus Nerve Stimulation), RNS (Responsive Neurostimulation), and DBS (Deep Brain Stimulation).

VNS via electrical impulses was the first therapy to be approved, so there are already some patients who have been treated with it. Data show that this technique improves seizure control by 50% or more in

more than half of the patients treated. It also decreases the risk of SUDEP (Sudden Unexpected Death in Epilepsy), which is the leading cause of epilepsy-related death. However, only a small percentage of patients, less than 5%, achieve seizure freedom [7], [8], [11].

RNS is a technique developed that allows monitoring of brain waves and when the device detects unusual or seizure-like waves it stimulates an electrical pulse. This pulse is stimulated in one or two areas of the brain where seizures begin, as this approach is intended to prevent seizures. Data report that patients treated with this technique have a reduction in mean seizure frequency of 82 % and a better quality of life. However, there are some side effects, particularly related to the surgical process, as this is a brain surgery, as well as to the process of stimulating electrical pulses [12], [13].

DBS is another technique recently approved that is restricted to severe epilepsies. It has had a huge boost in recent years, since by implanting intracranial electrodes in the areas of the epileptic foci, it is possible to stimulate electrical pulses directly, in response to increased rhythmicity, changes in frequency or amplitude of the EEG signals related to seizure generation. These electric neuromodulation devices have already demonstrated their efficacy in clinical trials, however the benefits may be overestimated as there are still inherent study limitations as well as methodical weaknesses, so further optimization and clinical investigations are required [7], [8], [14].

1.1.3 Optical Neuromodulation

Optogenetics, on the other hand, is a relatively new technique that controls neuronal activity through light. Compared to electrical stimulation, it is much more precise in modulating neurons in a brain area, since only cells with light-sensitive ion channels, pumps, or enzymes are stimulated or inhibited. However, it is a technique that still requires a lot of study in terms of being a technique used for the treatment of epilepsy, as there is a need to develop an implantable light stimulation system, demonstrate the safety of viral transfection of ion channels in the human brain, and also verify long-term neural control without producing unexpected changes in brain function [11], [15], [16].

Therefore, there are still many unsolved questions, so it is crucial to study other treatment alternatives, in particular thermal neuromodulation.

1.1.4 Thermal Neuromodulation

Thermal neuromodulation has been extensively studied in the last decade, since by decreasing the temperature in a certain area of the brain it is possible to inactivate neuronal activity in a reversible way [17], [18].

In this sense, to study the viability of this technique for the treatment of epilepsy and, in the future, to apply it to humans, animal models of epilepsy that can have the disease due to either genetic or acquired factors are used. Currently, the most commonly performed *in vivo* experiments to analyze the effect of cold on epileptic seizures are those on rodents. Thus, the easiest way to perform these experiments is to develop thermal neuromodulator devices to implant in the rat's head.

Generally, the devices are based on a Peltier chip consisting of two parallel connected conductors that when supplied with electric current, one will cool down and the other will heat up. This electronic cooling phenomenon is called the Peltier effect. As mentioned, there is one side that gets hot, so it is necessary to attach a heat sink to this conductor. This is necessary so that when the device is implanted in the rat's brain and current is applied to cool one region, the other one does not reach temperatures that cause the death of the cells (it should not be higher than 43 °C). Therefore, the heat sink should be made of a material with high thermal conductivity. Often aluminum or copper is used. In addition, many times, in order to further dissipate the heat generated in the brain, authors place tubes connected to the heat sink to circulate a liquid, usually water. In this way, since the liquid is at a much lower temperature than the neuronal tissues, heat is transferred from the neurons to the water. The continuous circulation of the water in the tube is able to release the heat concentrated in the brain, and thus the brain is cooled without the other side getting too hot. This developed focal cooling device is shown in Figure 1 [2], [14].

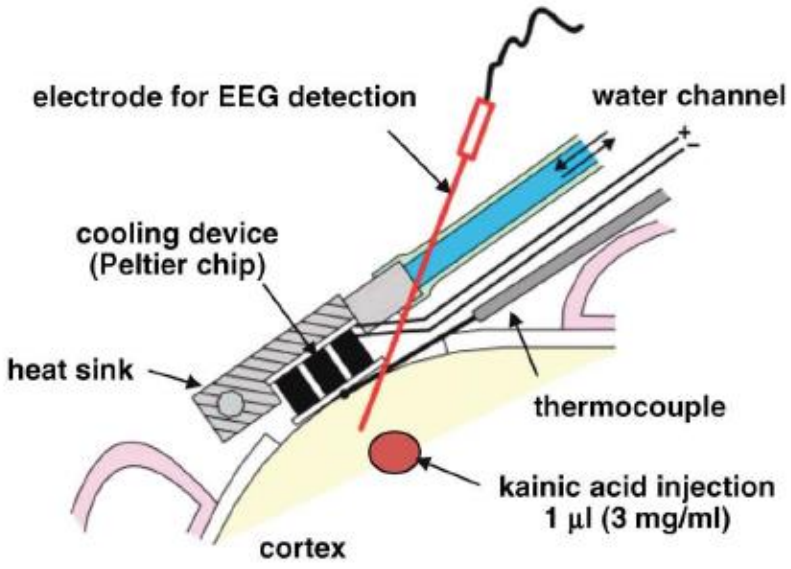

Figure 1 - Focal cooling device developed by Fujii et al. [14].

However, for experiments performed on rodents, this device is not very suitable, as they have large tubes. Since the rat is a small animal, the weight deposited on top of its head when implanting the device will create discomfort, so they tend to put their hands over their head to remove it. This will often make the tests unsuccessful, as they are able to pull out the tubes and wires, which compromises the continuity of the experiment. In addition, it is often necessary to re-surgery another rat to implement another device, which results in a delay in tests, since it is necessary to wait for the rat to recover from the surgery, and in a more expensive and material outlay, since the other devices become unusable. Therefore, it is essential to develop a small device that does not involve refrigeration liquids, in order to overcome the drawbacks detected in previously devices.

In this regard, and with a view to developing a device for human use to treat epilepsy in the future, it is essential to better understand the process involved in approving devices on the market in order to achieve this goal.

1.2 Medical Devices Development Methodology

The contribution from medical devices on patients' healthcare is extremely relevant, as it gives patients a better and longer life, by reducing or removing pain and suffering, by curing injuries, or even, by improving the quality of medical care and outcomes. Despite the recognized benefits, in order to achieve a device that can be used as clinical tool to improve the quality of epilepsy patients' lives in the future, it is first necessary to go through a series of steps. Actually, the process involved for the developing of a medical device is quite complex and, depending on the type of device, the steps for its approval may differ [19].

The FDA (Food and Drug Administration) is a USA (United States of America) federal agency created in 1906 that has the responsibility, to protect and promote public health. To this end, it ensures the safety and efficacy of human and veterinary medicines, biological products and medical devices. In addition, it also helps in the process of developing and innovating medical products, so that they become more effective, safe and affordable to maintain and improve the patients' health. FDA is one of the agencies that approves medical devices for the market, so it is necessary to know all the steps to be taken to accomplish this [20], [21].

The first step is for researchers to discover a medical need and then come up with an idea and a concept for an innovative medical device. Since the FDA classifies medical devices based on risk, it is necessary to see which class the device under development corresponds to, because the next steps are

different for each class. Given that what is intended to be achieved in the future is an implantable device for people with epilepsy, then it is assumed that it will belong to Class 3, General Controls and Premarket Approval [21].

Therefore, and given that the concept is workable, it is possible to move on to the next step, which is to design a device prototype. It should be noted that this prototype is not for human use, so firstly it must be made to test it in animal models. Only after getting results that prove that the concept is reliable, it is possible to develop a prototype for human use. After that, the next step is then the application for medical device approval. Note that it is easier to get approval if the device in question is similar to one already approved in the market, so it should be taken into account what has been previously developed [21].

In this sense, and following the process explained so far, it is crucial to develop a device to first perform tests on animal models to see if the concept applied to it really succeeds. If it succeeds, then the next step will be to create and design one for humans.

1.3 Motivation and Aim of the Dissertation

The motivation for this dissertation lies in the fact that, as previously mentioned, 30% of patients with epilepsy are resistant to antiepileptic drugs. Therefore, it is essential to find a way to control the seizures, and thermal neuromodulation is a strong candidate for this effect. In this way, the first step is to carry out tests in animal models, to later apply it for human use.

At the beginning of the year, an internship was held at Grenoble Institute of Neurosciences for two months, in order to evaluate the effect of thermal neuromodulation on epileptic seizures in GAERS (Genetic Absence Epilepsy Rats from Strasbourg). To achieve this, it was necessary to optimize a thermoelectric device that had been previously developed at UM. This consisted of a single Peltier coupled to a copper heat sink, since to cool one side it is necessary to remove heat from the other, hence the need for a heat sink with a high thermal conductivity. Once in Grenoble, two experiments were conducted with the developed device in order to evaluate the effect of cooling in two different ways. A total of 20 rats were used, of which 11 were male and 9 were female. Both experiments took 30 minutes, and four sessions were performed for each rat, on non-consecutive days. The rats were then divided into two groups, blocks and seizures. In both groups, an initial and a final baseline of 10 min was recorded, however, the middle 10 min of cooling was performed differently for each group. In the blocks group cold was applied every 2 min, meaning that a current was applied to the peltier every 2 min, and in the seizure group cold was only applied when the EEG showed that the rats were having a seizure.

However, one of the major limitations found during these experiments, was the fact that the rats had to be connected to wires to be possible to apply current on the peltier for cooling, and also to record the EEG. The use of this wire system brought some implications during the experiments, as the moving rats provided mobility artefacts in the EEG and, in addition, there were times when they pulled on the wires and the recording was disrupted. In this sense, it was possible to conclude that it is essential to carry out this type of tests with a wireless electronic device so that the results are as viable as possible. Furthermore, it will limit the risk of infection and improve the animal's mobility for prolonged periods of time when recording the EEG.

This was already known, so a wireless system had already been developed that allowed the application of thermal stimuli, but also the acquisition of an EEG. However, with the communication protocol used before, it was only possible to visualize one EEG channel. Since it is usually necessary to observe more than one channel, the aim for the dissertation project was to increase the binary throughput of wireless communication. In this way, it will be possible to observe signals with a higher frequency, as well as to observe several channels simultaneously.

In the end, a device will be obtained with a communication protocol that allows faster data transactions, as well as the control of a Peltier module and the recording of biosignals.

1.4 Contributions

In the ambit of the Master's Dissertation, a wireless communication of a thermal neuromodulator, meaning a device capable of cooling neurons as well as recording the EEG, was improved. This work contributed to make the *in vivo* experiments in rodents as less intrusive as possible, facilitating the tests performed. In addition, it also provided a greater knowledge in the process of developing electronic medical devices, namely wireless devices.

During the internship in GIN (Grenoble Institute of Neuroscience), it was possible to learn more about brain cooling, as well as the operation of in vivo experiments and their limitations. One of the major visible problems was the fact that, in order to be able to cool the rats' neurons and, at the same time, observe the EEG, it was necessary for the implanted device to be connected by wires to a current generator and to the EEG recording system, respectively. Often the rats pulled on the wires, which resulted in an immediate interruption of the experiment. Furthermore, it was not possible to carry out prolonged tests, as the rats were in a box, which was inside a Faraday cage, making the test rather unpleasant. Figure 2 shows that the fact that the mouse was connected to the wires limited its movements and, therefore, its

behavior, making the results of the tests unreliable. Yet another situation that occurred was the rats ripping the device off their heads, which led to them being discarded from the experiment. In this sense, and as a failure in an experiment with a rodent is not comparable to a failure in clinical practice, this experiment at the GIN only emphasized the need for a wireless neuromodulator device.

Figure 2 - Rat in the EEG recording cage setup.

In this sense, this dissertation contributed to improve a thermal neuromodulator system, more specifically the binary throughput of its wireless communication to provide a better efficacy of the system. Thus, the communication protocol was changed so that it was possible to exchange data from one module to another at a rate of 512 kbps. In the end, a system capable of sending data in two directions at a binary rate approximately 23 times higher than the previous one was obtained. In addition, an interface was also created on the computer to be able to receive and store the data that was received wirelessly by the receiver, and also send data back to the transmitter. Here it was possible to conclude that the possible binary throughput was about 368640 bps. Figure 3 presents a schematic of the developed system.

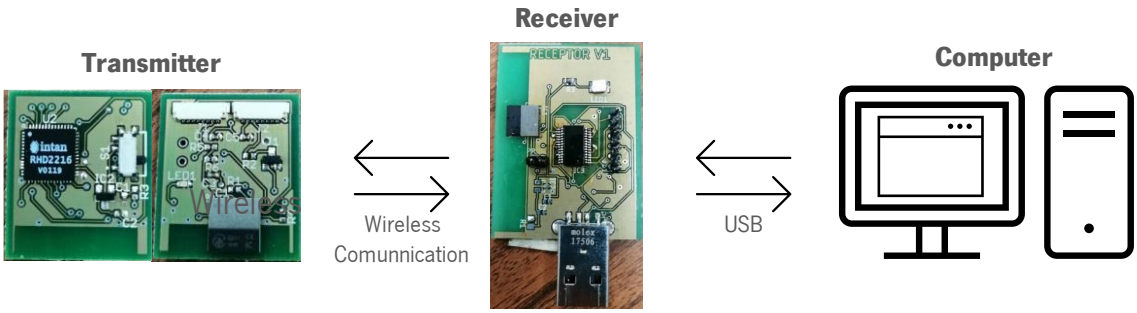

Figure 3 - Illustration of the system boards and architecture used for this work.

Therefore, with enhanced wireless communication from a device capable of cooling and collecting data, it will be possible to perform more experiments on animal models without having to sacrifice them too much. This is a big step towards the future being able to design and develop a similar device for human use.

1.5 Outline and Structure

The structure of this dissertation was organized into six chapters, in order to provide a better understanding of all the steps performed for the developed device.

Chapter 1 refers to the dissertation introduction, so it was first described the problem and, subsequently, the motivation, which provided the theme of this dissertation. Then, a brief description of the necessary steps to be followed to be able to develop a device was presented. Being thermal neuromodulation the promising technique for the problem in question, research of the already existing devices with this technique for rats was also carried out, with the intention of analyzing the needs for improvement and, therefore, trying to solve them. Additionally, it was also presented the contributions of this work.

In Chapter 2, the importance of developing a wireless device capable of cooling and recording EEG data to perform experiments was discussed. In this way, a literature review was conducted on all the techniques that have been studied so far for alternative treatments for epilepsy, as well as on potential systems that are used to record EEG in rats, to evaluate devices that can be used and/or modified to apply cold.

Chapter 3 describes the modules that were used during this dissertation. Additionally, the system architecture and software architecture of the PCBs is presented, in order to better understand what was at hand and what this would impact on the project's aim.

Chapter 4 consists in improvements made to the device that had been previously created, and therefore these enhancements were justified. Thus, it was explained in detail the wireless communication protocol used, as well as the software implemented to allow communication between the two modules. In addition, it was also presented the interface created for the computer to receive and save the data, as well as send it.

Chapter 5 presents the tests performed to validate the operation of the proposed device, as well as the discussion of the results obtained with it.

Finally, Chapter 6 included the main conclusions of the work, along with suggestions to be performed in the future.

CHAPTER 2 ELECTRONIC DEVICES FOR NEUROMODULATION ON EPILEPSY

The nervous system is one of the most difficult systems in the human body to comprehend, so when there are diseases associated with it, it becomes very complex to find effective treatments. Thus, efforts have been made in the research area to circumvent the limitations that exist in this system.

One of the major engines to better understand the complex mechanisms of the nervous system are the studies performed in animal models, since these provide good enough models of the brain and of human diseases associated with it. With these experiments, it thus becomes easier to develop new methods of diagnosis and treatment for neurological disorders. That said, what needs to be undertaken is to create devices that make such experiments possible.

In this sense, this chapter will conduct a literature review of the main treatments performed to treat epilepsy, as well as techniques that are being studied, in order to combat the lack of treatment for patients not responsive to drugs, nor candidates for surgery. In addition, the evolution of EEG recording systems in rodents will also be analyzed. Since to evaluate whether an antiepileptic technique works it is necessary to analyze the EEG of the animal model for long periods of time, it is important that this recording be as comfortable as possible for the animal so that it does not affect its behavior. Hence the need to do a state of the art of these systems to better understand how to implement a wireless EEG recording system in the device that will be developed.

2.1 Epilepsy Control Method for Drug-resistant Patients

When patients do not respond to medication, one of the treatments that can be performed is epileptic surgery which consists in the resection, destruction or disconnection of epileptic brain tissue. This requires a pre-surgical diagnosis, as not all patients are candidates to undergo these procedures, and it is also necessary to evaluate the advantages and disadvantages of the process [7].

The effectiveness of the surgery, that is, to be free of seizures, depends on the underlying pathology, the location of the epileptic focus, the precise delineation of the zone and the performance of the neurosurgical intervention. Risks and complications, on the other hand, consists of things inherent to interventions, such as unintentional brain damage due to hemorrhage or infections, as well as calculated risks related to the removal of specific brain tissue, such as memory deficits or cognitive impairment due to partial resection of the temporal lobe. In this sense, patients suffering from seizures that arise from the

eloquent cortex, or are multifocal, bilateral or generalized, are not candidates for surgery. In contrast, for cases in which the epileptic focus can be identified and the probable benefits of surgery overcome the associated risks, the surgical process is chosen, and the injured part of the brain is removed. The percentage of people who actually achieve seizure freedom after surgery ranges from 50 to 80 % [8], [10].

In view of this, there is still a large percentage of people who are both resistant to drug treatment and unsuitable for surgery, so they remain refractory to the various treatments. Thus, other alternative therapeutic approaches are needed. Currently, there is no proven therapy that prevents or reverses the epileptogenic process, however there are techniques that prove to reduce the number of seizures, thus providing a better quality of life for patients. In this sense, similar to what happens in other pathologies, such as Parkinson's, neuromodulation is a methodology that can be applied to patients with refractory epilepsy. This, through electrical, electromagnetic, optogenetic, thermal, or chemical stimuli, can alter neuronal activity, which is why it is a promising technique.

2.2 Electrical Stimuli Devices for Epilepsy Control

Over the years, researchers have demonstrated that it is possible to modulate the functional activity of neuronal networks by electrical stimulation in epileptic animal models [6]. Here, electrical pulses are applied to peripheral nerves or specific areas of the brain in response to increased rhythmicity, in order to stop the generation and propagation of seizures. This stimulation can be done in two different ways, either open-loop, i.e. in a programmed mode, or closed-loop, which is when stimulation is done in response to seizures [7], [8].

In this regard, electric neuromodulation techniques such as VNS, RNS and DBS have been developed and used in USA for the palliative treatment of patients who are resistant to antiepileptic drugs or are candidates for resection surgery, but this has failed, or for those who are not candidates for surgery [22]. Figure 4 presents a schematic of the treatment sequence for patients with refractory epilepsy.

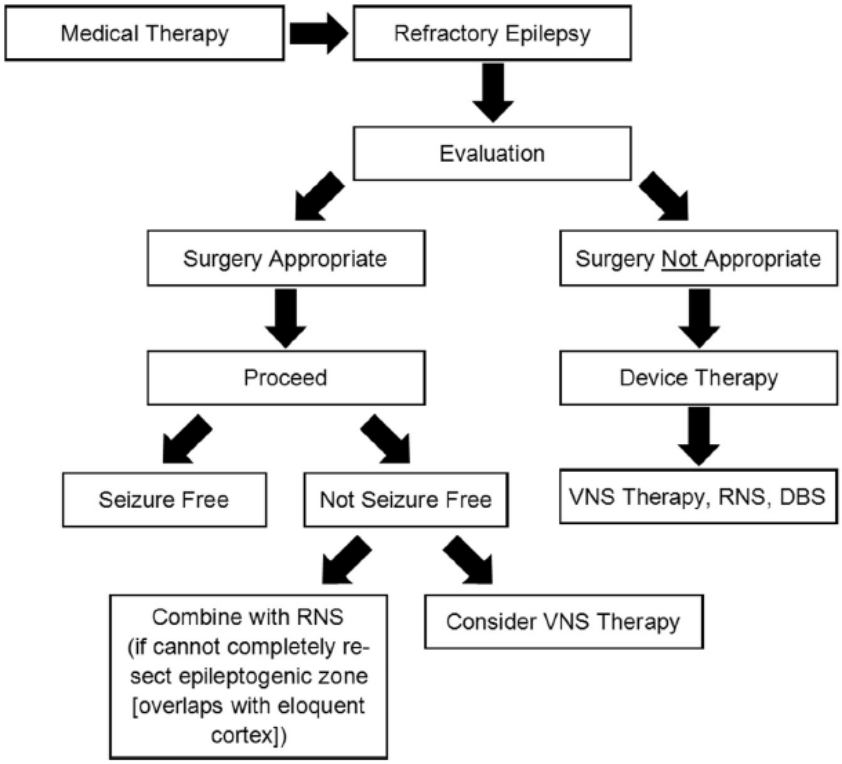

Figure 4 - Treatment sequence for patients with refractory epilepsy [22].

2.2.1 Vagus Nerve Stimulation

VNS is a therapy that was FDA approved since 1997, as it has been proven to help reduce the frequency of seizures for patients over the age of 12 who suffer from refractory seizures [23]. Data shows that by June 2018, over 100,000 patients worldwide have received this therapy as a form of treatment [22].

The VNS therapy system (Figure 5) consists of a pulse generator placed in the left subclavicular region that transmits electrical signals to the left vagus nerve through a bipolar VNS electrode coiled around it. Additionally, it also has a tunneling tool, handheld magnets and a programming wand with accompanying software for a handheld computer that allows the stimulation parameters to be read and modified. Currently, there are five models of VNS therapy generators, Demipulse Model 103, Demipulse Duo Model 104, AspireHC Model 105, AspireSR Model 106 and SenTiva Model 1000 [22].

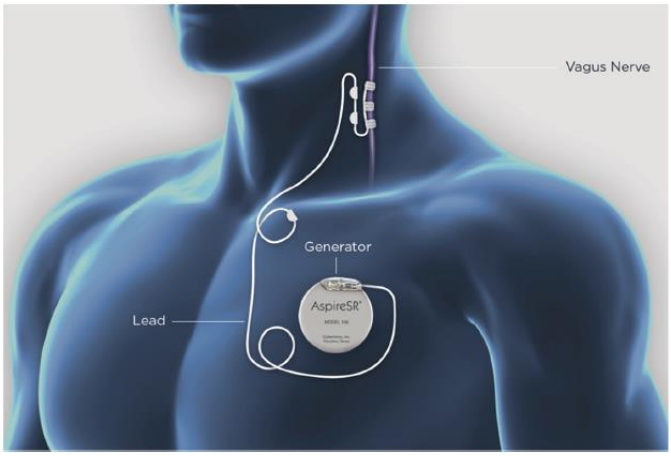

Figure 5 - VNS therapy system [22].

It should be noted that to evaluate device efficacy, it is usually determined the mean (50%) reduction in seizure frequency. In this sense, in two randomized, controlled trials, it was possible to determine the efficacy of VNS against sham stimulation in patients with drug-resistant focal epilepsy.

One of the studies (EO3) included 114 patients over 12 years of age, and in all of them the same VNS device was implemented. While the active treatment group received standard VNS of 30 Hz, 500 μs, 1.5 mA, 30 s ON, 5 min OFF, the control group was subjected to supposed sham stimulation of 1 Hz, 130 μs, 1.25 mA, 30 s ON, 90 min OFF. After three months of VNS therapy, a mean reduction in daily seizure frequency of 24.5 % was observed in the active treatment group and 6.1 % in the control group ($p < 0.05$) and furthermore 31 % of the treatment group had a 50 % reduction in seizure frequency while the control group had a 14 % reduction. The second study (EO5), on the other hand, was conducted with the same design and stimulation parameters, but with 198 patients, with 95 receiving effective stimulation and the rest receiving sham stimulation. Again, the results showed that there was a greater decrease in both the mean daily frequency of seizures and the mean reduction (50 %) in the frequency of seizures in the group with the effective stimulation, which was 27.9 and 23 % compared to 15.2 and 16 % in the control group. Therefore, in general, it is possible to state that such therapy succeeds in reducing the number of seizures. In addition, it also decreases the risk of SUDEP, and three out of four patients report that they had an improvement in their quality of life. However, despite being a safe technique and usually well tolerated, there are associated adverse events [22], [24], [25].

Collateral events can be acute or chronic, and these are related either to the surgical intervention or to the electrical stimulation itself. Acute side effects that have been reported are wound infection, left vocal cord paralysis, lower facial paralysis and, very rarely, severe bradycardia/asystole. In study EO5, there was a 3 to 6 % incidence of wound infection, where in 3 cases the device even had to be removed, and in 2 cases left vocal cord paralysis occurred, which was eventually resolved. The left facial nerve

paralysis was related to the surgical incision placement, so with the changes in surgical technique this is no longer observed. Severe bradycardia leading to asystole (reversible) was related to immediate testing of the device at implantation, so it is current practice not to test the device immediately after implantation [22], [23].

In other hand, most chronic side effects of VNS therapy are related to activation of the pharynx and larynx. Thus, voice hoarseness (37.2%), hypophonia (37.2%), and cough (45%) have been reported due to recurrent activation of the laryngeal nerve and the superior laryngeal nerve. In addition, many patients report a constriction in the throat and change in voice that may persist for a long time. Occasionally, jaw pain, headache, and pain in the abdomen are reported. Rarely, high current output can cause hemiparalysis of the diaphragm and weakness of the mid-tongue. Finally, Horner's syndrome has also been reported after VNS therapy. [22], [23].

2.2.2 Responsive Neurostimulation

The RNS system (Figure 6 (a)) is a closed-loop brain-responsive electrical neuromodulation system designed to prevent seizures at their source. This system approved in 2013 by the FDA consists of a neurostimulator that is placed under the scalp and inside the skull, connected to electrodes that are positioned at the epileptic focus, as pictured in Figure 6 (b). In addition, it has a remote monitor used by patients to upload their data and an RNS Tablet and Patient Data Management System (PDMS) used by physicians. Thus, it is possible to continuously monitor brain activity via EEG and program the device to deliver a small amount of electrical current when a seizure or seizure-like activity is detected [26]–[28].

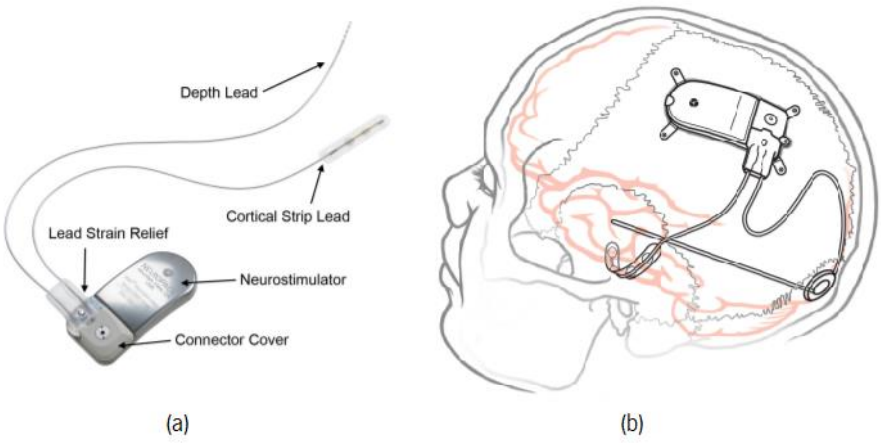

Figure 6 - RNS system: (a) RNS neurostimulator with two NeuroPace leads; (b) implanted RNS system [27].

Clinical studies conducted with this system on 150 patients showed that more than half of the patients had 50 % or fewer seizures than before implantation. In particular, there was an average reduction in

seizure frequency of 67 % at the end of one year of treatment, 75 % at the end of two years, and 82 % at the end of three or more years, the highest reported for a neuromodulation system. In addition, the study authors concluded that the RNS system provides lasting improvements in quality of life and that there is a decreased risk of SUDEP [12], [26], [28], [29].

On the other hand, this treatment also presents some risks, especially complications associated with the surgical intervention or even due to the performance and use of the device. The risks associated with surgery were infection after device implantation (7 %) and bleeding in the brain or under the skull (4.7 %). Other events that occurred in the studies were psychiatric symptoms (39.8 %), seizure changes (16 %), and seizure-related injuries (49.2 %), including seizure-related head trauma (10.5 %). Patients with epilepsy, especially severe epilepsy, were at risk of SUDEP, of whom seven eventually died. One of the great advantages of this device over anticonvulsant treatment is that it has no chronic side effects, and in addition it has no ongoing side effects compared to VNS therapy [12], [13].

2.2.3 Deep Brain Stimulation

DBS is a methodology already widely used for the treatment of Parkinson's disease. Thus, and due to its success, DBS has been developed as a treatment for other conditions. Although many still remain under investigation, in 2018, DBS of the anterior nucleus of the thalamus was approved by the FDA to function as an adjunct therapy for patients with refractory epilepsy [25], [30].

This consists of implanting electrodes in deep brain regions with the goal of controlling excess electrical activity in the brain using regular electrical pulses to reduce the frequency and severity of seizures. Therefore, depth electrodes are implanted using stereotactic techniques and are tunneled subcutaneously to an implantable pulse generator, which is usually placed in the subclavicular region, as can be seen in Figure 7. The generator enables control of the stimulation parameters, i.e., frequency, pulse width and voltage, by the patient or the physician, in order to achieve maximum effectiveness. It is important to emphasize that low frequency stimulation generally appears to excite nearby neurons, while high frequency stimulation may reduce local activity and thus induce reversible functional damage. However, this simplistic view of the mechanism of action has been challenged in recent years, and a more complete understanding is likely to facilitate better DBS treatments [25], [30], [31].

Figure 7 - Diagram of implanted DBS system [30].

In order to evaluate the efficacy of DBS of the anterior nucleus of the thalamus, a double-blind, randomized, controlled clinical trial (SANTE trial) was conducted. In this trial, during the 12 weeks prior to implanting the device, patients were analyzed for seizure rate, and after a 4-week recovery were randomized to receive or not a stimulus. This stimulus consisted of 1 min duration 90 μs pulses of 5.0 V at 145 Hz, separated by 5 minutes. Seizure rates were observed in a 12-week blinded evaluation phase, followed by a 9-month stimulation for all subjects in an open-label phase. Data point to a median seizure reduction of 35 % for the active group during the 12-week blinded evaluation, while the control group had 21.1 %. In the last month there was a median seizure reduction of 40.4 % for the active group versus 14.5 % for the control group. The 50 % response rates were 29.6 % and 25.9 % for the active and control groups, respectively. These were not statistically significant, so after post hoc analysis it was observed that they were significant for temporal lobe seizures during the 12 months (43.9 % active vs 29 % control), but not for frontal or other lobes [25].

In the long term, specifically after 1, 2, 5, and 7 years there was a median seizure reduction of 41.4, 55.6, 68.4, and 74.9 % and a 50 % response rate of 43.4, 53.7, 67.8, and 74 %, which again appeared better in the temporal lobe. In addition, there was also an improvement in the patients' quality of life, both after one year and after five years. On the other hand, adverse effects appeared, namely pain and paresthesias in approximately 20.9 %, local infection in 12.7 %, and lead mis-targeting in 8.2 %. As for possible adverse effects related to brain stimulation, 32.7 % were reported in depression (with one suicide within 4 years postimplant), and 25.5 % in non-serious memory loss. It should be noted that 66 % of the subjects reporting depression had a previous history [25].

In summary, although electric neuromodulation techniques seem to be able to increase anticonvulsant efficacy in both adults and children, they still require further assessments. Table 1 shows the main differences between the three electrical neuromodulation methods previously discussed.

Table 1 - Differences between VNS therapy, RNS system and DBS [26], [32]

	VNS therapy	**RNS system**	**DBS**
Number of People Treated	More than 100,000 including 33,000 children	Approximately 1,000	Not reported
Approval ages	12+ years old	18+ years old	18+ years old
Seizure type	Partial-onset	Partial-onset with frequent and disabling seizures (motor partial, complex, and/or secondary generalized)	Partial-onset (with and without generalized seizures)
Responsive therapy	Yes (in models 106 and 1000)	Yes	No
Implant procedure	Outpatient procedure, usually, 1-2 hours	Invasive brain surgery typically for 2-4 hours, overnight hospital stays	Invasive brain surgery, overnight hospital stays
Estimated battery life	4.9 – 10+ years (for model 1000)	5.1 – 9.4 years	3 - 5 years

2.3 Non Electrical Stimuli Devices for Epilepsy Control

Non-electrical stimulation, as the name implies, consists in modulating neuronal activity without the need to provide electrical pulses. Through optical mechanisms or even the application of cold, it is possible to control epileptic activity, and so these are excellent alternatives to be considered as techniques for the treatment of patients with refractory epilepsy.

2.3.1 Optical

An alternative approach, popular among the neuroscience communities, is to gain enhanced specificity through cell-type or circuit-specific manipulations in order to interrupt or prevent seizures while relatively preserving endogenous network activity. In this way, optogenetics emerges, as it can achieve rapid and reversible control of specific neural circuit components through the expression of light-activated ion channels, most commonly cation channels known as channelrodopsins. More specifically, through light intensity and wavelength it is possible to alter the potential of a membrane [16], [33].

In contrast to electrical neuromodulation that indiscriminately stimulates all cells within the electric field and cannot be applied in a hyperpolarizing manner to silence neurons and circuits, optogenetics offers bidirectional control, thus allowing silencing or activation of target neurons through different optogenetic probes [16].

In 2009, it was first reported that inhibition of glutamatergic neurons in the cortex was sufficient to reduce seizure activity in a rodent model of epilepsy [34]. Therefore, it demonstrated that optogenetics had the potential to act as a therapy for epilepsy. Already in more recent studies, it was shown that closed-loop optogenetic inhibition, specifically of thalamocortical neurons, could efficiently abort ongoing seizure activity in a rodent model of post-injury epilepsy. In the same year, one study showed that either optogenetic inhibition (hyperpolarization) of glutamatergic neurons, or "activation" (rhythmic depolarization) of GABAergic interneurons, was able to rapidly abort seizures in a rodent model with temporal lobe epilepsy. Additionally, another study suggested that optogenetic activation of a mixed population of GABAergic interneurons would be more efficient in aborting ongoing seizure activity [33].

While data suggests that combining closed-loop technology with optogenetic techniques would be an effective strategy in human patients with cortical epilepsy, much more research is needed to identify the stimulus parameters, cell types, and genetic delivery methods that will facilitate translation to human patients [33]. In addition, there are three major problems with this technique, namely practical, biological and functional problems. One of the practical problems is that it is necessary to place a light stimulation system in the appropriate places in the brain. The biological problems are that foreign proteins are used in the brain, which can lead long run to problems with immunogenicity. Finally, the functional problems consist in the fact that there is not yet enough information about cell populations producing unexpected changes in brain function in long term [11], [15].

These problems will delay clinical translation, but this technique is proving to be a critical tool in furthering the understanding of networks, seizures, and epilepsy, and even if it does not translate directly into a therapy, it will certainly allow the development of new approaches to the treatment of epilepsy [15].

Therefore, a very promising option for the treatment of epilepsy is thermal neuromodulation, more specifically focal brain cooling.

2.3.2 Focal Brain Cooling

Focal brain cooling is an attractive and non-destructive alternative strategy for patients with epilepsy, as it allows suppression of focal seizures through inhibition of synaptic transmissions. This, as the name implies, consists of applying cold to selected brain areas, either a superficial or deeper structure, in a specific and targeted way [3], [35], [36]. Compared to other forms of treatment, thermal neuromodulation has several advantages, including the adjustability of temperature modulation parameters required at the cooling site, its reversibility due to the possibility of interrupting or reducing cooling, thus controlling the adverse effects resulting from excessive temperature decrease, the ability to intervene in multiple foci by acting in a single target site, and the fact that cooling is a potential neuroprotector [37].

Therapeutic hypothermia is one of the oldest techniques, since researchers concluded as early as the late 19th century that it could reduce neurological functions on a systemic level. Therefore, the potential clinical use of cooling has been discussed since the 1940s. Baldwin was the first to demonstrate that systemic hypothermia can suppress epileptic discharges in the temporal lobe of epileptic primates [3], [14]. Already in a study developed in 1970 by Sourek and Trávníček [38] it was shown that the combination of a device called Autohypotherm for general hypothermia, with local extravascular irrigation with localized cold saline solution in 25 patients with refractory epilepsy, achieved a body temperature of 29-32 °C and a brain surface temperature of 5-6 °C. During 1 year of disease surveillance, and under administration of antiepileptic drugs, the patients reportedly had a reduced number of epileptic seizures and did not evidence any associated morbidity, with four of them experiencing no seizures at all [38], [39].

Although early studies demonstrated that brain cooling has the potential to end seizures, it was not until the late 20th and early 21st century that it gained more attention due to engineering technological advances. Thus, in further studies it was shown that irrigating the brain surface with cold Ringer's solution rapidly interrupts focal seizure activity and the use of thermoelectric devices terminates epileptic discharges in the neocortex. In this regard, in 2010, Fujii et al. proposed a cooling system that combined these two methods in order to perform more experiments in animals [14].

Recently, more specifically in 2019, Hata et al. developed a recirculating coolant cooling system (Figure 8) with the purpose of conducting clinical trials. This system is intended to target patients who have the epileptic focus in the gyrus, as this is an easily accessible area, whereas for cases of neocortical

epilepsy in functional areas surgery is more challenging. The process involved in this system is to cool, with a Peltier device, the coolant in a tank outside the body which then flows, under the pressure of a pump, to a titanium cooling device implanted on top of the epileptic focus where heat is removed from the brain and then is cooled again outside. In this sense, the patient will have to wear a jacket with two chest pockets that contains the Peltier device, the heat sinks, the saline tank, the pump, and the battery. It should also be noted that since this system includes parts implanted inside the patient that are connected to the outside, there is a risk of infection [2].

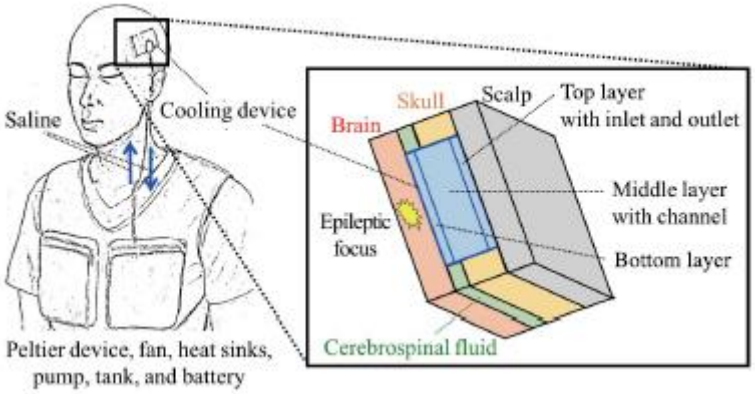

Figure 8 - Focal brain cooling system with recirculating coolant cooling [2].

As already mentioned in the previous chapter, cooling with coolants has numerous drawbacks, due to the fact that large and long tubes are required, which makes the total system implantation very complex. Therefore, for a mental disorder like epilepsy, the use of a cooling device based on low temperature fluid circulation methodologies is considered impractical for its treatment [35].

Thus, and with the evolution of thermoelectric devices and the necessary supporting technology the opportunity arises to re-evaluate cooling as a viable therapy for some forms of epilepsy.

2.3.3 Miniaturized Thermal Neuromodulators

The Peltier effect observed in 1834 was crucial for the creation of thermoelectric devices since this effect consists in generating a temperature difference at the junction between two different conductors when an electric current is applied [35]. In this way, and with advances in the development of modern semiconductors, typically alloys of bismuth, tellurium, selenium, and antimony, it has become possible to manufacture small thermoelectric devices. With the microelectronics industry in mind, more recently these devices have begun to be fabricated with thin film technology in order to make them even smaller. Thus, it is now possible to have a thermoelectric device with a thickness of less than 200 μm (Figure 9

(a)), whereas before they were typically 1.5 mm (Figure 9 (b)). It is worth noting that despite being smaller they have not lost the capability to pump heat, on the contrary, they have up to ten times more [36].

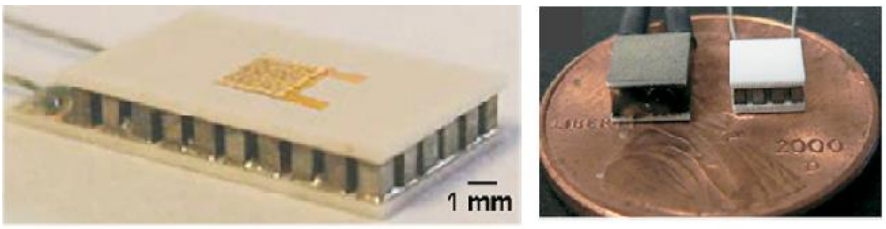

Figure 9 - Thermoelectric devices: (a) Peltier device fabricated by thin film technology; (b) Commercially available Peltier devices.

However, it was not always possible to have such small devices, so over the years, several studies have been carried out both *in vitro* and *in vivo* experiments with the thermoelectric devices available at the time, in order to evaluate whether it was possible to stop epileptic seizures.

The first study that demonstrated that cooling with a thermoelectric device could suppress seizures was developed by Hill et al. in 1999. To prove that this was possible, they conducted *in vitro* experiments with hippocampus slices that had epileptic activity induced by 4-aminopyridine, a blocker of voltage-gated potassium channels and a well-established convulsant agent. The slices were placed on the surface of a 5 × 5 × 2.4 mm Peltier module attached to the base of a perfusion chamber, and a current ranging from 0.6 to 0.8 A was used to activate the Peltier device. After activating it, they found that at a cooling temperature of 21 °C, the seizures ended in 8 seconds as opposed to the seizures in the control slices that lasted about 35 seconds. So, in order to prove that this was due to the thermal effect and not due to the result of the electric field generated by the Peltier device, another experiment was conducted in which the device was not in contact with the slices. In this case, since there was no cooling, the epileptic seizures were not attenuated, corroborating that seizures can only be attenuated when the device is in direct contact with the slice [40].

For first evidence on the use of thermoelectric devices, the results were quite promising encouraging the researchers to move on to *in vivo* studies. Thus, Yang et al. went on to investigate the effect of rapid cooling on seizures *in vivo* in anesthetized rats as a potential therapy for neocortical epilepsy. A window of the frontal bone was removed, and focal epileptic discharges were produced by microinjection of 4-aminopyridine into the motor cortex 0.5 mm below the pial surface. After injection, animals developed recurrent seizures that persisted for 2 hours. Two Peltier chips, each with 3.5 × 3.5 × 2.4 mm, were used to provide effective cooling. In addition, a copper rod attached to the thermoelectric devices was added

to serve as an excellent heat sink, and a 0.13 mm thermocouple was placed on the surface of the devices to be able to measure the temperature. Figure 10 shows the device created for this study [35], [41].

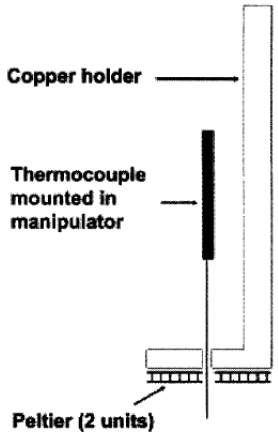

Figure 10 - Diagram of device constructed by Yang et al. to measure temperature below Peltier device [41].

Once the thermoelectric device was placed in direct contact with the neocortex and a current of less than 0.8 A was applied to it, they found that there were significant differences in the duration of the epileptic seizures [41]. The results obtained from this experiment are demonstrated in Figure 11.

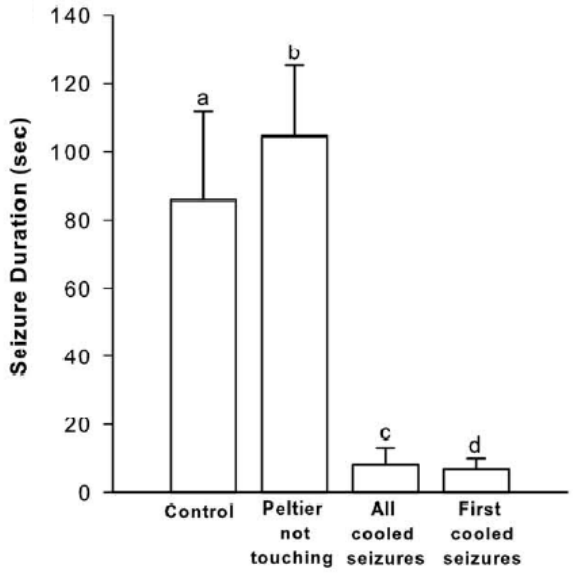

Figure 11 - Mean duration of seizures with the respective standard deviation: (bar a) Control group, without cooling application; (bar b) Peltier implanted but not located in the epileptic focus; (bar c) Cooling applied in all seizures; (bar d) cooling applied only in the first seizure [36].

It is easy to understand by observing the graph above that the mean duration of seizures in the control group (Figure 11 (bar a)), i.e., in the group that had no cooling, was 85.7 ± 26.2 seconds. On the other hand, in the group that was cooled to 20 °C, the mean seizure duration decreased to 8.4 ± 5.0 seconds. Applying the focal cooling treatment only after the first seizure, Figure 11 (bar d), or extending the treatment to all verified seizures, Figure 11 (bar c), shows similar mean seizure duration, thus proving that cooling positively influences the epilepsy progression right after the first actuation. Once again, it was

also shown that when the Peltier is not in direct contact with the epileptic focus, seizures are not attenuated, as can be seen in Figure 11 (bar b). In fact, it can even be affirmed that it has the opposite effect, so the Peltier's positioning in the epileptic focus is crucial for the attenuation of seizures. It was also shown that the duration of the seizures remained reduced even after rewarming, that is, after ceasing brain cooling. Moreover, in the histopathological examination of these animals there were no abnormalities after 2 hours of exposure to temperatures as low as 5 °C, a result which leaves the researchers optimistic that the effect of cooling will be exquisitely focal and well tolerated [3], [36].

Another conclusion also drawn from this preparation was that there is only a progressive decrease in seizure duration below 26 °C, however there was no significant difference between 20 and 22 °C, as can be seen from the graph in Figure 12. However temperatures below 20 °C were not tested [35].

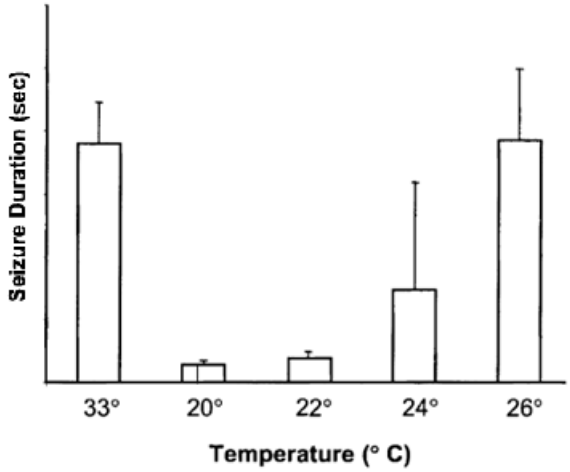

Figure 12 - Effect of cooling at different temperatures [35].

The same group conducted further experiments that revealed other attractive aspects of focal brain cooling. Using a thin thermocouple inserted in a 30-gauge needle, the researchers mapped the cortex below the thermoelectric device. There they found that the cooling only extended to a depth of about 4 mm, allowing them to conclude that its effect must be localized only to a small region of the neocortex below the pia [35].

These results show promising performance regarding the use of thermoelectric devices without fluidic channels. However, this device that has been developed is only effective in application on anesthetized animals due to the heatsink size. In this regard, it is still necessary to develop more miniaturized devices so that further tests can be carried out on rodents.

2.4 Closed-loop Control for Epilepsy

Behavioral assessment in rodents is critical for brain function research, so *in vivo* neurophysiological recordings are fundamental to mechanistically dissect the neural pathways that underlie behavioral changes and serve as markers for the dynamics, efficacy, and safety of potential therapeutic approaches [42]. So, as the intent is to analyze the effect of cooling on epileptic seizures, it becomes essential that at the same time that cold is applied on rodents the EEG is also observed. However, most *in vivo* recording systems require associated wires, so it is essential to develop a wireless closed-loop device capable of applying cold as well as recording the EEG of the area where cold is being applied.

EEG is a very important tool in medicine, since it allows the electrogram recording of the brain's biopotentials, that is, its electrical activity. The basis of this test consists in the amplification of voltage differences between an electrode placed in an area of interest and a reference electrode located at a distance. This distance can change according to the experiment that is being performed. In experiments related to the nervous system, the EEG is often used because it allows researchers to better understand sleep mechanisms, to diagnose sleep disorders, to detect neurological malformations, to diagnose concussions, to detect seizures and their location, among others [43], [44].

EEG recording can be conducted in two different ways: non-invasively, through electrodes positioned on the scalp, or invasively, with electrodes in the brain parenchyma or with epidural electrodes (stainless steel screws implanted through pre-drilled holes). It should be noted that intracranial electrodes can be as simple as stainless steel wires, or as sophisticated as multi-contact microarrays that penetrate the brain at predetermined depths, or multi-unit recording systems inserted via microcannula. Generally, invasive techniques are used in experiments, as they provide greater detail in quick oscillations, but extracranial recordings can be performed in situations where monitoring of cortical activity is necessary [43].

As far as data acquisition is concerned, there must be a direct connection between the implanted electrodes and the recording system, or else use miniaturized radio-telemetry transmitters implanted in the subject or carried in backpacks specially designed for the purpose [43], [44]. In fact, this has been the main focus in EEG recording systems, as the use of wired devices is one of the biggest hindrances of this test in rodent experiments. Thus, with scientific and technological advances, researchers have adapted these systems in order to provide answers to the desired needs and requirements.

2.4.1 Wireless EEG Recording Systems for Rodents

In the last decades, radiotelemetry has become a widely used and highly recognized approach for *in vivo* experiments, as it allows the measurement of a variety of behavioral and physiological parameters in conscious, free-moving animals. In addition, nowadays there is a concern to limit the number of animals in experiments as well as their suffering. Therefore, with the use of wireless systems this can be achieved without compromising the scientific studies that must be performed [45].

Currently, there are several commercially available systems for radiotelemetry deployment, so the choice of these should be made depending on the specific requirements of the laboratory and the objectives of current and future research and experiments. It should also be considered whether the telemetry configuration is compatible with other electrophysiological systems so that both can be connected or extended [45].

Lundt et al. presented a simple, quick and efficient technique for intraperitoneal and subcutaneous pouch implantation of a standard radiofrequency transmitter in mice and rats. Since they wanted to measure EEG, physical activity and temperature in rodents, they used a one-channel PhysioTel transmitter TA10ETA-F20 as well as a two-channel TL11M2-F20EET from DSI (Data Sciences International). These have a weight of about 1.9 and 3.9 g, an input voltage range of ± 2.5 and 1.25 mV, a channel bandwidth of 1-200 and 1-50 Hz, a nominal sampling rate of 1000 and 250 Hz, and a guaranteed battery life of 4 and 1.5 months, respectively. In terms of common specifications, both have a volume of 1.9 cm^3, a temperature operating range of 34.0 to 41.0 °C, and a magnetically actuated on-off mechanism. It should be noted that subcutaneous implantation of these transmitters should be done for animals with a minimum weight of 20 g, while for intraperitoneal implantation they should be considerably heavier. The transmission of the acquired signals is given by capturing the telemetered data from the implant through a receiver, RPC-1 also from the DSI, which then forwards this data to a Data Exchange Matrix (DSI), the latter serving as a multiplexer. Figure 13 shows the system used, as well as the procedure for collecting the data [45].

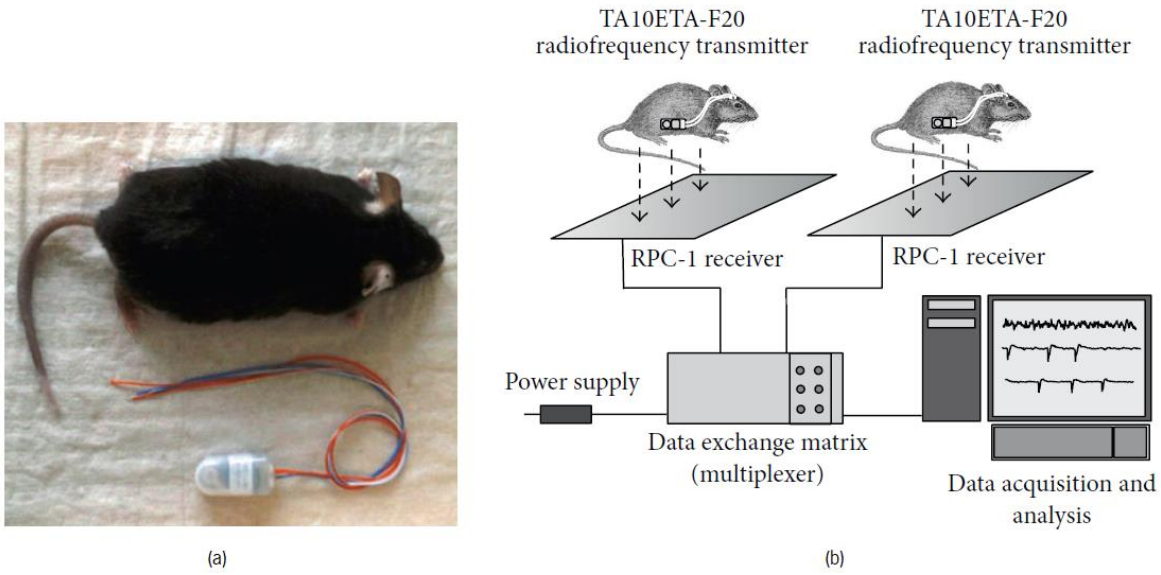

Figure 13 - EEG radiotelemetry system and radiofrequency transmitters: (a) Rat and the device that will be implanted on it; (b) Diagram of the system used to perform animal experimentation [45].

Zayachkivsky et al. addressed the need to develop devices capable of continuously recording EEG in immature rodents, since until now there were no devices small enough to be implanted in them. Thus, they designed a miniature wireless telemetry system with four goals in sight. It should be minimally invasive, have low power consumption of the unit allowing months of continuous monitoring without surgical re-implantation, be able to record high quality EEG waveforms with minimal motion artifacts, as well as allow the immature mice to be housed after surgery with their dam and littermates. Depending on the battery life, wireless transmitters have different characteristics, so three transmitters capable of amplifying two channels of EEG or local field potentials from deep brain structures, such as the hippocampus, have emerged for implantation in the immature mice. For a battery life of 2 weeks and a footprint of 5 × 7 mm, the transmitter weighs about 0.6 g and has a volume of 0.3 cm^3. If a 2 month battery life is desired, the footprint is 7 × 9 mm, and the transmitter weighs 2.3 g and has a volume of 0.8 cm^3. For a battery that lasts 6 months, the footprint is 7 × 12 mm, making the transmitter weigh 4 g and 1.4 cm^3. All the electronic components and the battery are encased in medical grade epoxy to make the device waterproof and rugged, thus preventing damage caused by chewing on the transmitter that could render the device inoperable. Unlike radio-frequency transmitters, the telemetry system uses capacitive coupling between the transmitter and a receiver antenna that sits under the animal's cage, allowing the animals to remain in their houses, as can be seen in Figure 14 [46].

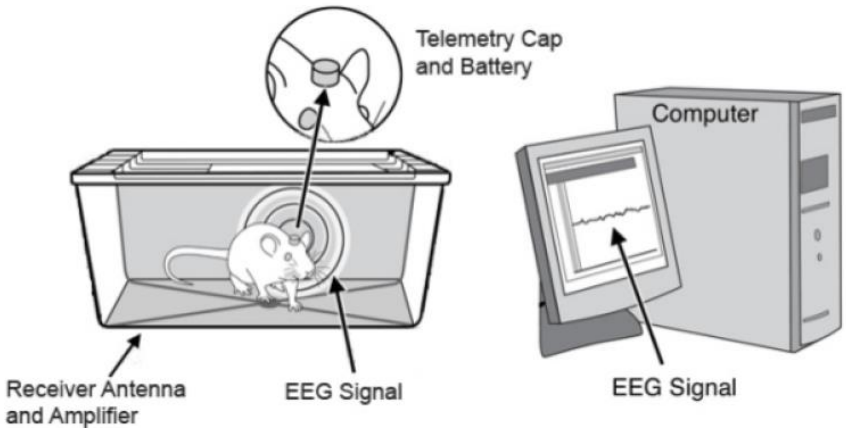

Figure 14 - Schematic of the recording system [46].

One of the great benefits of implanting these systems is the fact that only cyanoacrylate gel is required, as opposed to other existing systems that often require drilling holes to fix the device to the brain. In addition, it provides reduced risk of infection, increased animal mobility, as well as reduced morbidity and mortality that otherwise increase the time, money and number of animals needed for an experiment [46].

More recently, Cheng and Murari have developed an open-source, stand-alone electrophysiology recording system for rodents. This, which does not require any cables or receivers, consists of stand-alone, autonomous recording with two channels, a reference and a ground, which acquires, amplifies, filters and stores data by itself. The system design was implemented on three 0.6 mm thick vertically stacked PCBs (Printed Circuit Boards), one for the analog components, one for the digital, and one for the battery. In addition, it has a six-pin male connector to be able to connect the system to the mouse with implanted electrodes connected to a coupling receptacle cemented to the skull. The whole system measures (13 × 8 × 8) mm^3 and weighs 1.8 g. In Figure 15 it is possible to see the system, as well as its implantation in the mouse [42].

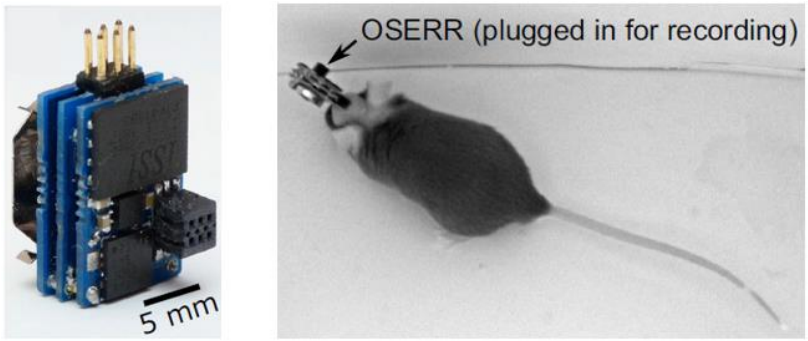

Figure 15 - Open-source standalone electrophysiology recording system: (a) A photograph of the system; (b) System plugged into an implanted electrode socket on a mouse [42].

The analog component of this system consists of a coupled instrumentation amplifier, a Sallen-Key high-pass filter, and a multiple feedback low-pass filter, providing third order attenuation (60 dB / decade) on both sides of the passband. On the other hand, the analog component includes a microcontroller that digitizes the signal in 10 bits at a firmware-programmable sampling rate and stores it on a flash memory chip. The system is powered by a 30 mAh CR927 lithium cell, which is regulated to a supply voltage of 2.5 V and a virtual ground level of 1.2 V. In addition, it also has a simple reader and a graphical user interface to transfer data from the device to a computer [42].

To demonstrate that the autonomous system worked, they performed an experiment where they measured EEG signals during induced seizure, prolonged recording, anesthesia, and social interactions in mice. Here they configured the amplifiers with a gain of 60 dB, leading to a scaled input of ±1.25 mV and a least significant bit (LSB) of 2.4 μV. The passband was set from 1 to 120 Hz and the sampling rate was fixed at 240 S/s. A 256 Mbit memory was used, giving an operation time of 15.5 h. After data collection they concluded that the system was a success [42].

2.4.2 Device Miniaturization

As the purpose of this dissertation is to develop a wireless device to apply thermal neuromodulation in rodents as well as record the EEG, a survey was conducted on devices that have these two characteristics. This way, it will be possible to analyze eventual flaws or limitations and therefore ensure a better response capacity for the device that will be developed.

Hou et al. presented a wireless, batteryless, RF (radiofrequency)-powered biomedical microsystem with versatile sensors for neuronal recording and actuators for epilepsy suppression based on focal brain cooling technique. Since it has no battery, the RF-powered design makes the presented microsystem small in volume and lightweight. Figure 16 shows the electrical architecture of the proposed microsystem that provides parts of the microsystem for biopotential, chemical, temperature measurement, and thermoelectric cooler actuator drive capability [47].

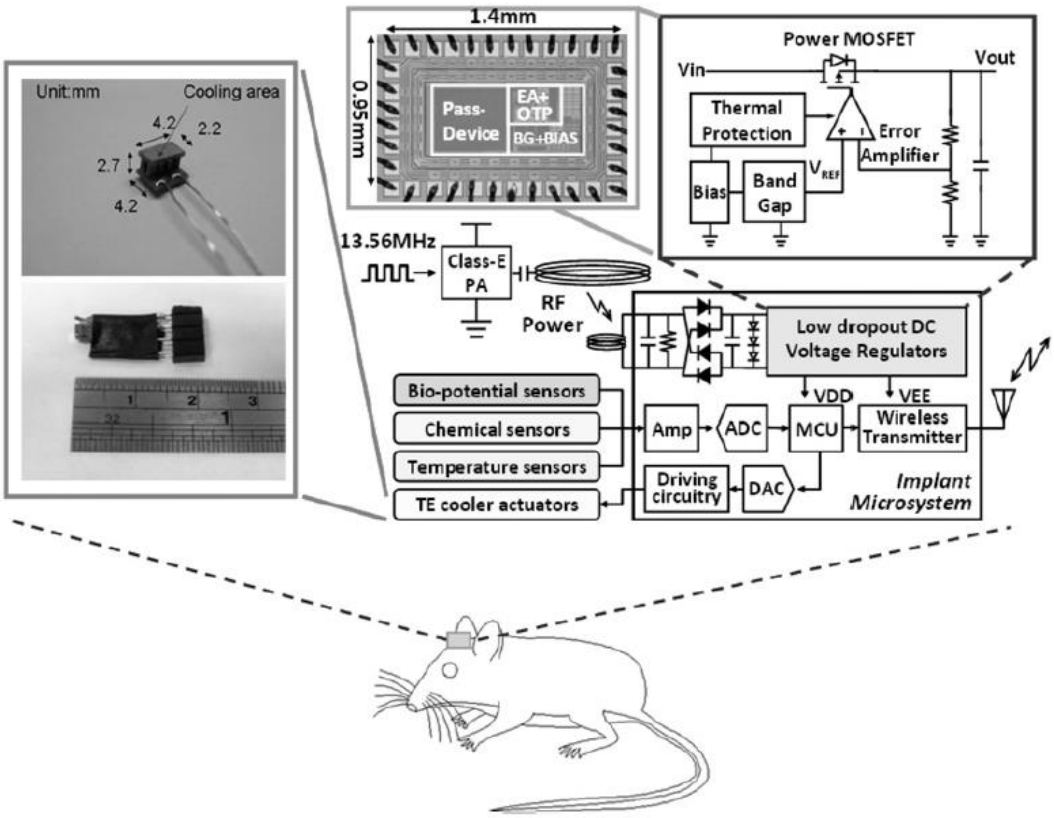

Figure 16 - System structure of the wireless powering device in an implant microsystem [47].

For this to act as a wireless power module, miniaturized spiral coils were then used that convert RF signals into a DC voltage. This will make it possible to control the various pairs of cooling units in the rat's head. In fact, since the thermoelectric cooler has multiple units, the cooling area is 4.2 × 2.2 mm which is quite a large area. These coolers together with electrodes that allow the EEG recording, form a module that is placed inside the rat's head, which is fixed with a biocompatible material. To be able to transmit the data, a Bluetooth module was also utilized, namely the BTM-182 (Rayson Technology Co., Ltd., Taiwan) with a Bluetooth v2.0 BC-417 chip (CSR Bluetooth, USA) and a PCB antenna [47].

Although this proposal meets the requirement for no wires, the fact that it has no battery makes the device only viable in controlled environments, as the rat needs to be at a certain distance for the device to be charged, thus making the rat's movements limited [47]. Also, and although the device seems to be ready to perform experiments, it does not seem that there is enough evidence that this device has actually been made, as there are no pictures of the final device. Only a schematic of the components that the supposed device and real pictures of the thermoelectric cooler are given. Therefore, the need to develop a wireless, battery-powered device capable of cooling the rodents' neurons as well as recording the EEG continues to arise.

2.5 Systems Features to Date

Thermal neuromodulation devices must take into account the brain's fragility and the difficulty of access to certain neuronal areas, so they must be small in size and equipped with tools that allow access to very specific locations. In this sense, and with the significant evolution in the fields of science and technology, most of the current implantable microsystems integrate requirements such as wireless technology with lower power consumption and biocompatibility.

In order to summarize the characteristics of all the wireless EEG recording systems for rodents mentioned so far, Table 2 was created. Thus, it is possible to observe the main differences between the systems that have been developed to date, namely weight, dimensions, battery, bandwidth, binary throughput, sampling rate, current, among others.

Table 2 - Systems features

Systems	Weight	Dimensions	Battery	Bandwidth	Frequency Band	Binary Throughput	Sampling rate	On/off magnetically	Current
Hou et al. [47]	N/A	(4.2 × 2.2 × 2.7) mm^3	13.56 MHz RF-power supply	N/A	N/A	N/A	N/A	N/A	0 – 200 mA
Zayachkivsky et al. [46]	0.6 g / 2.3 g / 4 g	0.3 cm^3 / 0.8 cm^3 / 1.4 cm^3	2 weeks / 2 months / 6 months	0.1 – 100 Hz	N/A	N/A	500 Hz	N/A	N/A
Lundt et al. [45]	1.9 g / 3.9 g	1.9 cm^3 each	4 months / 1.5 months	1 – 200 Hz / 1-50 Hz	N/A	N/A	1000 Hz / 250 Hz	Yes	N/A
Cheng and Murari [42]	1.8 g	(13 × 8 × 8) mm^3	50 h (240 S/s)	1 – 120 Hz	N/A	10 Mbps	240 S/s	Yes	210 µA

CHAPTER 3 IN HOUSE WIRELESS RECORDING SYSTEM

Typically, the required bandwidth for EEG applications is from 0.5 to 60 Hz. However, in other cases, frequencies up to 500 Hz may be required. Since often more than one channel is needed to measure the EEG signal, it is essential to effectively handle high data rates for high performance EEG recorders. For example, a system with 64 channels, where one sample is 16-bits, at 1 kS/s, requires a payload data rate of 1 Mbps [48].

The focus of the dissertation is to improve the binary throughput of a wireless recording system, so it is necessary to understand the entire system that was previously developed in [49].

The fact of using a system that has already been created means that any modifications that may be needed are limited to the components that have been used. In this sense, this chapter will cover the system and software architecture and how this will influence the improvements that are intended to be made, namely the wireless protocol and the maximum binary rate.

3.1 System Architecture

The system consists in two modules that were previously developed in house. These modules were developed with the intention that one of them could be carried by a rodent, in order to cool the neurons and acquire the EEG signal and the other one would receive the EEG data wirelessly to be stored in a computer. Figure 17 shows the two modules, Primary Transmitter (PTX) and Primary Receiver (PRX), that were used during the development of this work.

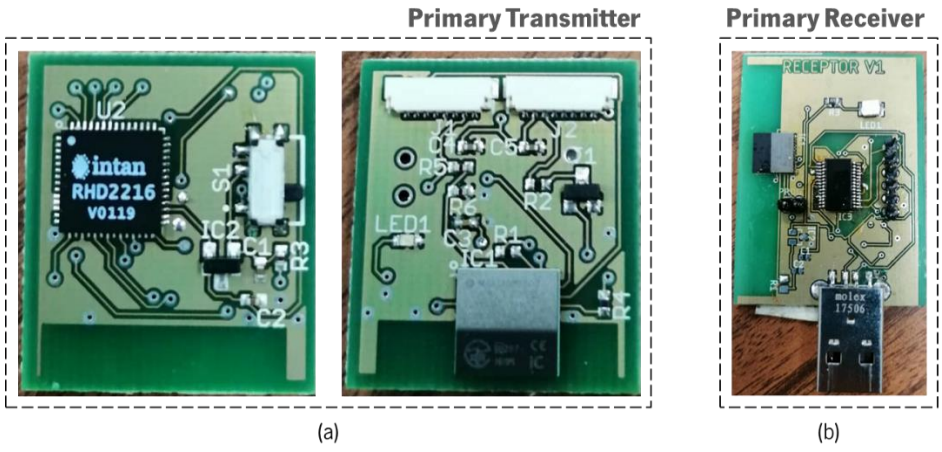

Figure 17 - PCBs previously developed in [49]: (a) Both sides of the PTX; (b) PRX.

The PTX contains a high performance Bluetooth 5 Ready, NFC (Near Field Communication) and ANT (Advanced Network Technology) low energy module (ISP1507) which integrates a Nordic NRF52832 MCU and an antenna, a low power electrophysiological signal acquisition chip (RHD2216) and a Peltier module. Since this is going to be carried by rats, this device should be as lightweight and small as possible so that tests can be performed under the best possible conditions. Hence, this device was developed with only a total weight of 2.5 g with a volume of (2.3 × 2.6 × 0.5) cm^3. However, since it requires a battery to supply power to it, the system is left with a weight of about 7.73 g, since the battery used weighs around 5.23 g. This is in accordance with the literature, as rats weighing more than 250 g are able to carry 60 g on their backs and still move freely [50].

Regarding the PRX, it has an ISP1507 module, similarly to the transmitter, and, in addition, a USB to serial UART interface device (FT232RL). In this way, it is possible to connect it to the PC and receive data that is being sent wirelessly by the transmitter. This one measure 3.6 × 6.5 × 0.2 cm, but since it is for plugging into the computer and will not be attached to the rat, it doesn't need to be any smaller.

In Figure 18 is presented a schematic of the main PCB components, to better understand how the whole system works.

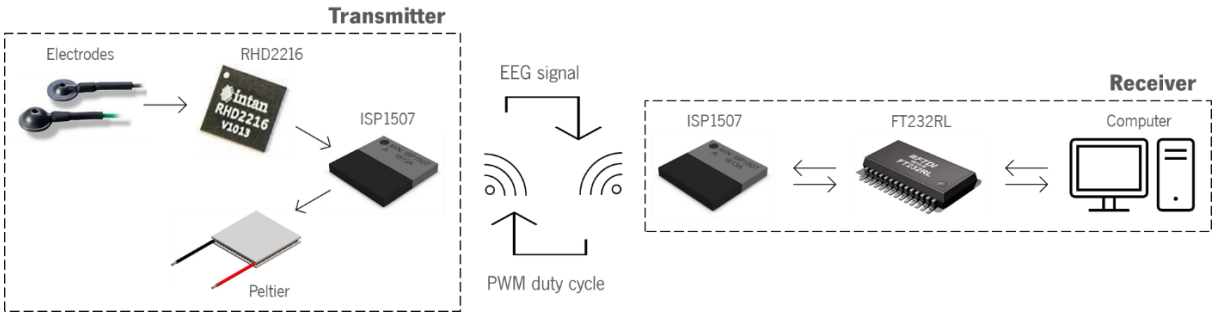

Figure 18 - Schematic of the whole system.

In this sense, it was necessary to study some components in more detail in order to understand their functioning and limitations. One of the most important is the wireless communication module, ISP1507, since it is from it that all other components will be programmed and is the one that will dictate the protocol chosen. However, it was also essential to understand the functioning of the RHD2216 chip, since it is the one that will acquire the electrophysiological signals and for that reason there are several parameters that need to be established.

3.1.1 Wireless Communication Module

The ISP1507 is based on nRF52 series devices from Nordic Semiconductor, and more specifically, about nRF52832 since the module used was the ISP1507-AX. In this sense, it was essential to know more about the nRF52 series and more specific nRF52832 [51].

The nRF52 series offers pin-compatible device options for BLE (Bluetooth Low Energy), 2.4 GHz solutions, and ANT solutions giving the freedom to develop a wireless system using the technology that best suits the application at hand. The unique system of memory protection and hardware resources allows applications to be developed on devices with embedded protocol stacks running on the same processor, without any need for stack bonding or strenuous testing to prevent the application and stack from interfering with each other [52].

Although the size of the ISP1507 is very small (8 × 8 × 1 mm), the module integrates a 2.4 GHz transceiver, 32-bit ARM Cortex-M4 CPU, decoupling capacitors, 32 MHz and 32.768 kHz crystals, charge capacitors, DC-DC converter, RF matching circuitry and antenna, as well as the wireless SoC (system-on-a-Chip). Its power consumption is exceptionally low as it uses a sophisticated on-chip adaptive power management system. For example, with advanced power management it is possible for a battery to last up to several years on a coin cell battery. In addition, it offers generous memory availability for Flash and RAM. A block diagram of the ISP1507 is shown in Figure 19 [51], [53].

It is important to note that the ISP1507 can be used in Central, Peripheral or both roles for BLE and for both ends of other proprietary protocols [51].

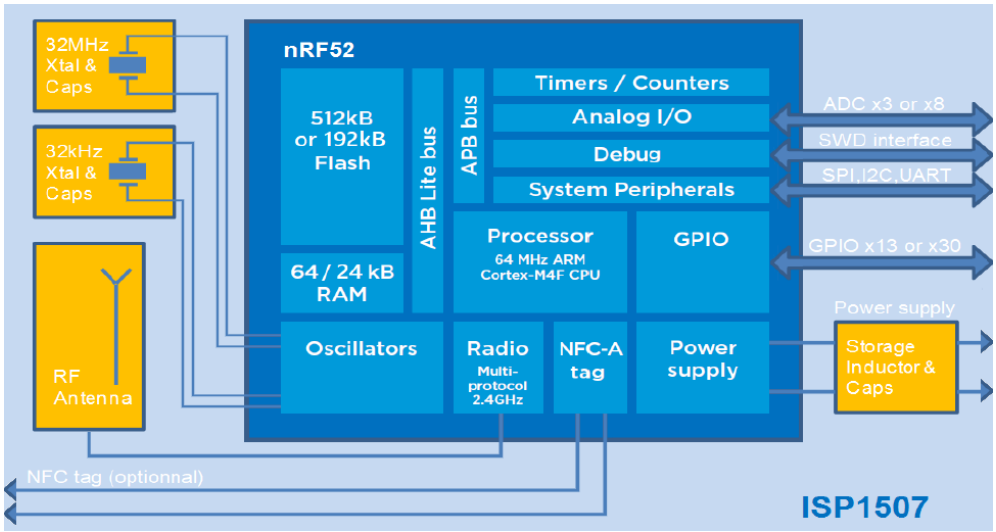

Figure 19 - Block diagram of the ISP1507 [51].

3.1.2 Digital Electrophysiology Interface Module

The RHD2216 is a microchip that belongs to the RHD2000-series from Intan Technologies. It is a complete low-power electrophysiology signal acquisition system. This device contains low-noise amplifier arrays with programmable bandwidths and are suitable for a wide variety of biopotential monitoring applications. The innovative architecture of these circuits combines amplifiers, analog and digital filters, a 16-bit multiplexed analog-to-digital converter (ADC), and a flexible electrode impedance measurement module on a single 8 × 8 × 1 mm silicon chip. This module can sample 16 channels of differential amplifiers at a maximum rate of 30 kS/s [54].

The RHD2216 chip communicates through a standard SPI (Serial Peripheral Interface) bus consisting of four signals, namely an active-low chip select (CS), a serial data clock (CLK) with a base value of zero, a MOSI (Master Out, Slave In) data line to receive commands from the master device and a MISO (Master In, Slave Out) data line to send pipelined results from prior commands to the master device. It is important to emphasize that the RHD2216 chip always functions as the SPI slave device, hence ISP1507 is going to be the master device [54].

This bus can be configured in two ways: standard CMOS signaling and low voltage differential signaling (LVDS). Since this has to be decided when creating the PCB to make the necessary connections, this was a choice that was previously made. The one chosen was the standard CMOS signaling which consists of a single wire to send a high or low voltage digital signal, 3.3 V and 0 V, respectively. Only when the output pin changes state, a burst of current is drawn from the supply to change the capacitance of this pin. It is important to highlight that these sudden changes in current strength create high frequency noise in the power supply that can interfere with the information being sent [54].

The way the chip communicates is through 16-bit data words transferred in each direction. This uses a pipelined communication protocol, that is, each command sent over the MOSI line generates a 16-bit result that is transmitted over the MISO line two commands later. In this way, the RHD2216 responds to five basic commands, namely CONVERT(C), CALIBRATE, CLEAR, WRITE(R,D) and, finally, READ(R) [54]. Figure 20 shows a schematic of the communication with the chip.

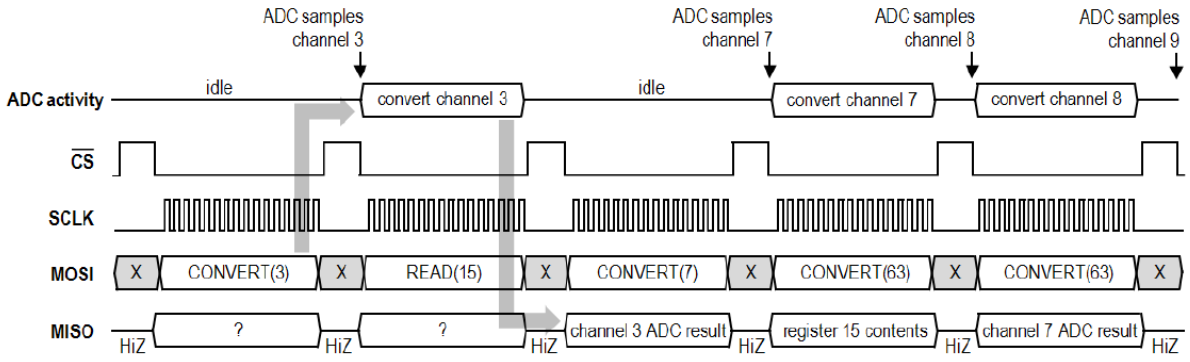

Figure 20 - Diagram of the communication with the chip RHD2216 [54].

The CONVERT command consists in ordering an analog-to-digital conversion of an analog channel C. Since this chip contains 16 differential amplifiers, then C will take values from 0 to 15. As it can be seen from the above picture, the result of this command will be sent by MISO two commands later, as mentioned before [54].

As for the CALIBRATE command, it performs an ADC self-calibration routine. This command should be done only once, after the chip is turned on and all register settings are finished. This calibration takes several CLK cycles to execute, so it is necessary to send 9 random commands to generate the CLK cycles needed for calibration. Note that the commands can be random, as the chip ignores them until the calibration is complete [54].

CLEAR, as the name implies clears the calibration parameters from the ADC. This command is not necessary to execute in normal chip operation [54].

On the other hand, WRITE writes an 8-bit data byte D into a RAM register R. In response it sends to the master, in the lower byte the data byte D so that the correct reception of the value can be confirmed. In the upper byte every bit is set to one [54].

Finally, READ reads the contents of a RAM or ROM register R. As a result, it sends in the lower byte what it has read from that register, while the upper byte is all zeros [54].

The chip contains 18 eight-bit RAM registers that configure various aspects of the chip's behavior and several eight-bit ROM registers that store basic chip properties. Therefore, it is necessary when powering up this chip to configure the RAM registers via the SPI master device. This configuration should be completed at least 100 μs before the ADC calibration, since some registers define parameters that optimize the ADC operation [54].

3.2 Software Architecture

In view of the system architecture analysis, it was also necessary to conduct the software architecture analysis. This is the critical link between the design and the engineering requirements, as it identifies the main software components, their external properties, and their relationships with other software.

In order to program the two modules of the system, Keil µVision5 IDE was used. This combines project management, runtime environment, build facilities, source code editing, and program debugging in a single powerful environment. The µVision Editor allows you to edit code, which can be written in Assembly or C/C++. The programming language used was C, since it is a general purpose programming language that allows the development of operating systems, databases, applications, among others. This language is commonly used in computer architectures ranging from the largest supercomputers to the smallest microcontrollers and embedded systems [55].

When it comes to the part of making the connection between the proposed electronic device and the users, MATLAB was the tool of choice. This is a programming and numerical computing platform used by millions of engineers and scientists to analyze data, develop algorithms, and create models. More specifically, it is possible to use MATLAB to control systems, process signals, wireless communications, machine learning and deep learning, among many others [56].

In this way, and using the tools explained so far, it was effectively analyzed what was necessary to program in each one of them. Figure 21 shows a schematic of the software architecture for this system.

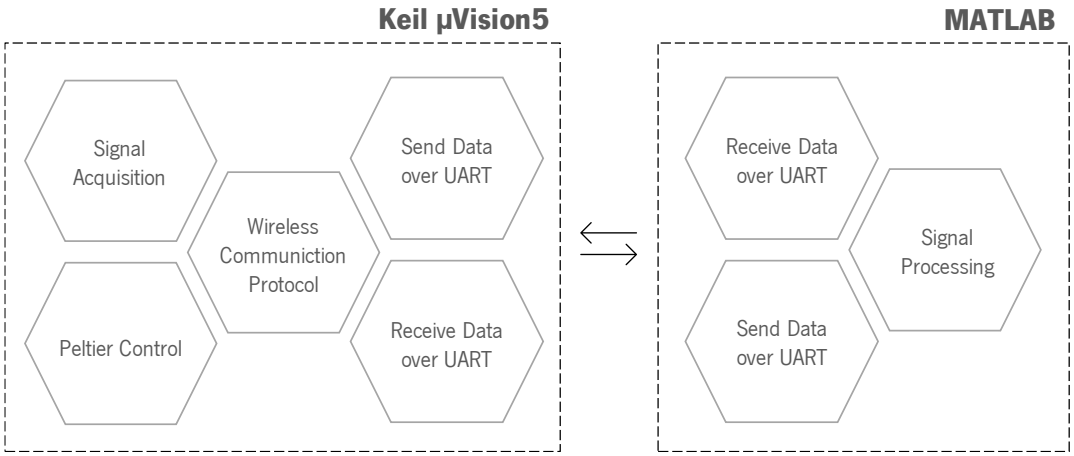

Figure 21 - Software architecture.

Since the goal was to obtain a device that could acquire electrophysiological signals, as well as control a Peltier to cool the rats' neurons, it was necessary to program the two modules to do this. Then, functions were created in Keil µVision5 so that both could communicate with each other wirelessly. Afterwards, a function was also created in the PTX that allowed the acquisition of signals and another that received

information from the PRX with the duty cycle value and applied it to the Peltier. In the PRX, in addition to the wireless communication function, functions were created to enable data transaction through the UART to be able to send data to the computer and also receive data from the computer.

In MATLAB, in the same way as the PRX it was necessary to make functions to receive the data coming from the PRX and send data back. Also, since the data received are samples of the input signal, it was necessary to do digital signal processing to be able to observe the signal as it is.

These functions described for each of these platforms will be explained in more detail in section 4.3 from the next chapter.

3.3 Data Rate Bottleneck Characterization

Given what has been mentioned so far, it should be noted that there are a number of factors that will limit the data transmission rate and that will not be able to be circumvented.

One of the crucial components is the RHD2216 chip. The chip's ADC can operate up to 1.05 MS/s, however, because there are 16 channels of differential amplifiers, the sampling rate of each channel decreases. Since the ADC allows it to sample voltage signals from the amplifier matrix as well as various sensors and auxiliary inputs across the chip, in the end the sampling rate per channel will be 35 times lower, i.e., 30 kS/s. Since this samples 16-bit words, for one channel this will correspond to a binary throughput of 480 kbps. This requires a protocol that can send bits at least at this rate. Previously, Bluetooth 5 had been used for the two modules to communicate with each other, which in theory could transfer data at a more than sufficient rate, however in practice it was not possible due to various factors that the protocol required. Thus, the wireless communication protocol had to be changed.

One of the critical factors in the choice of wireless communication protocols is the fact that only one of the protocols that runs on the ISP1507 can be chosen. Depending on the protocol chosen, the binary rate will be the maximum data rate that the protocol can achieve. For this, and before proceeding to the final implementation of the chosen protocol in the two modules, an example provided by Nordic Semiconductor was tested in order to analyze the maximum data rate obtained by the ESB (Enhanced ShockBurst) protocol. It was found that it was possible to send 32 byte packets every 500 µs in both directions, which results in a binary rate of 512 kbps. Therefore, it was concluded that this protocol would be sufficient to send the desired data.

However, as mentioned above, several channels are normally required, which leads to the conclusion that for the maximum sampling rate of the ADC, it is only possible to send data from one channel, because

the transmission rate of the wireless communication protocol is not enough for more. If more than one channel is desired to be sampled simultaneously, the sampling rate needs to be reduced. For the example mentioned above where an EEG signal with a frequency of about 500 Hz needs to be observed, the sampling rate of each channel will have to be at least 1 kS/s, i.e., 16 kbps. Since the communication protocol allows 512 kbps to be sent, it would be possible to observe 32 channels, but the RHD2216 chip only has 16 channels, so this would be the maximum possible. However, for the PCB used it is only possible to observe two channels, since there is only connection for two. So, in case both channels are intended to be seen, the sampling rate of each channel should be lowered to about half of the maximum frequency, that is 15 kS/s.

CHAPTER 4 SYSTEM ANALYSIS AND PERFORMANCE IMPROVEMENT

The wireless communication between the modules becomes essential for this system to work properly and to be able to perform tests on animal models in a more efficient and comfortable way.

The protocol previously used was Bluetooth 5 (BL5) which, in theory, would have a more than sufficient rate to transmit the data. However, due to factors such as the inter frame space (IFS) between packets, the requirement to send empty packets between each packet sent, the limit on the number of packets per connection interval, the connection interval itself and the sending of bytes in a packet that are not used for the application payload, the theoretical values for the throughput altered. Thus, they only achieved a system with a data rate of 1,331 Samples/s, which corresponds to 21,296 bps [49].

In this sense, one of the main modifications made in this system was the wireless communication protocol, so that data could be sent from one module to another at a much higher rate. Note that the protocol would have to allow, like the previous one, bidirectional data sending so that it would also be possible to send a duty cycle value to control the Peltier.

Therefore, in this chapter it will be discussed which protocols could be used with the supplied module and why the chosen protocol for wireless communication is the best option. Afterwards it will be explained in detail how the communication protocol works, as well as the changes that had to be made in the software in order for the two modules to work with the new protocol at the rate that was desired. In addition, the process of creating the interface that allows data to be received and stored on the computer and sent back information if the user wishes will also be explained.

4.1 Alternative Faster Wireless Links

In order to change the wireless communication protocol, it was necessary to analyze a protocol that could also transfer data bidirectionally yet could transfer data more quickly. Since it was intended to use the PCBs that had been previously designed and created, it was also crucial to choose a protocol that was compatible with ISP1507 module. Since, as mentioned earlier, it supports Bluetooth LE, including the high-speed 2 Mbps feature, NFC, ANT and 2.4 GHz solutions, it was among these that a protocol was chosen [53].

Given that Bluetooth had been used before, a decision had to be made between NFC, ANT and 2.4 GHz proprietary. NFC was discarded, as it only works over shorter distances, around 10 a 20 cm, which

is not practicable for the tests that are to be carried out with the rats. In addition, the data transfer rates are low, around 106, 212 or 424 kbps, and its procedure is quite complex compared to other protocols [57]. ANT, on the other hand, was also not chosen, since this protocol was designed for low bit rate, low power sensor networks. When in burst mode it only allows a maximum throughput of 60 kbps, which is not an advantage since the goal of this project is to increase throughput as much as possible [58], [59].

In this way, of all the possibilities, the protocol that seemed most appropriate for the problem was one of the two Nordic 2.4 GHz proprietary protocols. Although 2.4 GHz proprietary development does not offer the interoperability that comes with standards like Bluetooth, it can offer special abilities to adapt both ends of a communication link for maximum efficiency [60]. Table 3 shows the differences between the two 2.4 GHz proprietary protocols from Nordic Semiconductor.

Table 3 - 2.4 GHz proprietary protocol features [60]

	ESB	**GAZELL**
Frequency agile	No	Yes
Max. throughput	1.3 Mbps	230 kbps
Pairing options	No	Yes
Configurable TX	Yes	Yes
Synchronous communication	No	Yes
Auto-acknowledge	Yes	Yes

Since the main goal of the application was to increase the binary rate, the ESB protocol was the one chosen, given the fact that it had a higher maximum throughput.

4.2 Enhanced ShockBurst

ESB is a wireless communication protocol from Nordic Semiconductor that allows a device from the nRF52 series to communicate with one from the same series or one from the nRF24L series. Thus, since both modules have an ISP1507 from Insight Sip that is based on the nRF52832 chip, then it is possible to implement the ESB protocol. ESB provides bidirectional data packet transfer, including packet buffering, packet acknowledgement, and automatic retransmission of lost packets. Another advantage of this protocol is that it offers low power consumption radio communication which is crucial for the application that is being developed [61].

The ESB supports a star network topology where typically there is one PRX (Primary Receiver) and up to 8 PTX (Primary Transmitter) [61]. In this application, only one receiver and one transmitter will be needed.

As for the packet transaction, it is initiated as soon as the payload is added to the FIFO (First-In-First-Out) and successfully completed when the PTX receives an ACK (acknowledgment) packet from the PRX. It should be noted that the ESB only allows you to send a maximum of 32 bytes of dynamic payload length. In case it is intended to transmit data from the PRX to the PTX, then the only way is for in PRX to add a payload to the ACK packet. To achieve this, in the same way as in PTX, the payload is added to the TX FIFO and when PRX receives a packet from PTX, then the payload is sent along with the ACK packet. To better understand this process, an illustrative schematic is presented in Figure 22 [61].

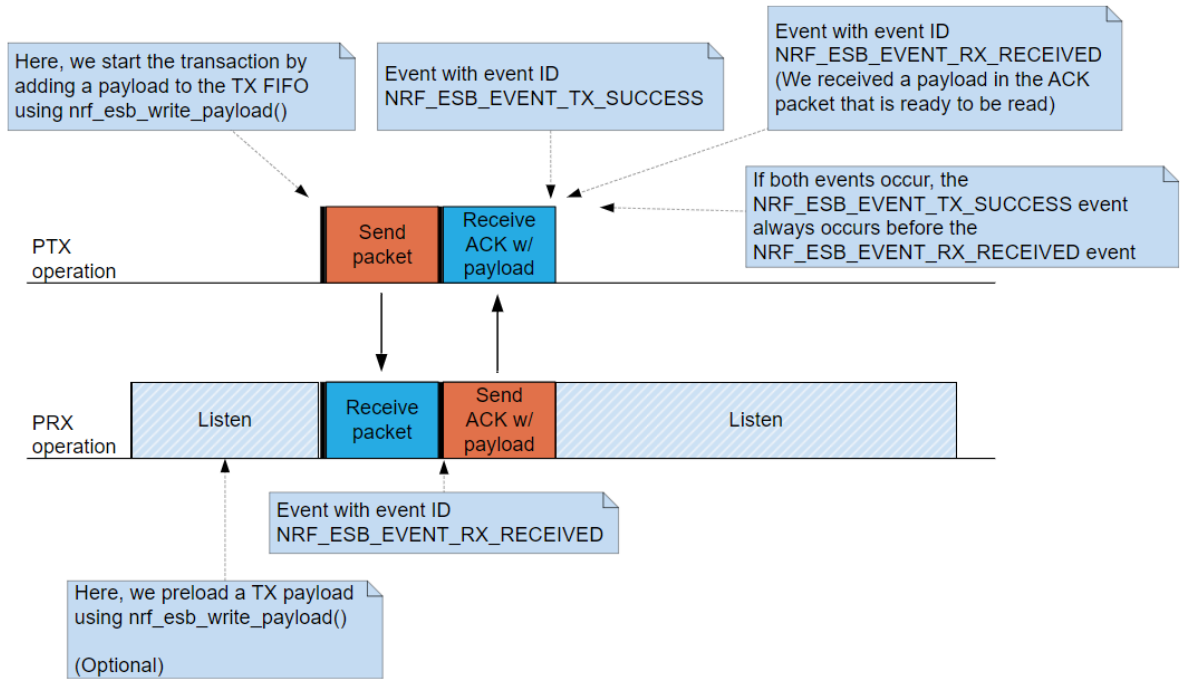

Figure 22 - Packet transaction [61].

Another feature of this Nordic protocol is that when the PTX does not receive the ACK packet, it retransmits the packet until the ACK is received. This prevents data packets from being lost. However, this also causes a delay in sending the next packet of data, so in this application it is set to not retransmit any packets. It should be noted that ACK could be disabled so that there would be no delays, but as mentioned before, sending ACK packets is the only way to send information from the PRX to the PTX, so ACK must be enabled [61].

It is important to emphasize that any packet that is transmitted from a PTX to a PRX is uniquely identified by a two-bit PID (Packet ID) field in the packet header along with the packet's CRC (Cyclic Redundancy Check) field. This PID is used to distinguish a new packet from the previous one in case they

contain the same payload. CRC, on the other hand, has the purpose of detecting errors. Therefore, with both of them the probability of a packet being falsely identified as a retransmission attempt and dropped by the PRX is reduced when several consecutive failed packet transmission attempts occur [61].

For the transmission and reception of packets, a pipe is used, which is a logical address on the nodes, mapping to one on-air address. Each on-air address is composed by a 2-4 byte long base address and a 1 byte prefix address. It should be noted that the nRF52 radio uses an alternating sequence of 0 and 1 as the packet preamble. Therefore, for packets to be received correctly, the most significant byte of the base address shouldn't be an alternating sequence of 0 and 1, in other words, it can' t be 0x55 or 0xAA. Pipe 0 has its own unique base address (base address 0), while the remaining pipes, 1 through 7, use the same base address (base address 1). For each of the 8 pipes there is a unique long byte prefix address. A schematic of the addresses of each ESB packet is in Figure 23 [61].

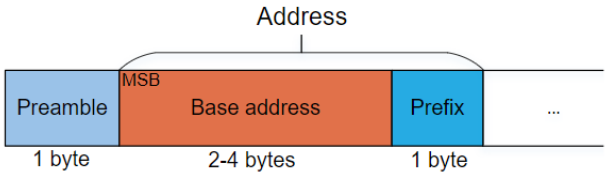

Figure 23 - Addresses from an ESB packet [61].

On-air, what is transmitted first is the most significant bit of each address byte, so the most significant byte of the 2-4 byte long base address is the first to be transmitted, while the prefix byte is the last. Furthermore, addresses cannot consist of a prefix 0x00 and an address in the format 0x00XXXXXXXX or 0x0000XXXX, as this will return the error code NRF_ERROR_INVALID_PARAM [61].

In short, when the ESB protocol is enabled in PTX mode, any packets that are uploaded to a TX FIFO will be transmitted at the next opportunity. In cases where an ACK is successfully received from a PRX, the PTX assumes that the payload was successfully received and added to the RX FIFO of the PRX. Thus, the transmit packet is removed from the TX FIFO so the next packet in the FIFO can be transmitted. If an ACK received by a PTX contains a payload, this payload is added to the RX FIFO of the PTX [61].

In regard to the ESB being enabled in PRX mode, all enabled pipes (addresses) are simultaneously monitored for incoming packets. If a new packet is received that was not previously added to the RX FIFO of the PRX, and the RX FIFO has space available for the packet, the packet is added to the RX FIFO and an ACK is sent back to the PTX. If the TX FIFO contains any packets, the next usable packet in the TX FIFO is appended as a payload in the ACK packet. Notice that this TX FIFO packet must have been loaded into the TX FIFO before the packet is received [61]. Figure 22 helps to better visualize the existing procedure in each of the modules.

4.3 New System Version Implementation

In this section it is explained in detail the software implementation made for both modules in order to increase the binary throughput as well as maintain the functionality of sending and receiving data in both directions. Furthermore, the result of the graphical interface created is also demonstrated, as well as the explanation of the code behind it. By creating this interface, it is possible to receive and store the data coming from the transmitter on the computer, as well as send the data back to the transmitter, in a more user-friendly format.

4.3.1 Transmitter Software

First, and before any code implementation was carried out, it was necessary to understand which components of the transmitter needed to be programmed. Since the transmitter has an Intan RHD2216 chip, an ISP1507 module and a Peltier as main components, it is necessary that these are programmed in a way that allows the RHD2216 to communicate with the ISP1507, and the ISP1507 with the Peltier. Following this, it was planned how to send and receive data. Therefore, a flowchart of the main function (Figure 24), which consists of sending the EEG data, as well as the function of receiving the PWM (Pulse Width Modulation) value to control the peltier was produced in order to help in the development of the software. It should also be noted that this code implementation and the consecutive programming of the transmitter components was conducted through the Keil µVision5 IDE.

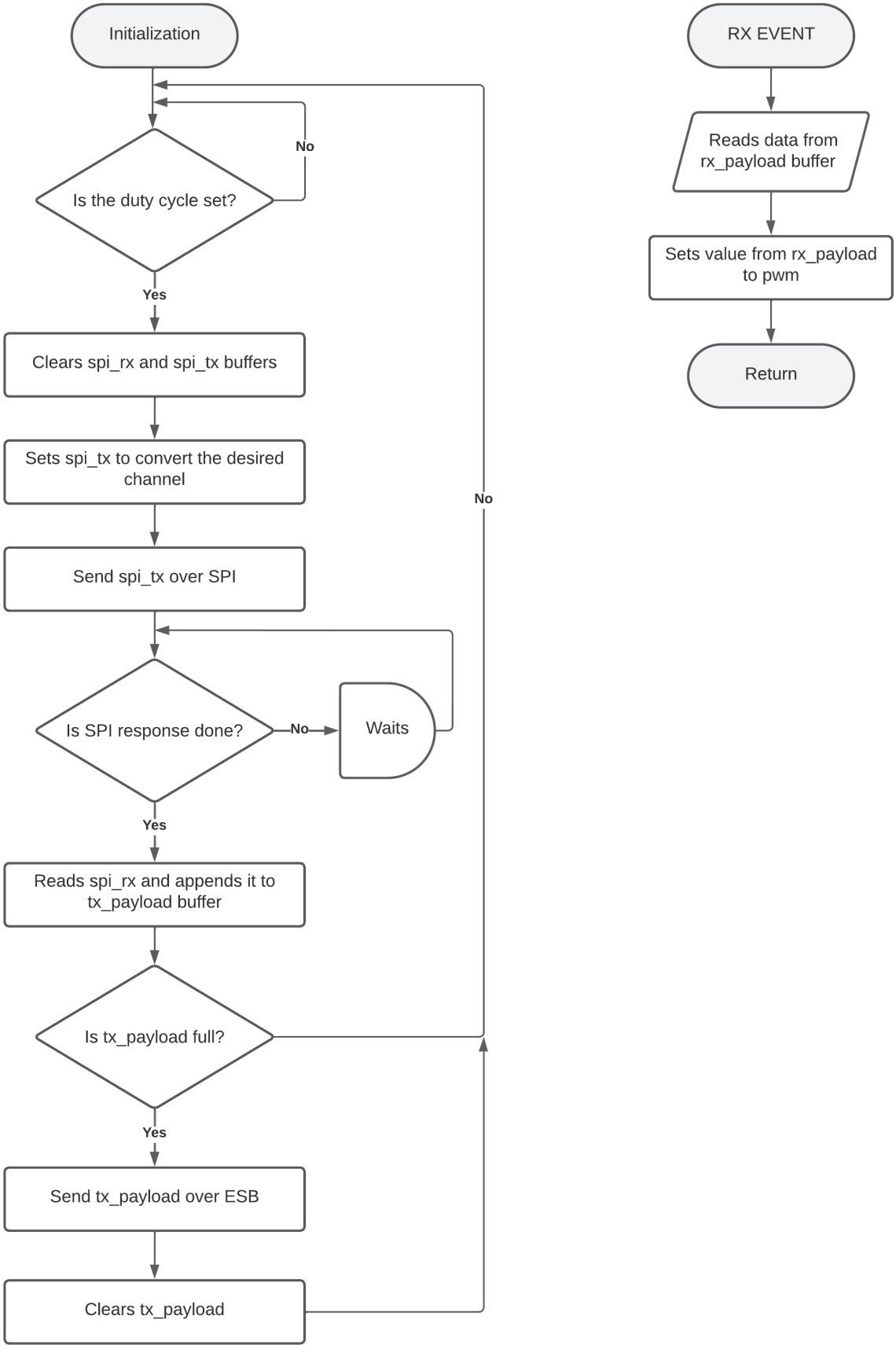

Figure 24 - Flowchart of the transmitter software.

Before the main function was performed, it was necessary to initialize some functions, requirements, and aspects so that all would work correctly. One of the main requirements was to enable the communication between RHD2216 chip and ISP1507 module. Another aspect to initiate was the PWM to control the Peltier current through a duty cycle. In [49] these two functions had already been created, however some changes had to be made to make everything work with the new communication protocol. Regarding the wireless communication protocol, everything related to BL5 was removed and the necessary functions for the ESB protocol were created. Next, each of these functions will be described in more detail.

- **SPI initialization**

For this function it was first necessary to configure the MOSI, MISO, CS and CLK pins, and then set 4 Mbps as the SPI bit rate. As for the clock mode it was defined the active state in the high state and the sampling on the clock's leading edge. As for the bit order it was defined that the most significant bit is first to be received and that SPI will work in thread mode. After this, it was then necessary to configure the RHD2216 chip in order to set the number of channels used, the filters, the ADC (Analog-to-Digital Converter) sampling, among others. To do this, it was required to send a series of data to configure all the registers as intended. Once the chip is all set up, it becomes possible to receive the ADC samples into the ISP1507.

- **PWM initialization**

Here, in this function, it was defined the number of channels to be used, the frequency of the PWM output and also the pin that will function as the PWM output. In addition, the polarity of the active state was also defined. After that the PWM is initialized and activated.

- **ESB initialization**

As for the initialization of the wireless protocol, we started by configuring the ESB settings, namely, putting it in PTX mode, setting the protocol with a dynamic payload length, a binary rate of 2 Mbps, no packet retransmission and finally enabling ACK. In addition, it was also created an event handler associated to it. Afterwards the protocol was initialized, and the base and prefix addresses were set up as required.

After this, the main function was implemented, based on the presented flowchart. However, it was required to create a buffer, named spi_tx, to store 2 bytes to send as code to the SPI, another buffer, spi_rx, to store 2 bytes regarding the response from the code sent and, finally, a 32 byte buffer, denoted tx_payload, to store incoming data from SPI and then be sent wirelessly.

Once all this is created, in the main function all the functions are initialized and then a cycle is started so that the data collected by electrodes is sent wirelessly to the receiver, as well as receiving the PWM value when it is sent from the receiver to the transmitter.

At the beginning of each cycle, the value of the PWM is checked, so that if there has been any change, it will follow. In this way, if there have been any changes, the PWM pin is set to the duty cycle corresponding to that change. After this, the spi_tx and spi_rx buffers are cleared so that 2 bytes can be sent through the spi_tx buffer to the RHD2216 chip with information corresponding to the convert command for the desired channel. When the sample conversion is over and the two bytes corresponding to that sample are stored in the spi_rx buffer, then a flag is raised that the data exchange is complete, and these two bytes are stored in the tx_payload buffer. Note that since it is only possible to send 32 bytes of useful data via ESB wireless communication protocol, then it waits until it gets 30 EEG samples to send. It should be highlighted that it only waits for 30 bytes, since the first byte of tx_payload will correspond to the decimal value 0 to know that it is a packet of EEG samples, and the second byte will correspond to a decimal value from 0 to 255, in this same order, to facilitate the calculation of lost packets and thus determine the associated error rate. This cycle described so far is repeated until tx_payload is full. After the 32 byte transaction is initialized, a success event occurs and then the tx_payload can be cleared.

This is repeated continuously and only interrupted when a value is received with the ACK. In the case of receiving payload along with ACK it is already known that it is the value corresponding to the duty cycle, so this value is placed in a variable called PWM so that at the beginning of the cycle when it checks if there is any change in this value, then the pin value corresponding to the duty cycle output changes to this received value.

4.3.2 Receiver Software

Similar to the transmitter, it was also essential to program the receiver components, with the intention that this device could receive the EEG samples and send them to the computer, and also receive from the computer the duty cycle value desired by the user and send it back to PTX. In this sense, and to help in the development of the algorithm, a flowchart was built, as shown in Figure 25.

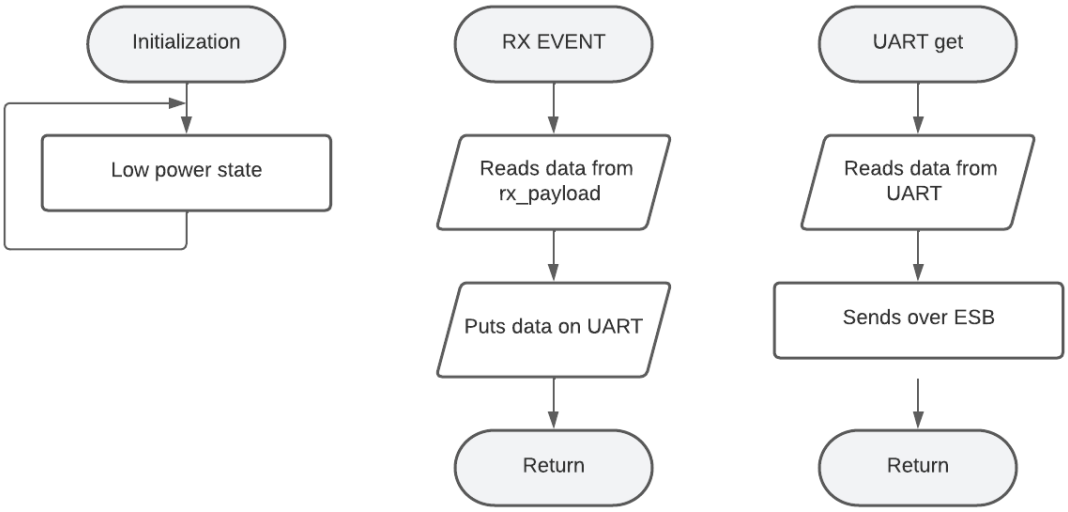

Figure 25 - Flowchart of the receiver software.

In the same manner, some functions and requirements were also initialized first, namely those concerning the ESB protocol and also the UART so that will be possible to transfer data to the computer and vice versa. These functions will be further explained below.

- **UART initialization**

Here it sets the UART pins, namely RX, TX, RTS and CTS, and the baud rate to 921,600 and disables flow control and parity. In addition, when the UART module is initialized, two buffers were defined, one for transmitting data and the other for storing received data, as well as a handler for UART events.

- **ESB initialization**

As for the initialization of the ESB protocol, it began by configuring its settings as default, having only changed a few. In the same way as the transmitter, the protocol was defined with a dynamic payload length and a binary rate of 2 Mbps, and a handler event was created. However, the protocol mode had to be changed to PRX. Once this was done, the base and prefix addresses were defined in the same way as previously explained and the protocol was initialized.

After all the functions and requirements are initiated, a main function loop is created in which the device will constantly be in its low power state unless events occur regarding the RX and the UART.

If an RX event occurs, then the receiver has probably received data from the EEG. So, since it is known that 32 bytes will always be sent per packet with EEG information, it is first verified if the incoming data has 32 bytes. If this is confirmed, then it is sent byte at a time through the UART to the computer

so that data can be stored later. Note that when the receiver receives a packet, an ACK is sent back to let the transmitter know that the data transfer was successful.

In case data is received by the UART from the computer, namely the duty cycle value that the user wants to apply, then this value is stored in a buffer called tx_payload. In this way, as soon as an ACK is sent back to the transmitter, this value is transferred along with the ACK.

4.3.3 Graphical User Interface Development

A Graphical User Interface (GUI) consists of bridging the gap between the user and the electronic devices in a more intuitive way. For this, MATLAB was used, more specifically the App Designer, since it is the recommended environment for building apps in MATLAB. This integrates two main tasks, first establishing the visual components and then programming the app's behavior.

Therefore, an interface was created to allow the visualization of EEG data in real time, as well as allowing the user to set the duty cycle to be applied in the Peltier. The GUI created is illustrated in Figure 26.

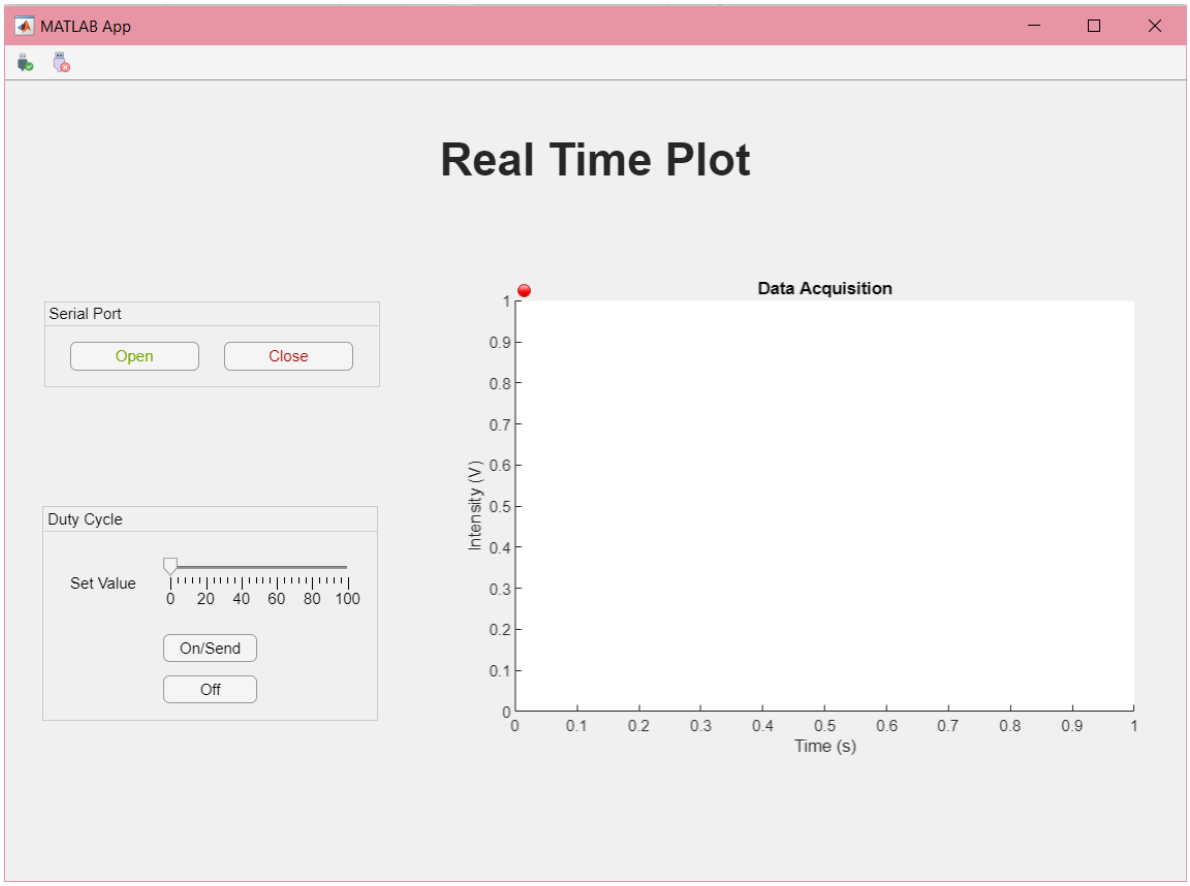

Figure 26 - GUI created in MATLAB.

The GUI is practically divided into three parts, parts related to connecting the serial port, acquiring and displaying the received data and choosing the duty cycle. The following is a short explanation for each of these parts.

- **Serial Port**

Here two push buttons have been created, so that the user can choose whether or not to connect the serial port. As soon as the "Open" button is pressed, the serial port opens and the data transaction starts, either from the receiver to the computer or from the computer to the receiver. When the "Close" button is pressed, the serial port closes and the data transaction stops.

- **Data Acquisition**

In this instance, a plot was created in order to be able to visualize the data from the rat's EEG.

- **Duty Cycle**

In this, a slider was created for users to select from a value from 0 to 100 the duty cycle they want to apply in the Peltier module. In this sense, it was also necessary to create two push buttons, one to send the value selected in the slider through the serial port to the PRX and that is then sent to the PTX and another to turn off, i.e., set the duty cycle back to zero. Note that in this last one the value zero is also sent through the serial port so that the Peltier module no longer has current.

The main code implemented for this interface is practically all in the function referring to the "Open" button, since it's only from there that the data transaction can begin. This way, and before proceeding to the implementation of the code related to opening the serial port, receiving data and plotting them, a flowchart (Figure 27) was made to help organize the code and really understand what was necessary to implement.

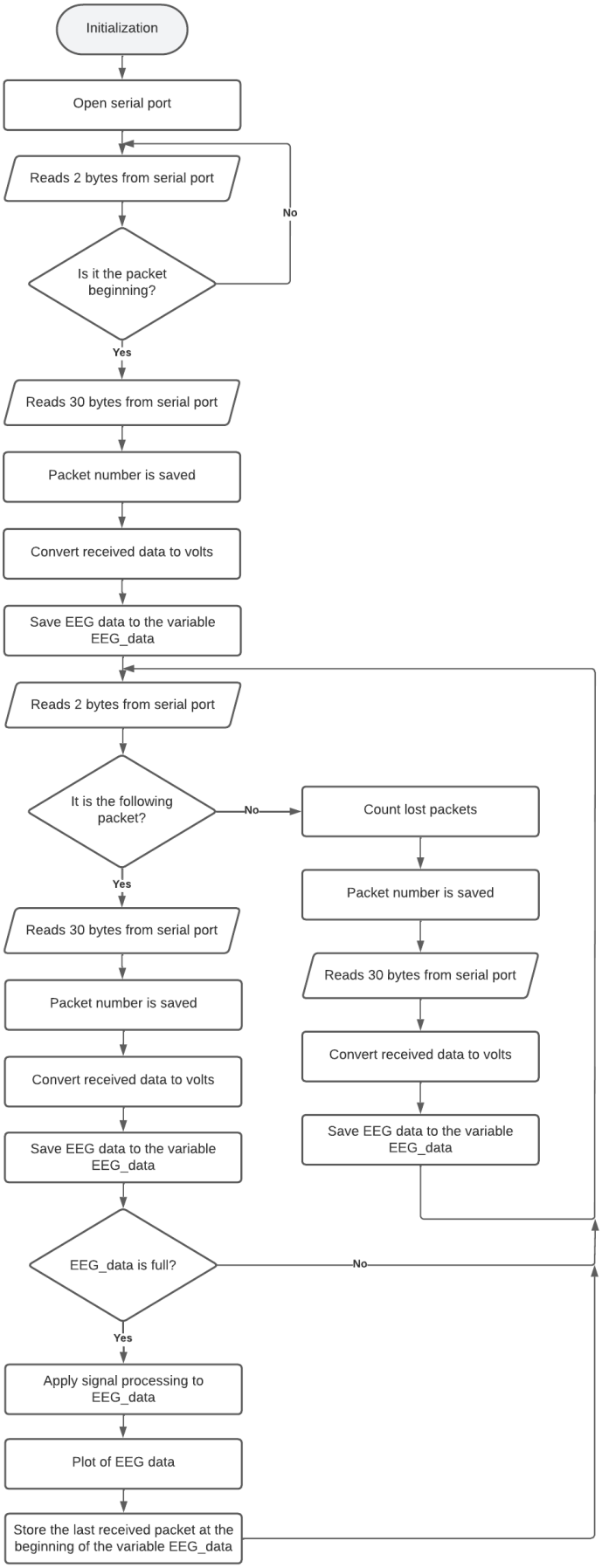

Figure 27 - Flowchart of the code implemented in MATLAB.

As soon as the "Open" button is pressed, the connection to the serial port is established at a baud rate specified in the code according to the one chosen when initializing the UART in the PRX and data starts to be received. As mentioned before, the data packet contains two bytes that do not refer to EEG samples. This was done to make it easier to control the number of packets sent. In this sense, first a start of a packet had to be found, and once this was found, the data that referred to the packet number and the data that referred to the sampled signal were split. After this the signal data was converted to volts and added to a variable called EEG_data.

Until this variable is full, data is added to it, always checking that the packet being received is the next relative to the previous one. If it isn't, then packets have been lost, so it saves in a variable how many packets have been lost, and it stores the value of this last packet to see if the next one is the next number.

Once the variable is full, signal processing is then done to recover the sampled signal and the processed data is plotted. It should be noted that signal processing is done to reconstruct the signal to make it easier to visualize, that is, to see the waveform just like the input. In this sense, it will be explained in more detail what was done to recover the signal in the next section.

4.3.4 Signal Processing

As the frequency of a signal increases, fewer samples will be taken in one period, so the signal will not be displayed as it is. More specifically, when working at the limit, that is, when the input wave has a frequency equal to half of the sampling frequency, the signal has only two samples per period, which makes it difficult to observe. In this sense, it becomes essential to perform signal processing so that the signal can be recovered and visualized as best as possible.

To recover a signal, it is necessary to apply a low-pass filter to the sampled signal. However, attention has to be paid to the frequencies used in the filter. A block diagram of a sampled signal reconstruction is shown in Figure 28.

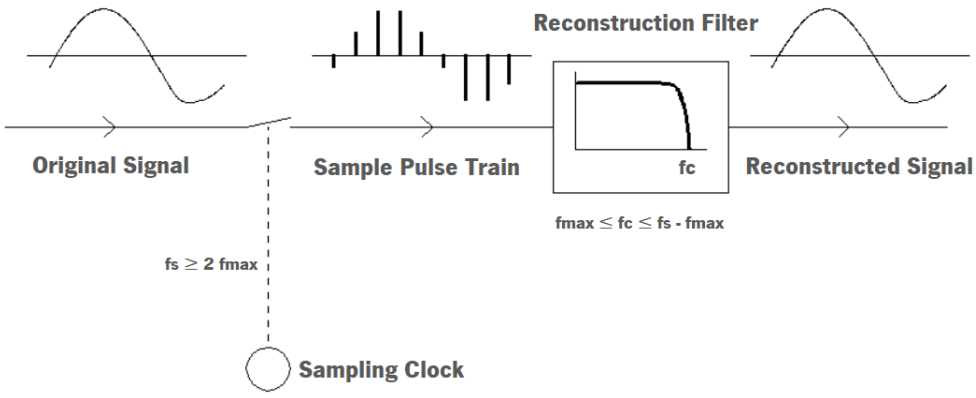

Figure 28 - Block diagram of a sampled signal reconstruction.

Based on Nyquist's Theorem, the sampling frequency (fs) must be at least two times the frequency of the signal being sampled. Therefore, when a signal is being recovered, the cut-off frequency (fc) of the applied low-pass filter should be between the maximum frequency of the input signal (fmax) and the difference between the sampling frequency and the maximum frequency of the input signal. In this way, the signal is fully reconstructed. It should be noted that if the filter has a frequency below the maximum frequency, then the signal is not fully recovered because some of the information will be lost. If the cutoff frequency is higher than fs - fmax, the filter will include information from the next period, which means that the waveform will no longer be recovered as it was initially since it has aliasing.

However, since we are dealing with digital filters, there are still situations where even if the low-pass filter is within limits, it cannot fully reconstruct the signal, so it is often preferable to do an interpolation. An example of this is when the number of samples per period is low and given that the digital low-pass filter does not increase the number of samples, the signal cannot be obtained as it is at the input. In Figure 29 is a block diagram of how to recover a sampled signal through interpolation.

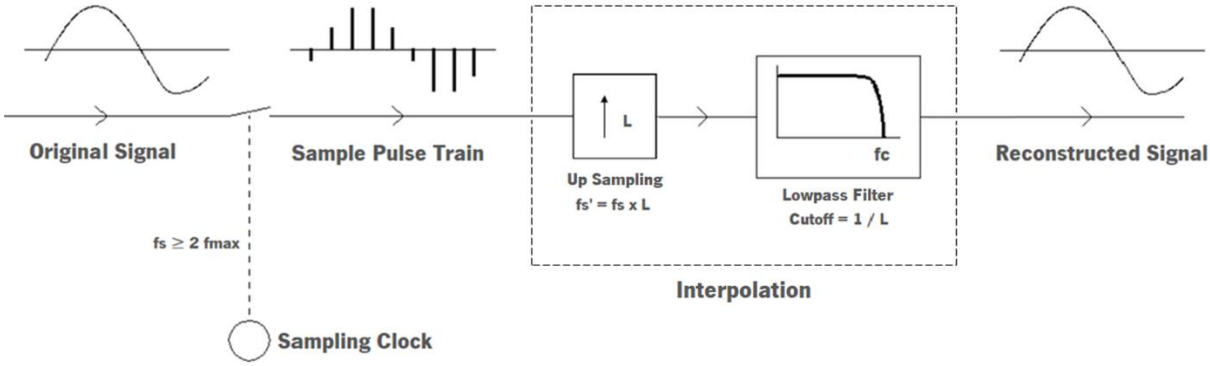

Figure 29 - Block diagram of a sampled signal reconstruction with interpolation.

The interpolation involves doing an up sampling, which consists of inserting L-1 zeros between two existing samples, and then applying a low-pass filter to estimate missing samples through the neighbors.

Thus, it can be concluded that interpolation estimates the missing samples from the original samples. In this way it is already possible to reconstruct the signal digitally [62].

To actually proceed with applying interpolation to the sampled signal received in the computer, we used MATLAB's *interp* command. This command follows three essential steps [63]:

1. expands the input vector to the desired length by inserting zeros between the original data values;
2. designs a special symmetric FIR filter that allows the original data to pass through unchanged and interpolate to minimize the mean square error between the interpolated points and their ideal values;
3. applies the filter to the expanded input vector to produce the output.

This method of digital signal processing was applied before the signal was displayed on the interface, in order to make the signal as close as possible to the input.

CHAPTER 5 TESTS AND RESULTS

With the intention of evaluating the operation and performance of the proposed system, it was necessary to carry out some tests. Therefore, in this chapter the results of each test will be demonstrated, as well as the conclusions reached.

To determine the exact sampling rate of the ADC, the time it takes to receive a sample was observed on the oscilloscope.

Likewise, the packet transmission rate between the two modules was also observed on the oscilloscope in order to know the binary throughput in real terms. In addition, it was also seen how long it took 32 bytes to be sent through the serial port.

Since we are working with a high binary throughput it was also essential to determine the error rate associated with the packet transaction.

Another important characteristic to determine is the system's power consumption. So, when the packets were exchanged, the current that was consumed was measured, and then the battery lifespan was calculated.

Finally, and in order to validate the whole system, a sine wave was applied to the connectors corresponding to electrodes and it was verified if in the interface created in the computer the same wave was seen as in the input.

5.1 ADC Sampling Frequency

The ADC sampling frequency is the number of analog samples an ADC can convert to digital in one second. The RHD2216 chip contains a 16-bit successive-approximation ADC with an integrated analog MUX (Multiplexer), allowing it to sample voltage signals from the amplifier array, as well as various sensors and auxiliary inputs across the chip. According to the datasheet, the ADC can operate up to a maximum of 1.05 MS/s which allows each channel to be sampled at 30 kS/s. Thus, for it to operate at a rate higher than 700 kS/s, it was necessary to set the ADC buffer bias variables to the decimal value 2 and the MUX bias variable to 4, from register 1 and 2, respectively [54].

In order to know exactly the sampling rate, an output pin was defined to change its state every time a sample was converted, and its behavior was observed in the oscilloscope (Figure 30).

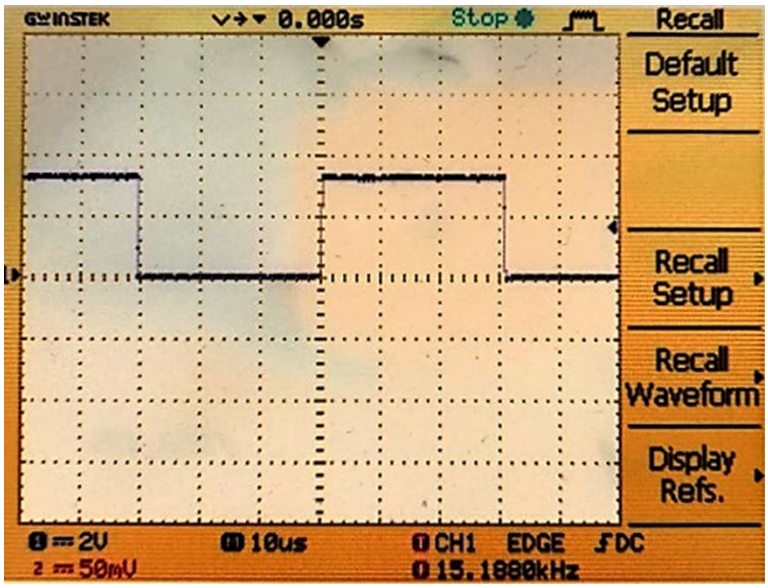

Figure 30 - Signal representative of ADC samples being converted.

Observing the image, it is possible to conclude that every 30 µs, the state of the pin changes. This means that every 30 µs a sample is converted. Hence, the actual sampling frequency of the ADC is about 33,333 Hz.

5.2 Data Rate Performance Assessment

The main purpose of this project was to increase the binary throughput between modules. In this sense, and after everything was implemented, it was necessary to know exactly how many bits were sent in a second. So, similar to the sampling frequency, an output pin was set in the PTX to commute at the end of a sent data packet. In Figure 31 it is possible to observe the pin behavior.

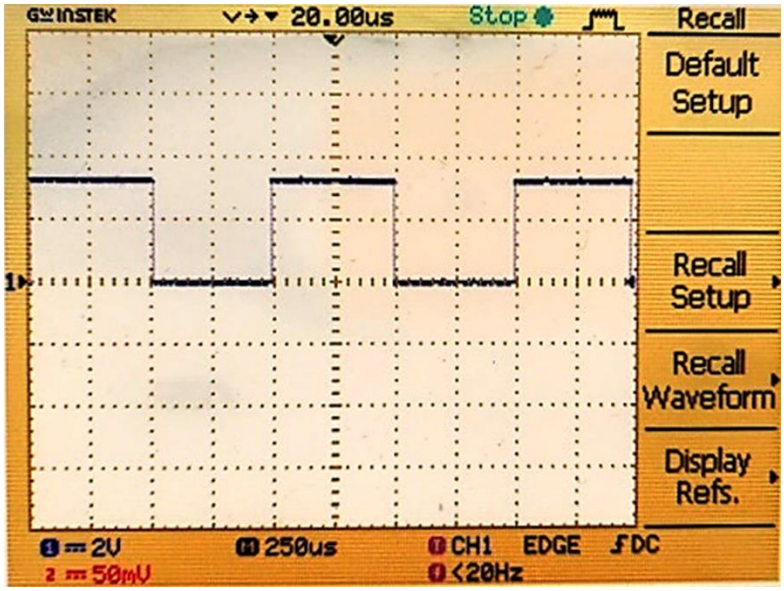

Figure 31 - Signal representative of data packets being sent from PTX.

From the previous figure it can be seen that every 500 µs the pin status switches, which means that a packet has been sent. Thus, it is known that in one second 2,000 packets will be sent. Since these packets contain 32 bytes, this means that the binary rate of wireless communication is 512,000 bps.

However, not all 32 bytes correspond to samples of the RHD2216 chip. As mentioned in the previous chapter, only 30 bytes correspond to samples coming from the ADC. Since one sample is 16 bits, i.e., 2 bytes, then per packet only 15 samples are sent. In terms of binary throughput, a rate of 480,000 bps is then obtained where all these bits correspond to signal samples.

Similarly to the PTX, an output pin was set on the PRX to validate that the packets were received at the same rate. The pin's performance is shown in Figure 32.

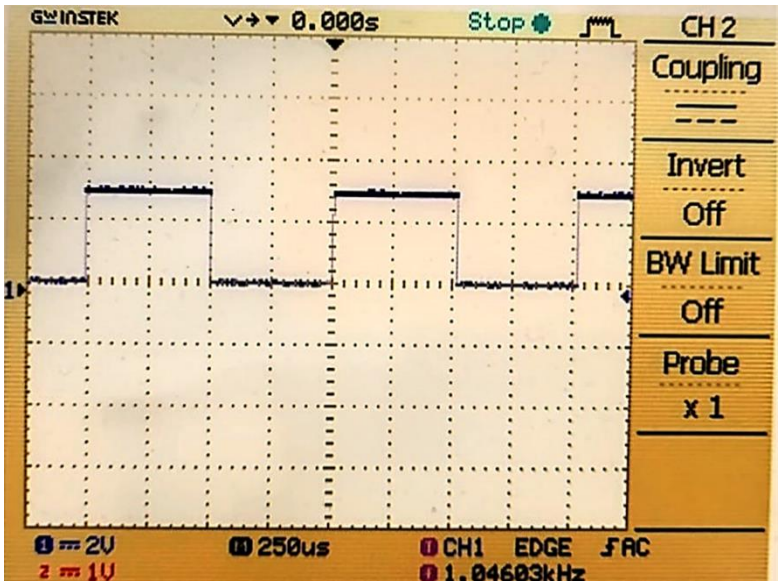

Figure 32 - Signal representative data packets being received at PRX.

As it can be seen data is being received at the same rate as it is being sent, which validates the functioning of the data transaction from one module to the other.

In addition, and to also validate the bytes being sent via UART to the computer, the TX was observed, it changes state depending on each bit sent. As the TX signal shows each of the bits, that is, it goes to a higher or lower state depending on whether the bit is 1 or 0, then bytes were sent in the form 0b01010101, 85 in decimal, to facilitate their observation in the oscilloscope. It is important to emphasize that each time a byte is sent over the UART, 10 bits are sent, because there is a start bit and a stop bit between the byte that is meant to be sent. The result obtained is presented in Figure 33.

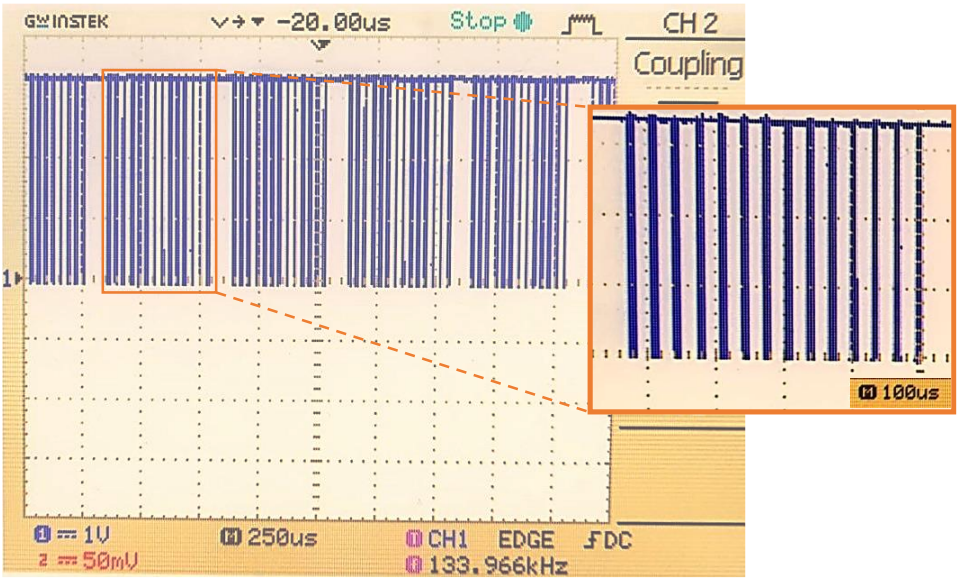

Figure 33 - Signal representative bytes being sent from the UART to the computer.

By observing the oscilloscope image, it can be seen that in about 430 µs, 14 bytes are sent over the UART. Since a baud rate of 921600 has been set, then 32 bytes of EEG data should be sent in about 347 µs, which would allow a complete sending of a packet within the time of receiving another one. However, this does not occur, as there is a time between sending of 10 bits, as can be seen in Figure 34.

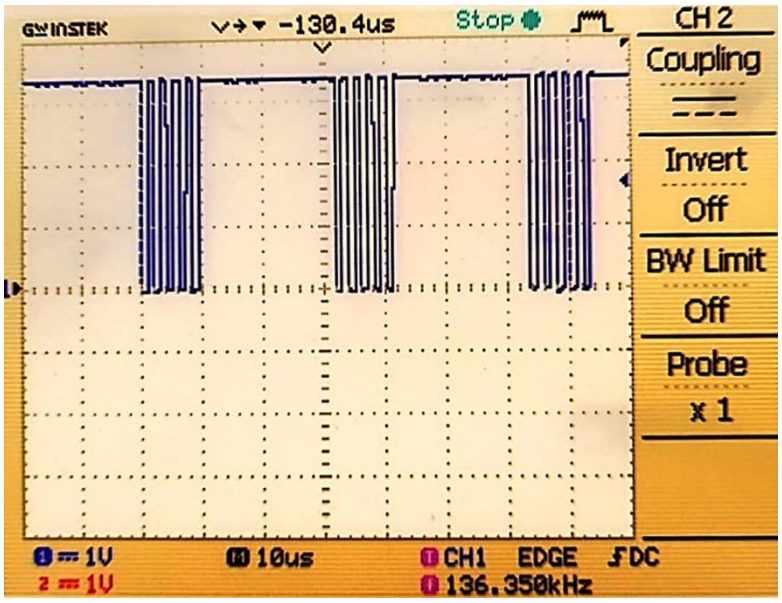

Figure 34 - Signal representative 10 bits being sent from the UART to the computer.

Looking at the figure above, it can be observed that 10 bits are sent within the time assumed for a baud rate of 921600, that is about 10 µs, but it takes about 22 µs until another 10 bits are sent. In this sense the data transmission will be delayed, which results in data loss, since every time a 32 byte packet

is received via wireless, the data sending through the serial port is interrupted. This explains why in Figure 33 it only shows 14 of the 32 bytes being sent within the time between packets.

It is worth noting that in order to analyze what was causing that time interval between bytes, the module was set to send only data through the serial port, in other words, the wireless communication was disabled. It was observed that there was about 10 μs of interval between bytes, however it was not possible to conclude why this interval increased to double the time when the wireless communication was activated.

So instead of getting a sending data rate to the computer of about 921600 bps, only approximately 312500 bps was achieved. Since, as aforementioned, the acquired signal data is being sent wirelessly at a rate of 512 kbps, this will result in a significant loss of information.

5.3 Bit Error Rate Performance Assessment

Since a very high binary throughput is being used, it is crucial to determine the error rate of the entire system. So, to determine the amount of data lost when communicating, the device was left for 2 minutes acquiring data and sending it to the computer. PTX was about one meter away from the PRX, which is a reasonable distance, since it is often not possible to be too close to the rats when acquiring EEG. It is important to emphasize that tests were performed at shorter distances and the results were the same.

After the two minutes of acquisition, the computer received about 2494432 bytes. However, about 234893 packets were expected, which corresponds to 7516576 bytes, since one packet contains 32 bytes. Therefore, 5022144 bytes were lost, which corresponds to a percentage of lost data of about 66.81 %. This percentage is considerably high, since more data is lost than is received, more specifically only about 40 % of the data is received. This means that the maximum throughput that can be achieved with the device in question and the computer used is $0.4 \times 921600 = 368640$.

In order to analyze potential problems that could be causing such a high error rate, the baud rate was reduced to 460800 and the error percentage was calculated. With this, 1549664 bytes of 1900448 bytes that should have been received have been received, so a much lower error percentage was obtained, about 18.46 %. However, doing the calculations, 0.8×460800 gives 368640, which is in line with what was determined earlier.

Hence, it can be concluded that in practice the binary throughput of the system is 368640 bps and not 512000 bps. Indeed, there is something that is slowing down the whole system, and as seen earlier

one of the factors is the communication over the UART. However, it does not imply that this is the only source of the problem, as there may already be errors associated with wireless communication.

5.4 Power Consumption

An essential feature to determine when developing wireless electronic devices is their power consumption. In this sense, it was measured the current consumption of the PTX when it is performing data transaction, with the help of an ammeter and a current consumption of approximately 15.5 mA was obtained. It should be noted that the system was powered at a voltage level of 3.3 V.

Since when conducting in vivo experiments on rodents, the PTX is placed on the rats' backs, then it is necessary to connect a battery to it so that it can be powered. Thus, it is also important to determine how long the battery will last in order to know the time window in which experiments can be performed without having to interrupt them.

In the case where the user only wants to be monitoring the rodent's EEG and assuming that the system is being powered at 3.3 V with a 250 mAh battery, then the duration of uninterrupted operation is going to be approximately 16 hours ($250/15.5 \approx 16$ h). However, most of the time during the experiments it is necessary to activate the Peltier in order for it to cool the rats' neurons, which results in an increase in total current consumption. Therefore, assuming that a current of about 100 mA is supplied to the Peltier, the system will consume a total current of 115.5 mA. This causes the battery lifespan to decrease to about 2 hours without interruption. Nevertheless, when the experiments were performed at GIN it was not necessary for the Peltier to be enabled all the time. In fact, in 30 minutes of experiment, only for 5 minutes the Peltier was activated, so the battery under the mentioned conditions would last about 7 hours and 42 minutes ($250 / (115.5 \times 0.17 + 15.5 \times 0.83) \approx 7.7$ hours).

From these results, it is possible to state that this battery is sufficient for the intended experiments, as it is possible for it to last for a day's work. Only at the end of the day would it be necessary to recharge it again.

5.5 System Validation

With the intention of validating the functioning of the whole system, more specifically the signal acquisition and wireless communication, a signal generator was connected to the electrodes of the PTX and this same signal was observed in the interface created.

A sine wave was generated with the signal generator since it was not possible to perform tests on animal models and observe their EEG. Thus, and given that the RHD2216 chip was programmed to have a cutoff frequency of 0.30 Hz and 10 kHz for high-pass and low-pass filters, respectively, it was defined as an input signal a wave with a peak-to-peak amplitude of 6 mV and a frequency of 2 kHz. The result generated in the interface is shown in Figure 35.

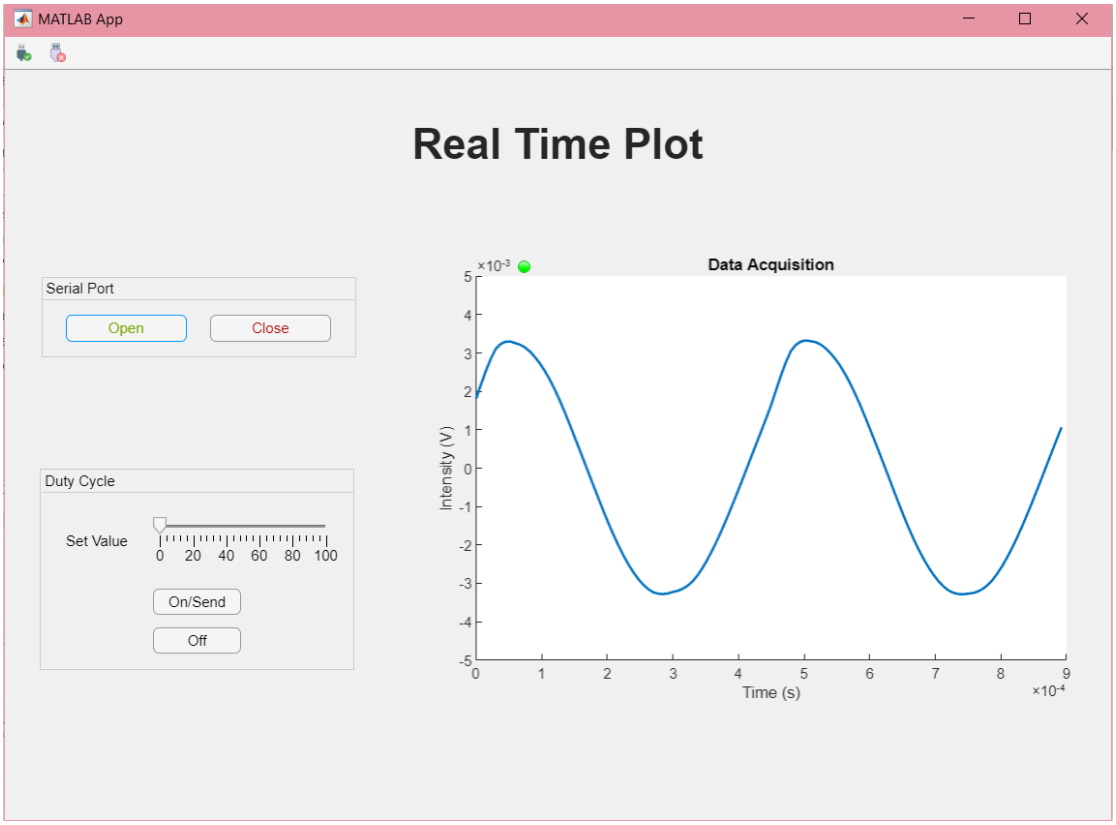

Figure 35 - Acquired sine wave on the created interface - 2 kHz.

By observing the figure above, it is possible to see that the acquired sine wave has a period of about 500 µs and a peak-to-peak amplitude of about 6 mV, so it can be concluded that it is exactly the same as the signal that was applied on the electrodes.

Another test was performed with the purpose of testing the filters implemented in the RHD2216 chip. In this sense, a sine wave with the same amplitude as the previous one, but with a frequency close to the cutoff frequency, was put on the electrodes. Since the cutoff frequency is 10 kHz, a frequency of 8 kHz was chosen. Figure 36 shows the result obtained by the interface.

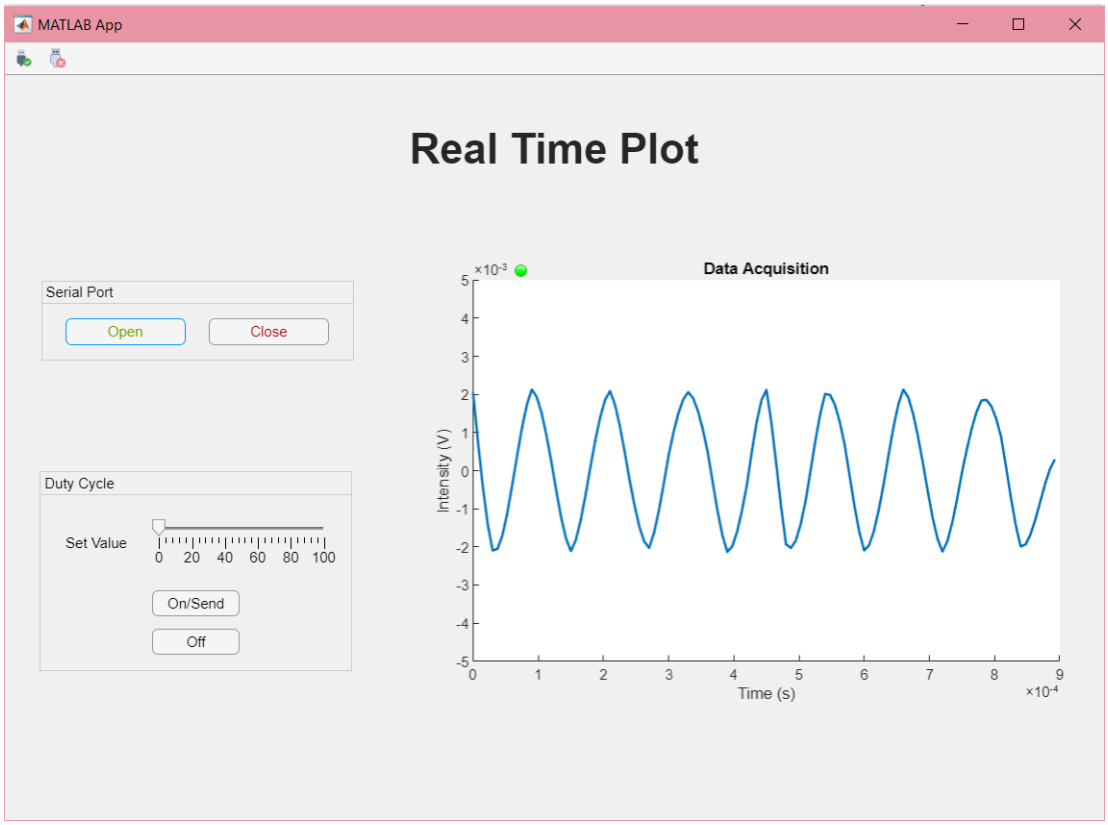

Figure 36 - Acquired sine wave on the created interface - 8 kHz.

From the image above it is possible to see that the signal obtained has the same frequency as the input, since one period is about 125 µs. Whereas the amplitude has decreased considerably, which means that the effect of the low-pass filter is already beginning to be noticed for a signal with a frequency of 8 kHz.

After the analysis of various signals that have been applied to the PTX electrodes, it can be concluded that the acquisition system is functioning properly, since it is possible to observe the input signal in real time in the interface created.

CHAPTER 6 CONCLUSIONS AND FUTURE WORK

In this chapter it is presented the conclusions taken during the development of the Master's Dissertation, as well as future work that can still be performed to improve this device further.

6.1 Conclusions

The main purpose of the Master's Dissertation project was to improve the wireless communication of an existing device.

The protocol previously used was Bluetooth 5.0 which, in theory, would be able to transfer data at a rate up to 2 Mbps, however due to several factors it was only possible to exchange data at 21296 bps. The sampling frequency, on the other hand, was performed at 1388 Hz, which is sufficient to acquire a rat's EEG. However, if it is necessary to acquire more than one channel simultaneously, this rate is no longer enough.

In this regard, the wireless communication protocol was changed to ESB which is a 2.4 GHz proprietary protocol from Nordic Semiconductor. With this protocol it was possible to send data in both directions at a rate of 512 kbps. Since the maximum sampling frequency that could be achieved on the RHD2216 chip was about 33,333 Hz, then it was possible to use ESB as the communication protocol. However, when performing the lost data percentage determination, it turned out that the binary throughput rate in practice was about 368640 bps.

In order to compare the characteristics of the new version of this system with the devices described in section 2.4, and also what had been done previously with this system, Table 4 was created.

Table 4 - Comparison of the present system with the previous system and those in section 2.4

Systems	Weight	Dimensions	Battery	Bandwidth	Frequency Band	Debit Throughput	Sampling rate	On/off magnetically	Current
Hou et al. [47]	N/A	(4.2 × 2.2 × 2.7) mm^3	13.56 MHz RF-power supply	N/A	N/A	N/A	N/A	N/A	0 – 200 mA
Zayachkivsky et al. [46]	0.6 g / 2.3 g / 4 g	0.3 cm^3 / 0.8 cm^3 / 1.4 cm^3	2 weeks / 2 months / 6 months	0.1 – 100 Hz	N/A	N/A	500 Hz	N/A	N/A
Lundt et al. [45]	1.9 g / 3.9 g	1.9 cm^3 each	4 months / 1.5 months	1 – 200 Hz / 1 - 50 Hz	N/A	N/A	1000 Hz / 250 Hz	Yes	N/A
Cheng and Murari [42]	1.8 g	(13 × 8 × 8) mm^3	50 h (240 S/s)	1 – 120 Hz	N/A	10 Mbps	240 S/s	Yes	210 µA
Previous in-house system [49]	10 g	3.6 cm^3	4 – 33 h	0.3 – 100 Hz	2.4 GHZ	21296 bps	1388 Hz	No	7.5 – 400 mA
New version of in-house system	7.73 g	(2.3 × 2.6 × 0.5) cm^3	2 – 16 h	0.3 – 10 000 Hz	2.4 GHZ	368640 bps	33333 Hz	No	15.5 mA

In comparison with the other devices, it can be noted that the sampling rate is much higher than the others, which makes it possible to acquire signals with much higher frequencies. In turn, the binary throughput also had to increase to be able to send all the sampled data. This led to a considerable decrease in battery life, since the current consumed by the device when it is acquiring signals and sending the data wirelessly was much higher than before.

In conclusion, a system was obtained that can perform two-way data transaction at a rate 17 times higher than the previous system, which will make it possible to observe signals at a much higher frequency.

6.2 Future Work

Beyond the improvements made to this device, there is still some future work that can be accomplished.

The first thing to improve is the communication over the UART, more specifically, to find out why there is a delay between the sending of bytes over the serial port and then, eliminate it if possible.

One of the most important and necessary improvement is the miniaturization of the module that is placed on the back of the rat. Although its volume and weight are within the parameters for the rat to be able to carry and move with it normally, it is still necessary to make the PCB even smaller, to make it as lightweight as possible. To this end, the PCB must be redesigned in order to accommodate more all the electronic components. A big help in miniaturization will be using the RHD2216 chip in its bare die form instead of the standard one. As it can be seen from Figure 37, the bare chip is much smaller, in fact it goes from a size of 8 × 8 × 1 mm to 4.8 × 4.1 × 1 mm, which will help in the system's miniaturization.

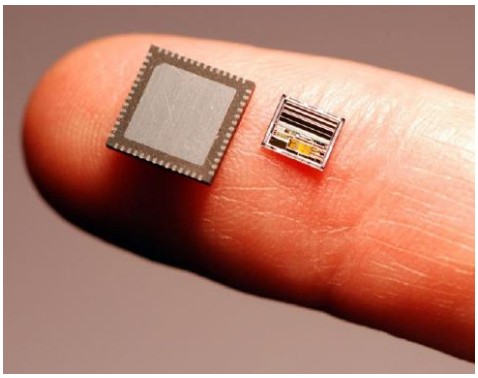

Figure 37 - Differences between the package size and bare die form of the RHD2216 chip.

Another improvement that can be made to make the device even more wire independent is the way it is charged. At the moment, it is necessary to remove the battery and recharge it, however, one solution

is for it to be charged by radio frequency. This way, it would not be necessary to remove the battery and therefore testing on animal models could be conducted for unlimited time.

In addition, an algorithm that detects epileptic seizures could also be implemented, so that the cooling process could be autonomous. In this sense, it would not be necessary for a person to be watching the EEG the whole time of the experiment to apply or not current to the peltier when a seizure occurs.

In short, there are still several improvements that must be made to this system in order for it to become "ideal" for conducting experiments in animal models. If after experiments it is proven to be successful, then as future work will be to create a similar system for clinical use.

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