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Implementation and testing of a platform for neuroprosthetic applications

Adele Macrì Panarese

Supervisors:

Prof. Danilo Demarchi

Prof. Silvestro Micera

MSc. Ivo Strauss

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A mio padre e mia madre

Abstract

The development of prosthetic devices increasingly focuses on approaches which allow amputees to control them in a physiological way. A great problem of actual most promising prostheses, myoelectric prostheses, is the absence of sensory feedback, so patients perceive the prosthetic device not as a part of themselves. Different types of electrodes have been proposed to directly interface with the peripheral nervous system at different degrees of invasiveness (i.e Cuff, TIME, tLife). The purpose is restoring motor and sensory activities, in amputees or patients with different kind of neurological injuries.

In this work, a system which combines stimulation, recording and elaboration functionalities has been implemented . After the stimulation of peripheral nerves, the EMG signal that comes from muscular contraction elicited is recorded and elaborated in real-time. For the realization of the system, a Tucker Davis Technology (TDT)'s Neurophysiology Workstation has been utilized, which includes: the RZ5D BioAmp Processor, a multi-channel stimulator (IZ2), and a multichannel amplifier (PZ5). These devices are controlled, based on processes that have been configured through circuit blocks developed in RP Visual Design Tool.

The software that manages the hardware devices communicates with a Graphical User Interface developed in Matlab® through which the user can control the stimulation properties and visualize results in real-time. The recorded EMG signals were elaborated and transferred to Matlab® for further processing.

The system has been tested in experimental sessions held on pigs, where hook, cuff and transversal, intrafascicular electrodes have been utilized to stimulate the sciatic nerve. We successfully showed that the system provides information about the level of activation of each of target muscle in respect of the charge injected and about the selectivity of stimulation of intraneural electrodes.

The implemented system allows the real-time elaboration of signals and it is customizable according to the applications by simply varying the hardware configuration and the signal processing chain. This platform provides a base for everybody who wants to perform experiments related to the characterization of neural electrode selectivity. The system can also integrate the recording of electroneurographic signal so that it can be utilized also in bioelectronic medicine applications that extrapolate information from the peripheral nervous system.

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Chapter 1

Introduction

1.1 Overview of actual prosthetic devices

When a person performs any action, in general adapts the motor command depending on the sensorial feedbacks received by the interaction with external environment and objects. In case of amputation, not only the motor control but also the sensory sensation is lost.

Nowadays the number of amputees reaches about 2 million in the United States and each year 185.000 new amputations are registered [1]. In Europe the incidence is even greater, with nearly 3 million of amputee patients with an occurrence of 290 thousands per year. Causes may be various, from vascular diseases to different kind of injuries. A variety of prosthetic devices have been developed over the years to solve the issues whom which an amputee person faces.

- The first model is represented by cosmetic devices, which only reproduce the amputee limb from an aesthetic point of view, without restoring any functionality;
- Active body-powered prostheses use the energy produced by persisting joint to allow the movement.
- Myoelectric prostheses where the EMG signal recorded from remaining muscles of limb is used to control the movement.

Myoelectric prostheses are surely the most advanced devices in the market for the restoration of movements. However, although emg signal is simple to be recorded and not invasive, myoelectric prosthetic devices have different disadvantages.

- As regard to the motor control, only one joint movement at time is possible: indeed the emg signal recorded from a couple of agonist and antagonist muscles is exploited. Furthermore, as the level of amputation becomes more proximal, the number of signal sources decreases. The high level amputee patients have to

learn to use their proximal remaining muscles to move their extremities in a not intuitive way.

- A great challenge still remains the possibility for patients to obtain a sensory feedback from their prosthetic device.

In comparison with a sane person, an amputee not only has a major physical difficulty, but also a greater mental commitment. Actually, the absence of sensory feedback forces the amputee patient to constantly monitor the task executed. The prosthesis is perceived as a foreign body by the user and this aspect contributes to increase the tendency of prostheses abandonment.

In order to overcome the enounced problems, an interesting prospective of last years is represented by the interaction with the peripheral nervous system. The purpose is restoring motor and sensory activities, in amputees or patients with different kind of neurological injuries. In this direction two are the solutions that are being investigated:

- Targeted Reinnervation which includes both Targeted Muscle Reinnervation (TMR) and Targeted Sensory Reinnervation (TSR).

TMR consists in taking nerves that innervated the muscles of the limb before amputation and to redirect them to alternative muscles: in this way, the electroneurographic signal (ENG) of residual nerves produces an electromyographic activity on the muscles reinnervated. The EMG signal generated is directly related to the motor intention of the patient, it has a higher amplitude and signal to noise ratio if compared with the associated ENG signal, so it is simpler to be reorded and analyzed.

Similarly, in TSR, the skin near the TMR site is reinnervated by afferent nerve fibers which innervated the limb before amputation. So in this area the amputees feels the sensation that normally perceived in the missing limb [3].

- implantable electrodes to directly interface the peripheral nervous system at different degrees of invasiveness.

The thesis focuses on the development of a system which makes it possible the use implantable neural electrodes to actuate the stimulation of peripheral nerves and to verify the performance (selectivity) of intraneural electrodes. A detailed description of actually developed neural interfaces is given in the chapter 1.3. Before that, the structure of peripheral nervous system is explained in the following chapter (1.2.2).

1.2 The peripheral nervous system

1.2.1 Action potential: Hodgkin and Huxley model

Nervous fibers communicate through the propagation of action potentials. Excitable cells are characterized by a rest potential, which is guaranteed by the presence on the cell membrane of ion selective channels that transport ions against electrochemical gradient by exploiting the ATP hydrolysis energy (Na-K pump) or the electrochemical gradient caused by the contemporary transport of other molecules (Na-C exchanger). The Na-K pump transports for each cycle 3 Na^+ ions outside the cell and 2 K^+ ions inside. This implies that the rest potential of the cell, defined as $V_m = V_i - V_{out}$, is negative. It has values from -50 to -100 mV on the basis of cell type.

Physiological or artificial stimuli cause a voltage difference across the membrane, which, in turn, modifies the structure of ion channels, allowing them to open or close. As a consequence, the membrane permeability changes.

A stimuli can generate an action potential: from the rest state (-90 mV in nerve fibers) the voltage becomes less negative until it reaches a positive value (depolarization phase), then the inverse process occurs and the initial condition is restored (repolarization). This process lasts less than 1 ms in nerve fibers (1.5 in skeletal muscle fibers, 200-400 ms in cardiac cells).

The action potential is followed by an absolutely refractory period in which the cell is not activated at all and by a successive period of limited activation (relative refractory period): in nerve fibers this time is about 2 ms . The depolarization phase is due to an increase in Na^+ ion channel conductivity so an influx of Na^+ occurs; then the conductance goes down while the K^+ pump conductance increases and so K^+ ions go out of the cell and the rest situation is restored after a condition of hyper repolarization.

The formation of the action potential has been modelled by Hodgkin and Huxley in 1952. The cellular membrane is representable as a capacitor that separates the inner cellular space from the extracellular liquid. In the Hodgkin and Huxley model three channels are considered: Na^+ , K^+ and a general leakage channel, R [4].

When a current I is applied through stimulation, it splits in a $I_c = Cdu/dt$, where u is the membrane potential (current which charges the capacitor C) and the other I_k ionic components that flows through channels. When channels are completely opened, they are characterised by conductances g_{Na} , g_K and g_L . The open/close behaviour of Na^+ channel is influenced by other two variables: m and h ; while the variable n influences g_K conductance. The sum of ionic I_k current has been formulated as:

$$\sum_k I_k = g_{Na} m^3 h (u - E_{Na}) + g_K n^4 (u - E_K) + g_L n^4 (u - E_L)$$

In Figure 1.2 it is indicated the dependence of variables m , h and n in function of the u potential membrane and the correspondent time constants. Note that m is a crescent

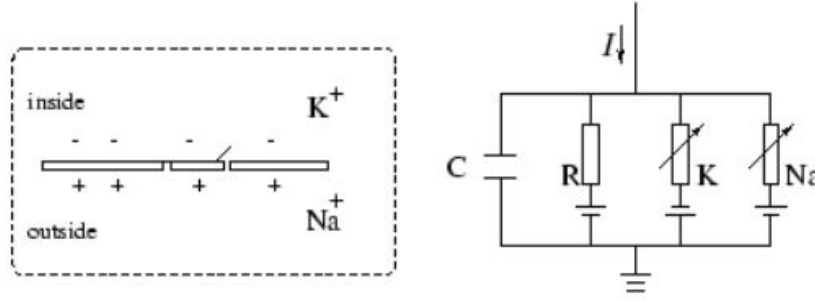


Figure 1.1: Hodgkin-Huxley model of an eccitacle cell membrane. The cellular membrane is representable as a capacitor that separates the inner cellular space from the extracellular liquid. Three trans-membrane channels are considered: Na^+ , K^+ and a general leakage channel, R . Reproduced by [4].

function and has a small time constant (it acts opening Na^+ channels), while h is a decrescent function and has a higher time constant (it acts closing the channels). This models the Na^+ channel behaviour characterized by a first increase in the conductivity and in the successive slower decrement. The variable n has the same time constant of h . It opens K^+ channels but in the opposite direction (K^+ ions go out the cell).

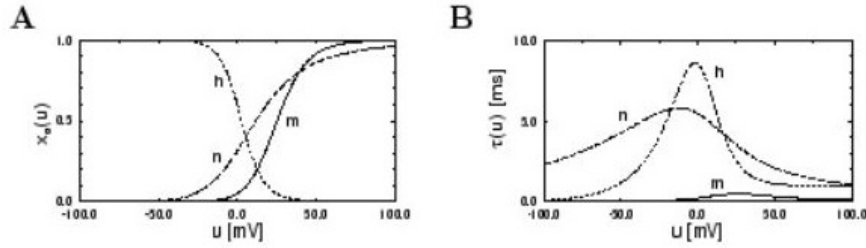


Figure 1.2: A) Equilibrium function for the variables m , h and n in function of the u and B) time constant in Hodgkin-Huxley model . Reproduced by [4].

The exponential factors used by Hodking and Huxley to model the ionic current have a physiological explanation as it has been demonstrated that K^+ channels are made up of 4 equal units (m^4) and Na^+ channels are made by four units, three of which of the same type (n^3) and the fourth unit is of a different type (h).

1.2.2 Anatomy of the peripheral nervous system

The peripheral nervous system is made up of ganglia (ensemble of cellular bodies of neurons) and nerves constituted by neuron axons. It allows the communication between the central nervous system, CNS (which includes the brain, the brainstem and the spinal cord), and the rest of organs and peripheral structures.

The peripheral nervous system (PNS), with reference to function, can be divided in:

- autonomic: which determines functions of internal organs (i.e heart or liver). It includes a preganglionic part made up of nervous cells that take origin from the spinal cord or cranium and a postganglionic part.
- somatic: which controls the activity of voluntary muscles and communicates with skin receptors. The soma of these nerve cells is near or inside (only for cranial nerves) the spinal cord or skull. It comprehends both motor and sensory nervous fibers. Distally it includes effectors and sensors (thermoreceptors for temperature sensation, nociceptors for pain sensibility and mechanoreceptors that provide sensations related to skin deformation, motion of joints, muscle length).

According to the structure of the Central Nervous System from which peripheral nerves of somatic system have origin, they can be classified in:

- cranial nerves, which exit from the brain and the brainstem;
- spinal nerves that take origin from the spinal cord. In turn, spinal nerves can be distinguished in:
 - nerves belonging to **motor department**, also called **efferent fibers**. They leave the ventral section of the spinal cord (**ventral root**) and reach neuromuscular junctions in skeletal muscles.
 - **afferent** or **sensory fibers**, which have their origin in the **dorsal root** of the spinal cord and terminate in different kinds of receptors situated in muscles, skin or tissues.

Cranial and spinal nerves allow a double-sense communication: stimuli originated from the central nervous system spread from the center toward the periphery, enabling the muscular contraction; while sensorial stimuli, collected by receptors, propagate in the opposite direction. A representation of spinal nerves is reported in Figure 1.3.

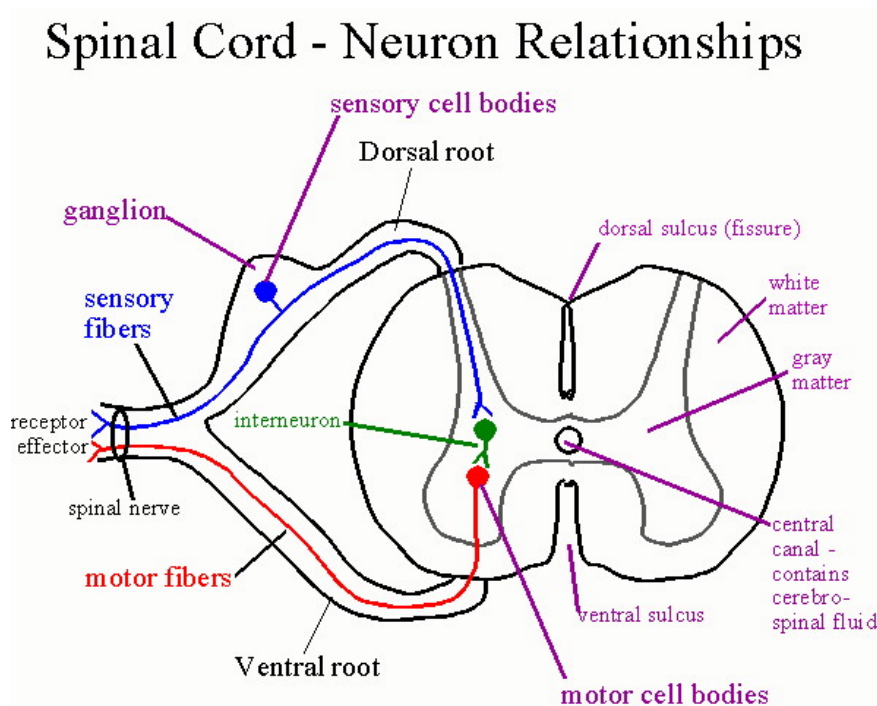


Figure 1.3: Representation of spinal nerves which include motor fibers which exit from ventral root and sensory fibers which originate from dorsal root[5].

An additional distinction between nerve fibers is possible taking into account the myelination state. Myelin is an insulating sheath made of lipids that circles an axon. In the peripheral nervous system, it is constituted by Schwann cells.

- Myelinate axons are characterized by a greater conduction velocity of the action potential since the signal spreads in a saltatory way passing from one Ranvier node to the subsequent one. As consequence, myelinate fibers are used to execute high speed tasks (i.e contraction of skeletal muscle) or in fine sensory discrimination.
- Conversely, in unmyelinated fibers the action potential propagates in a continuous and slower way.

Peripheral nerves are characterized by a concentric layer structure, they include:

- neuron axons of both autonomic and somatic nervous system;
- Schwann cells (if present) and a basement membrane that enclose the axons;
- ganglia of neurons that come from dorsal root (motor division) and ventral root (sensory division).

These components are encapsulated in the **endoneurium**, an ensemble of fibroblasts and collagen fibers.

Different nerve fibers are collected together and enclosed in a **perineurium** cellular membrane. This structure represents a **fascicle**. In each fascicle, fibers have a wavy pathway, which allows a limited stretching of nerve before causing damage. Within a fascicle, fibers which innervate a particular target are collected, even if proximally fascicles tend to fuse. The perineurium is the middle layer and it carries out some important functions: it provides tensile strength to the nerve, stabilizes the fluid pressure inside the endoneurium and acts as a diffusion barrier. In turn, fascicles are embedded in a collagenous of connective tissue structure named **epineurium** which give support to the nerve. Also blood vessels are enclosed in this outermost layer in order to supply nerves with the required energy. [6], [7]. A representation of peripheral nerve structure is reported in Figure 1.4.

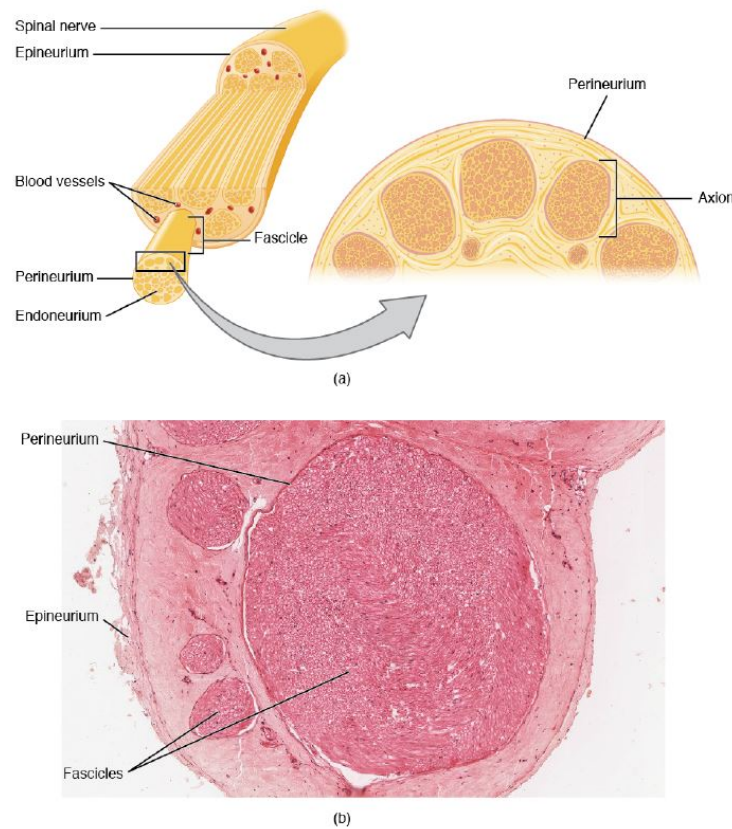


Figure 1.4: (a) Schematic representation of peripheral nerves organization which includes epineurium, perineurium and endoneurium layers, (b) microscopy of a peripheral nerve section. Figure readapted from [8].

1.3 Peripheral nerve system interfaces: State of the Art

In the realization of PNS interfaces there are several requirements which must be taken into account [9]:

- Selectivity in the stimulation.
- Selectivity in the recording.
- Invasiveness: to obtain a major selectivity is required a greater invasiveness. It is closely connected to the degree of damage produced on the nerve. A compromise between selectivity and invasiveness is needed.
- Biocompatibility: materials used for the realization of the neural interfaces must not release substances in a toxic concentration. They must produce a limited foreign-body response of the organism in order to have a thin encapsulation connective tissue. In the chronic implants, a crucial aspect is the stability of electrodes over the time. Important characteristics are the resistance to corrosion and to the biological environment. Several materials have been tested. Nowadays, the most utilized materials are Platinum, Tungsten, Iridium, Platinum-Iridium alloy, Iridium-oxide and stainless steels for the realization of the conductive sites, while silicon, polyimide, elastomers, polytetrafluoroethylene (PTFE) as insulating substrates [10].

The production of electrodes based on thin-film substrates made of polymers (as polyimide) is becoming an increasingly widespread procedure. Infact, the fabrication can be made through well-established micromachining techniques, so fast production and repeatability are possible. In this case the fabrication includes different steps:

1. construction of the polyimide substrate by the deposition of the polymer on a silicon wafer through the techniques of spin coating or chemical vapour deposition.
2. realization of the conductive components. Standard photolithography can be used. So on the substrate a layer of a photosensible resist is deposited, again by spin coating. A mask containing the desired pattern is used to transfer the geometry after the UV exposure and resist development.
3. Fabrication of conductive paths in order to link the electrode's active sites with contact pads. Sputtering is used at this purpose.
4. Covering of conductive parts by deposition of an additional polyimide layer.
5. Reactive ion etching is executed in order to produce the access holes to electrode's sites and pads.

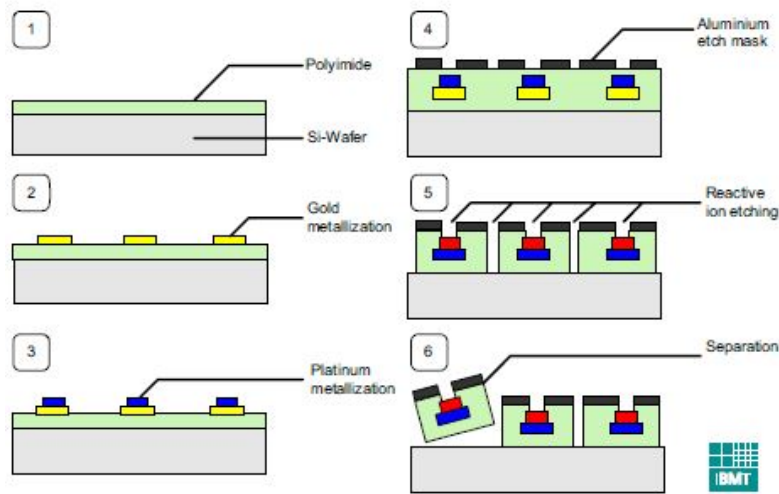


Figure 1.5: Fabrication step of a thin film based electrode. 1. deposition of polyimide substrate. 2-3. Realization of conductive sites and paths. 4. Covering of conductive component and mask deposition for next reactive ion etching step. 5. Reactive ion etching to produce access to conductive components. 6. Separation of electrode from silicon wafer. Figure readapted from [15].

6. mechanical separation of the electrode from the silicon wafer.

This section gives an overview of most used present peripheral nervous system interfaces, which have been developed with the aim to obtain nerve fibers stimulation and recording. The presentation follows a growing invasiveness order.

Extraneural electrodes

They are applied on the outermost membrane of the nerve, the epineurium. If compared with intrafascicular electrodes, they are characterized by an easier implantation procedure and a less probability of nerve damage. The minor invasiveness respect to intrafascicular electrodes ensures a greater longevity [11]. The mainly used variants are:

- **Cuff electrode:** Conductive electrodes sites (in a number greater than two) are positioned at the inner surface of an insulating cylindrical enclosure. The latter is realized in a flexible material which allows the electrode adaptation to the nerve structure. Different configuration of the active site are possible:
 - bipolar: one electrode is used as cathode and the other one as anode;
 - tripolar: the central electrode is the cathode and the external contacts are used as reference anodic electrodes: this configuration allows the reduction of electrical noise.
 - multipolar: the tripolar configuration is reproduced along the electrode

structure allowing a greater selectivity between the fascicles that are inside the nerve.

The selectivity depends on the configuration used. However, cuff electrodes act (record from or stimulate) on many axons in different nerve fascicles, predominantly external ones.

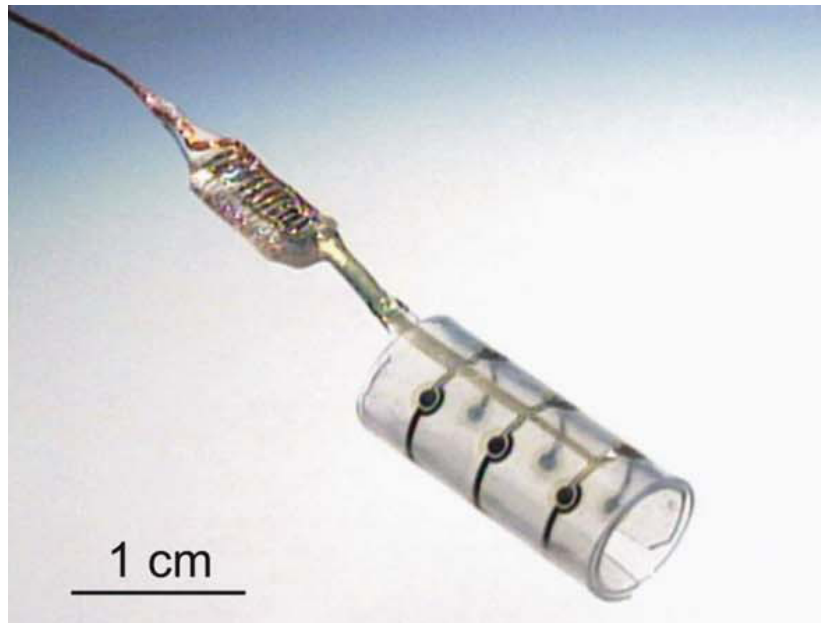


Figure 1.6: Cuff electrode used in peripheral nervous system. Figure readapted from [12].

- **Flat interface nerve electrodes (FINE):** They are similar to cuff electrodes but have an elliptical configuration. Therefore, fascicles within the nerve are more accessible since they are forced to lie in a smooth plane. [13] The flat configuration permits a greater selectivity than in cuff electrodes but, on the other side, the probability of nerve impairment increases as tensile stresses, caused by the particular geometry, grow up.

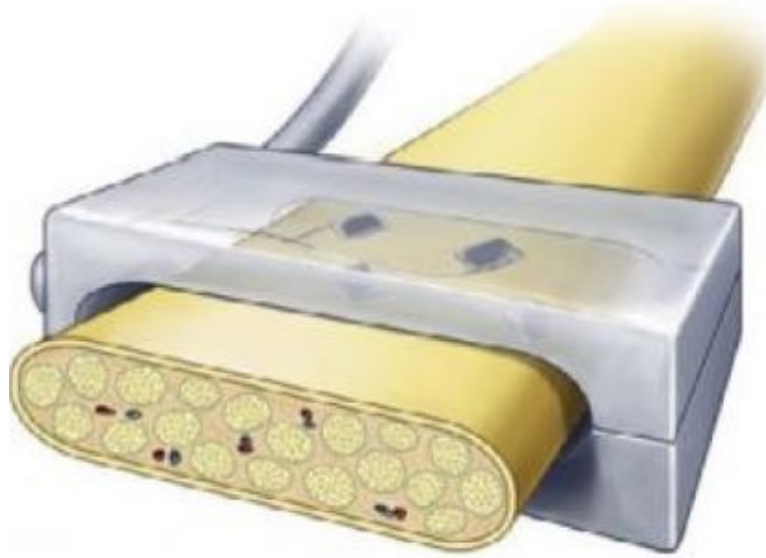


Figure 1.7: A schematic representation of Flat Interface Nerve Electrode (FINE). Figure readapted from [14].

Intrafascicular electrodes

Intrafascicular electrodes are applied within the nerve fascicles. Respect to extraneural electrodes they are characterized by a higher selectivity in fibers stimulation and by a lower intensity current. Furthermore, the motor fiber recruitment is more stable. With regard to recording performances, an encrease of signal-to-noise ratio has been observed when compared to extraneural electrodes [7]. According to the clinical application, intrafascicular electrodes can be implanted in several configurations within the nerve fascicles. As consequence, different typologies of electrodes have been developed.

- **Longitudinally Implanted intrafascicular electrodes (LIFE):** They are applied inside a single fascicle longitudinally to the nerve fibers. The insertion is made by means of a driving wire, which allows the positioning of the active sites of the electrode at the desidered portion of the fascicle.

LIFEs electrodes consist of conductive fibers, usually in Pt-Ir or Kevlar®, which are coated by an insulating layer (i.e Teflon) with the exception of the zone dedicated to recording and/or stimulation. Pt-Ir electrodes result rigid respect to nervous tissue. This causes a reduction in signal-to-noise ratio of the the recorded signal due to the drift of the nerve fibers [16]. The longitudinal implantation enables the contact with a low portion of the fascicule, so it is difficult to achieve a selective recording or stimulation. [17]. Multiple electrodes implantations are necessary to stimulate fibers that belong to different bundles in the nerve.

- **thin-film LIFEs (tfLIFE):** They represent a variation of LIFE electrodes and consist of a thin polyimide structure on which are situated eight contact sites.

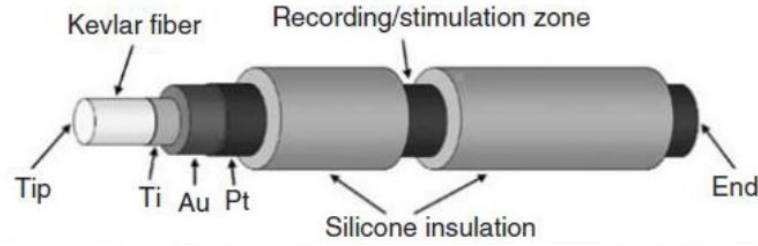


Figure 1.8: Representation of a polyLIFE: Longitudinally Implanted intrafascicular electrodes based on polymer. The polyLIFE is made up of a Kevlar fiber, coated with different metal layer of titanium (Ti), gold (Au), and platinum (Pt) and insulated with silicone. The recording/stimulation zone consists of approximately 1 mm non-insulated portion of the metallized fiber. Extracted from [7].

- **Transverse Intrafascicular Multichannel electrodes (TIME):** They are inserted transversally to the nerve axis. In this way it is possible to activate different subsets of axons in different nervous fascicles. Several Platinum electrodes are arranged on a thin, strip-like substrate in polyimide. Then, the insulating polyimide structure is folded so that the electrodes sites produce an arrow configuration at the tip of the electrode

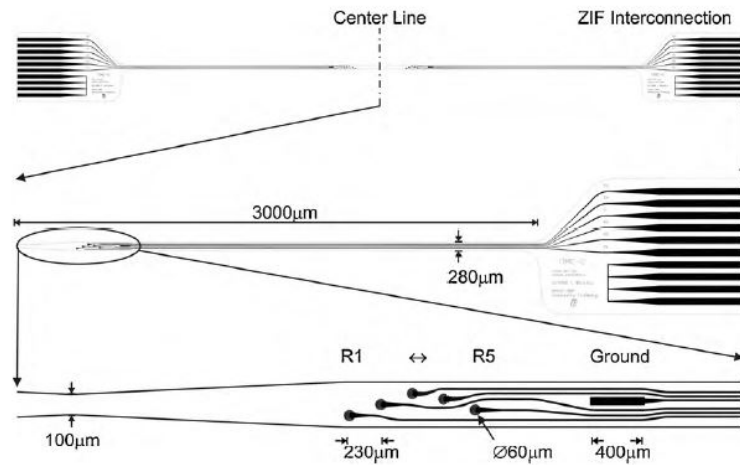


Figure 1.9: Representation of TIME electrode structure. Upper image: TIME electrode before folding the structure at the center line level. Middle image: TIME after bending. Bottom image: zoom of electrode site at the tip of the electrode. Extracted from [18].

In the Figure 1.10 the different modalities of cuff, LIFE and TIME electrodes are illustrated.

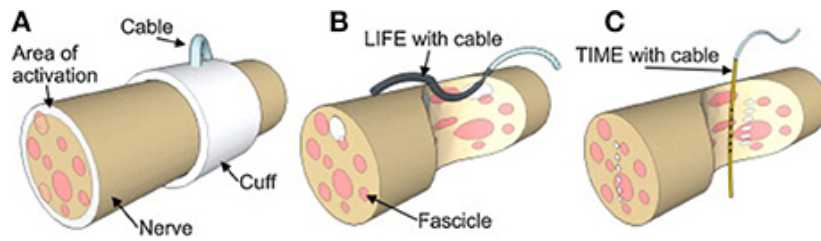


Figure 1.10: Representation of implantation of Cuff, LIFE and TIME electrodes. Extracted from [19].

Chapter 2

Materials and Method

The purpose of this thesis is the development of a system which allows to test the performances of different types of neuronal interfaces applied to the peripheral nerves. For each typology of electrode used, the selectivity performances in the stimulation of the nerve fibers have been evaluated through the recording and analysis of the EMG signal coming from target muscles. To this end, the system must combine stimulation, recording and elaboration functionalities.

2.1 Structure of the used platform: its components and their interaction

The system has been developed using a Tucker Davis Technology (TDT)'s Neurophysiology Workstation. It includes three main components: the RZ5D BioAmp Processor, a multi-channel stimulator (IZ2-MH), and a multichannel amplifier (PZ5). In addition, a high performance Computer with the necessary tools to the working of the system is present. Stimulation and recording electrodes complete the platform. The schematic representation of the system is reported in Figure 2.1.

The detailed explanation of the different components is given in the following sections. Here the macro interactions are presented.

A first important aspect to take into account is the physical connection between the devices: the PC is linked to RZ5 processor by means of an optic fiber. In turn, the RZ5 communicates, again with two dedicated optic fibers, both with the IZ2 stimulator and with the PZ5 amplifier [20].

Secondly, all devices have to be configured according to the intended application. Device programming has been implemented with circuit files developed using TDT's RP Visual Design Studio (**RPvdsEx**). Through a TDT run-time application, **OpenEX**, circuits are loaded on the RZ5 processor and from it, configuration parameters are sent to the stimulator and multichannel amplifier. The circuits created in RPvdsEx can include

parameters which can be changed dynamically during the execution of the circuit file, parameter tags, and which are accessed by **OpenWorkbench Server**.

A Matlab® GUI Interface allows the user to dynamically interact with the circuits developed on RPvdsEx, enabling the stimulation parameters definition and variation. Optic fibers permit the transmission of data also in the reverse direction. So, once devices configuration has been completed and stimulation executed, the EMG signal is recorded and transferred from PZ5 amplifier to RZ5 processor, and it is sent to Matlab® environment for further elaboration and the visualization of the results in the GUI.

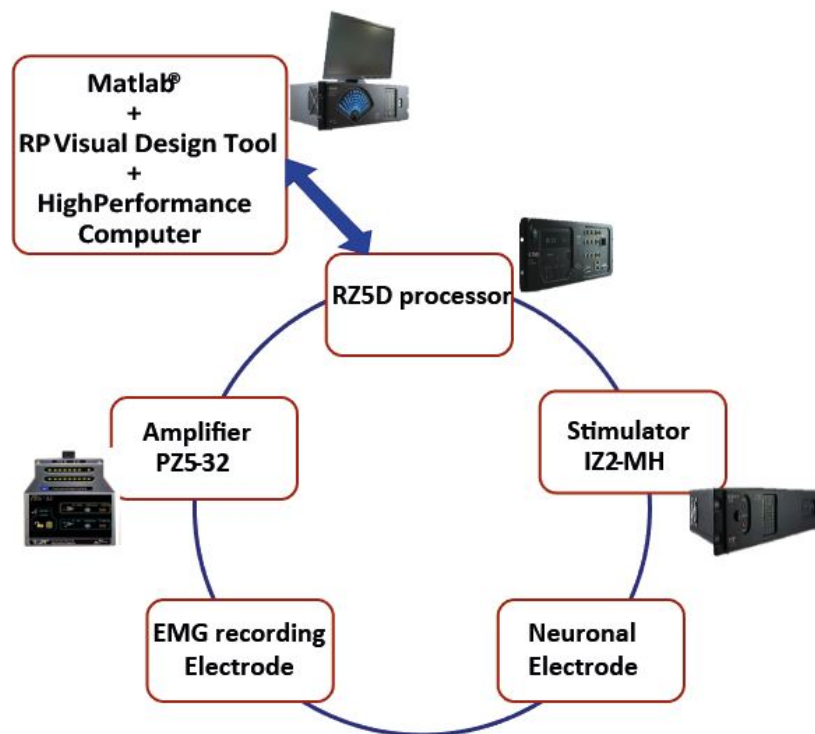


Figure 2.1: System's components. A High Performance Computer communicates with the RZ5D processor through which the management of both stimulation block (right side of the circle) and recording block (left side of the circle) is possible.

2.2 The developed platform: Hardware description and its software configuration

In this section the technical characteristics of devices included in the system and the relative RPvdsEx blocks for their configuration are presented. The RZ5D Processor represents the key component which allows the management of both stimulation and recording circuit.

2.2.1 RZ5D BioAmp Processor

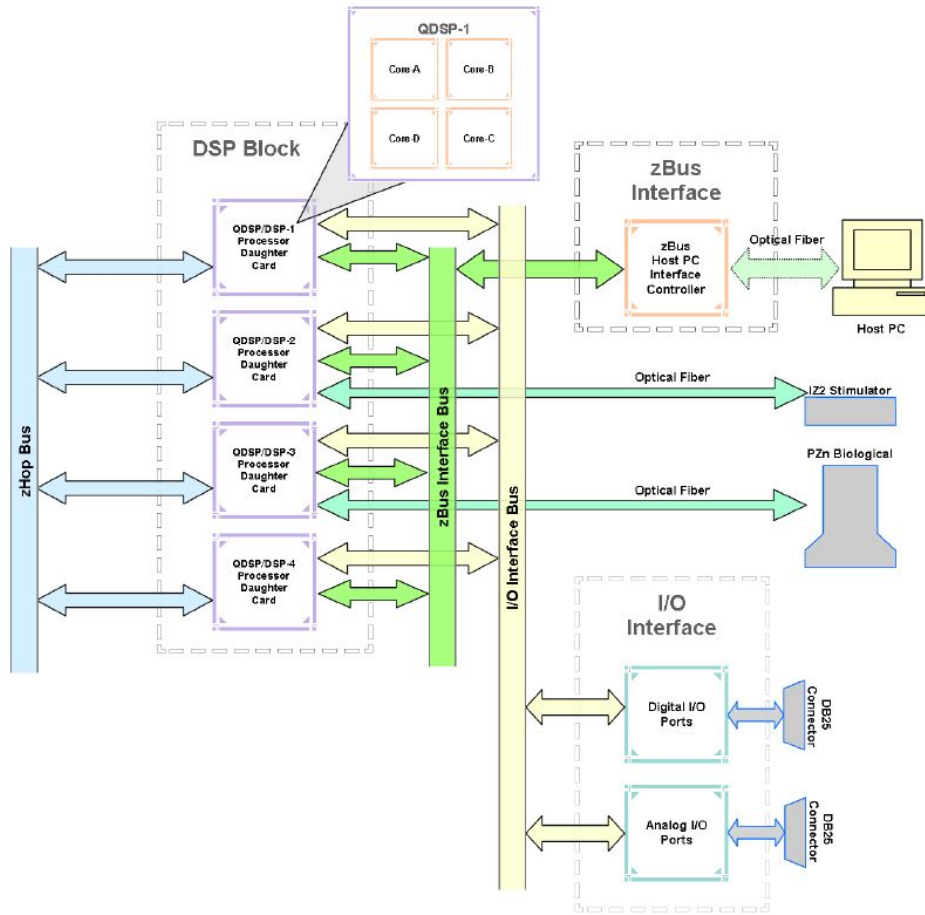


Figure 2.2: Schematic representation of RZ5D processor's architecture. Extracted from [20]

The RZ5D BioAmp Processor allows the parallel stimulation, real-time acquisition and processing of signals.

- **Acquisition:** a maximum of 32 channels of neurophysiological signals can be acquired and processed in real-time at a sampling rate of up to 50 kHz. Data can be input from a PZ amplifier.

- **Stimulation:** the RZ5D can allow stimulation through up to 128 channels at a maximum sampling rate of 50 kHz if used with TDT's IZ2 stimulator.

In Figure 2.2 it is reported the general architecture of the RZ5D device and the way in which the different components communicate is showed.

As visible in the Figure 2.2, three fundamental blocks can be identified in the RZ5D's architecture:

- **Digital Signal Processor (DSP) block:** each DSP is connected to three data buses, one of which (zHop Bus) enables the data handling and transfer between DSPs; a second data bus connects the specific DSP with I/O Interface Bus; the third one connects the DSP with a zBus Interface which communicates with a Controller located on the Host PC.

Futhermore the **second DSP** is linked through an Optical Fiber to the **IZ2 Stimulator**.

The **third DSP** is linked through an Optical Fiber to the **PZn Amplifier**.

This hardware structure is reflected in the construction of circuit files developed in the RP Visual Design Studio (RPvdsEx): at each DSP is associated a particular sheet in the RPvdsEx environment. As consequence, blocks that interact with the stimulator have been assigned to DSP-2, while those ones which communicate with the amplifier have been placed on the sheet associated to DSP-3.

- **zBus Interface:** it enables the bidirectional communication with the host PC. Indeed, PC control commands and Data are exchanged between the PC workstation and the DSP Block.
- **I/O Interface:** it lets the transfer of output data and the acquisition of data from the PZ amplifier and from other Digital and Analog Ports connected to external devices.

In Figure 2.3 the RZ5D specifications are summarised.

RZ5D Technical Specification	
DSP	Three or four standard DSPs and/or quad-core (QZDSP)
D/A	4 channels, 16-bit
Maximum Sample Rate	Up to 48828.125 Hz.
Imposed Sample Rate	6103.52 Hz
Frequency Responce	DC – 0.44*Fs (Fs = sample rate)
Voltage Out	+/- 10.0 Volts, 175 mA amx load
S/N typical	82 dB (20 Hz- 20 Khz at 9.9 V)
Output Impedance	10 Ohms
A/D	4 channels, 16 bit
Maximum Sample Rate	Up to 48828.125 Hz.
Imposed Sample Rate	6103.52 Hz
Frequency Responce	DC –7.5 KHz
Voltage In	+/- 10.0 Volts
S/N	82 dB (20 Hz- 20 Khz at 9.9 V)
Input Impedance	10 KOhms
Fiber Optic Ports	
Stimulator (IZ2)	One output for IZ2, up to 128 channels
Preamplifier (PZ)	One input for PZ5,PZ2,PZ3 or PZ4
Digital I/O	8 programmable bits: 3.3 V, 25 mA max load
	2 programmable bytes: 5 V, 35 mA max load

Figure 2.3: RZ5D Technical specification. Extracted from [20]

2.2.2 The stimulation circuit

The stimulation circuit includes different components which are:

- the RZ5D processor: on the DSP-2 of the processor is loaded the circuit, developed in RPvdsEx environment, which allows the configuration of the IZ2-MH stimulator and the generation of digital stimuli. Stimuli are produced on the basis of stimulation parameters that the user defines in the Matlab® environment through the GUI interface.
- the IZ2-MH stimulator which converts digital stimuli in analog signals which can be erogated in form of current or voltage stimuli according to the IZ2-MH configuration.
- a Zero Insertion Force ZIF-Clip® headstage adapter which allows the transfer of stimuli from IZ2-MH banks to an Omnectics connector.
- a 36- pin female Omnetics nano dual row connector, to which stimuli are sent to a printed circuit board (PCB).
- on the PCB a 36-pin male nano dual connector is linked to a 20-pin SAMTEC Connector
- finally the stimulation electrode whose pins are welded on the Samtec connector.

The Figure 2.4 represents how the enumerated components are connected starting from the IZ2-MH stimulator to Samtec Connector. The following sections describe the IZ2-MH stimulator technical characteristics and the code which has been used to interact with the hardware configuration of the stimulation circuit.

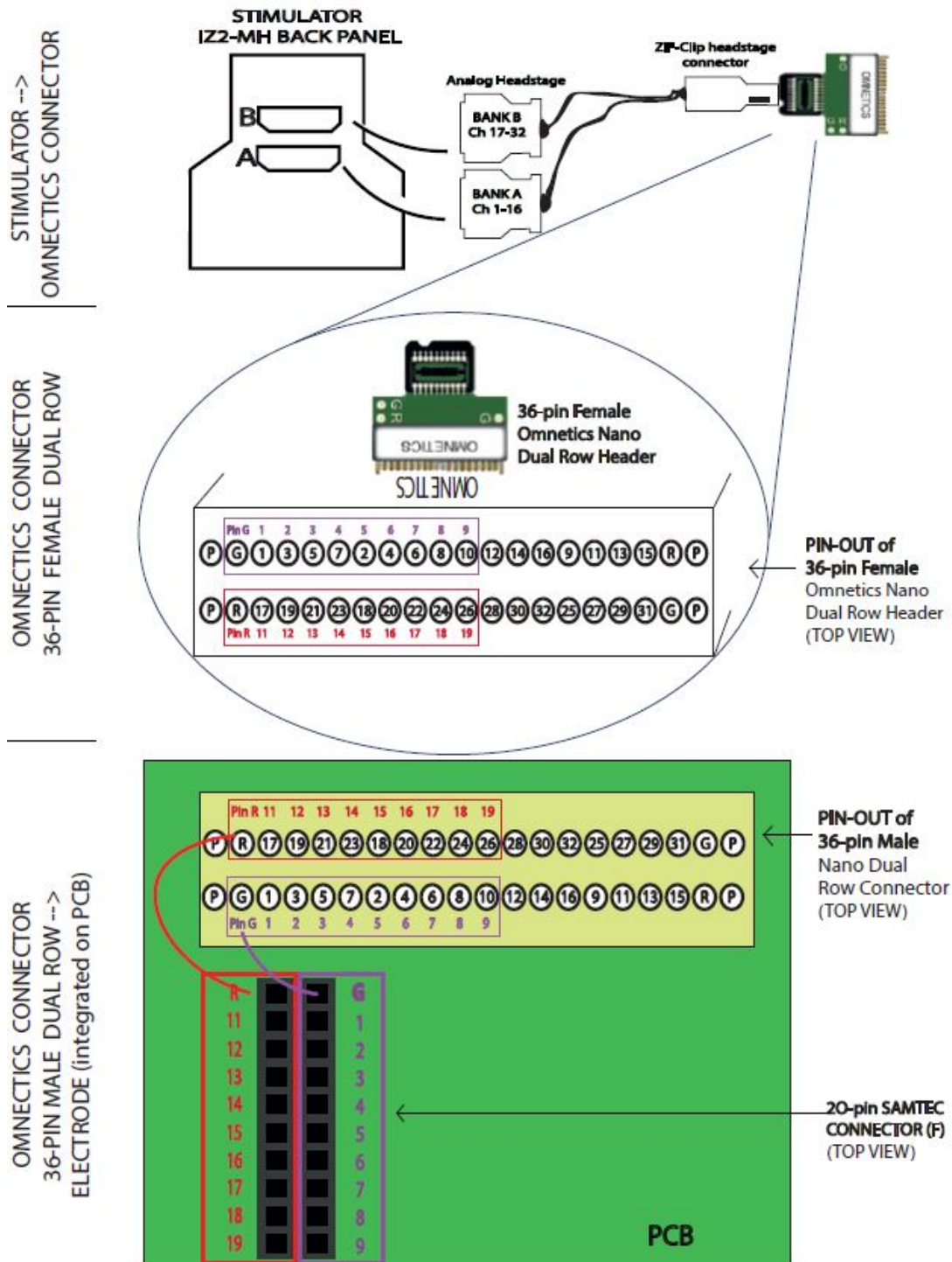


Figure 2.4: Hardware components of the stimulation circuit. From up to bottom: stimulation banks of IZ2-MH stimulator are connected by means of analog headstages to an Omnetics connector. Omnetics connector is inserted into a specular connector welded on a printed circuit board (PCB). From this connector conductive paths are realized to reach a Samtec Connector in which pins of the peripheral nerve electrodes are inserted. The electrodes are finally used to stimulate nerve fibers.

2.2.2.1 IZ2-MH Stimulator

It includes 32 stimulation channels that are organized in banks of 16 channels. For each channel a maximum of 3 mA can be supplied and up to 12 V on up to 10 electrodes [20]. The sampling rate of the stimulator is the same which has been defined for the RZ5 base station, 6 KHz.

Software configuration

The circuit which controls the IZ2-MH stimulator is associated to DSP-2. The Control block used to configure the IZ2-MH is reported in Figure 2.5. The defined waveform and the information related to the stimulation channel enter the IZ2_Control block through the port indicated as *StimSignal* [21]. In addition, the following settings have been defined:

- Stimulation Mode: Current (in μA);
- Number of possible stimulation channels: 32. However, stimuli are sent through a channel at time.



Figure 2.5: IZ2_Control block used to configure the IZ2-MH Stimulator and to send the desired stimulation waveform through its channels

The user chooses the active sites of the neural electrodes from which the current stimuli have to be erogated. However, stimulation elecrodes are physically connected to a 20-Samtec connector whose pinout is different from the previous block in the stimulation chain, represented by the Omnetics Connector. More specifically, in Figure 2.6 it is possible to see the 36-pin Omnetics Connector [22] and the 20-pin Samtec connector pinouts. It is possible to note that pin1, pin2, pin3 of Samtec Connector are associated respectively to pin1, pin3, pin5 of Omnetics Connector and so on. The latter pinout reproduces the physical distribution of channels in TDT's IZ2-MH stimulator. This aspect has been taken into account in the construction of the circuit that manages

the erogation of stimuli and a remapping of active sites has been done directly in the RPvdsEx environment. This means that, when the user decides to erogate stimulation from a particular active site of the electrode, current is output from that channel of the stimulator which is associated to the specified site on the electrode (i.e active site 2 is chosen, so current is erogated from channel 3 of the stimulator).

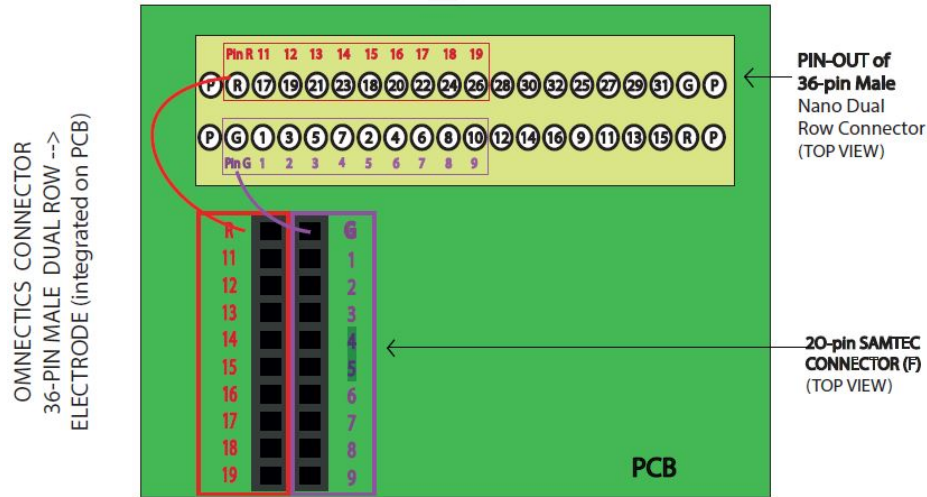


Figure 2.6: Correspondence between Omnetics Connector pins and SAMTEC connector pins.

In Figure 2.7 a part of the circuit implemented in RPvdsEx which associates electrode's active sites (on the left) to stimulator channels (on the right) is reported.

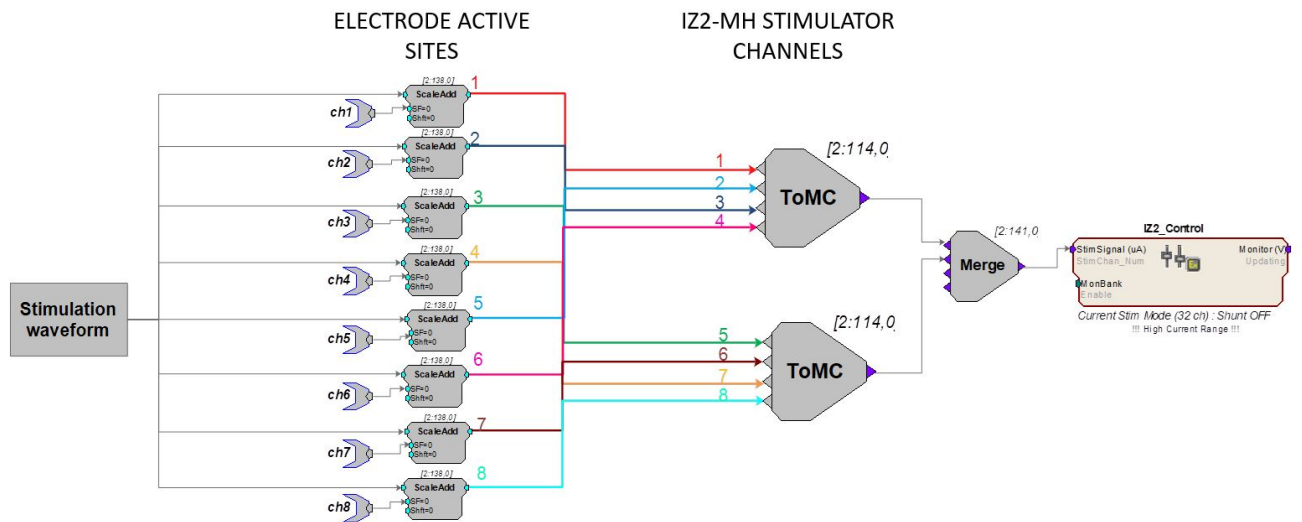


Figure 2.7: RPvdsEx circuit to remap IZ2-MH stimulator channels (right side) to the 1-8 active sites of electrode used for stimulation (left side)

2.2.2.2 Peripheral nerve interfaces: stimulation electrodes

Different types of electrodes have been used to test the system:

cuff electrode with two conductive platinum foils and an insulating substrate in PDMS.



Figure 2.8: Extraneural cuff electrode used to test the system

tf-life electrode: The structure of a portion of the electrode is reported in Figure 2.9. The whole electrode has 2 mirrored halves identified as (a) and (b). The reference sites have the same dimensions of active sites. The dimensions are indicated in Figure 2.10.

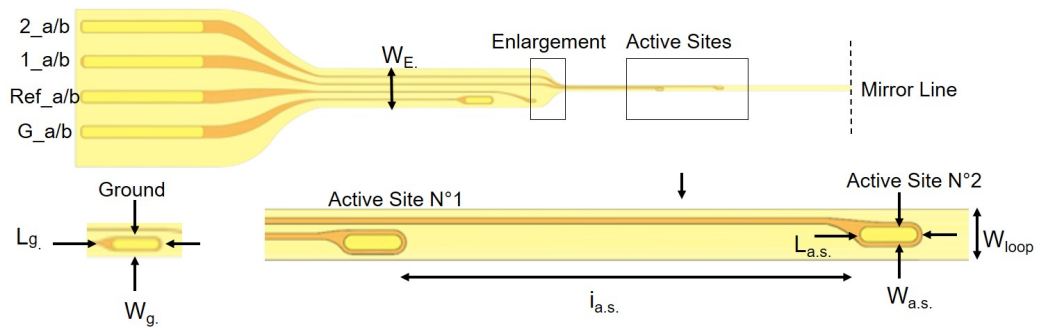


Figure 2.9: Intraneural tf-life electrode used to test the system

		[mm]
$L_{a.s.}^*$	Active Sites Length	0.2
$W_{a.s.}^*$	Active Sites Width	0.08
$i_{a.s}$	Inter-distance between active sites	1.5
W_{loop}	Loop width	0.16
W_E	Enlargement width	1.1
L_g	Ground Length	0.8
W_g	Ground Width	0.2

Figure 2.10: Dimensions of intraneural tf-life electrodes used to test the system

2.2.3 The recording circuit

The recording circuit includes different components which are:

- EMG electrodes: superficial adhesive gel Ag/AgCl electrodes have been used.
- Splitter-box with 32 available channels to connect EMG electrodes to the PZ5-32 Amplifier.
- PZ5-32 Amplifier;
- the RZ5D processor.

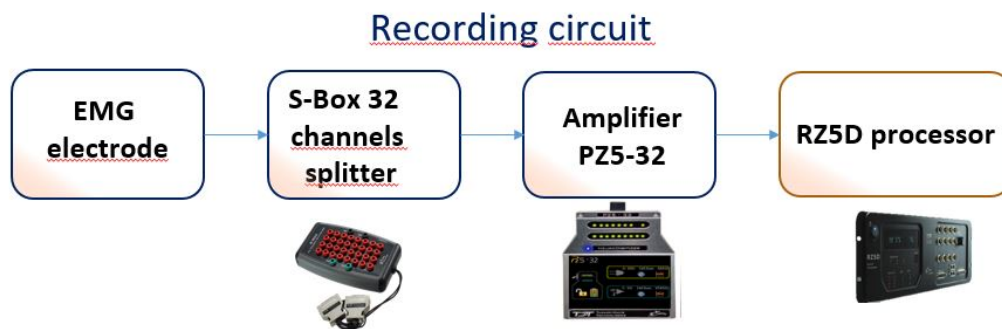


Figure 2.11: Schematic representation of the Recording circuit

2.2.3.1 PZ5-32 NeuroDigiter Amplifier

The PZ5-32 NeuroDigiter Amplifier has the following characteristics [20]:

- 32 analog inputs arranged in 16-channel banks. Each bank has its own ground and reference. So the PZ5-32 has two banks or logical amplifiers, A e B, accessible at the back-panel through DB26 connectors which are used to input signals from external devices.
- 2 or 4 digital inputs.

The signal sampling rate range is comprised between 750Hz - 50 KHz.

According to the signal to acquire, each amplifier bank can be set in one of four configurations characterized by appropriate sampling rates and Referencing modes:

- **EMG (Electromyography)**: it has Differential Referencing Mode, AC Coupling and a sample rate of 750Hz. The differential mode uses odd channels to record (+) and each even channel serves as an individual reference (-) channel for the preceding odd channel. AC coupling implements a high pass filter with a cutoff frequency of 0.4 Hz.
- **EEG (Electroencephalography)**: It has Shared Referencing Mode, AC Coupling and a sample rate of 750Hz. In Shared mode, all banks have the same reference, that is the reference (pin 5) of the first bank .
- **LFP (Local Field Potentials)**: it has Shared Referencing Mode, AC Coupling and a sample rate of 3kHz.
- **SU (Single Unit)**: it has Local Referencing Mode, AC Coupling and a sample rate of 25kHz. In Local reference mode, each bank uses its input (pin 5) as reference.

In order to obtain a greater signal-to-noise ratio, onboard filters are located in the PZ5 architecture to further reduce the noise by down-sampling the signal that is above the frequency range of interest (the down-sampling percentage goes from 10% to 45% of sampling rate imposed). Analog signals are converted to digital signals and transferred to the RZ5D processor through an optic fiber.

Software configuration

The configuration of Logical amplifiers is set through the PZ5_Control block (represented in Figure 2.12) which is assigned to DSP-3.

Since a maximum of 8 differential EMG channel is acquired, only one Logical amplifier has been used. The EMG configuration has been chosen and the following parameters have been imposed:

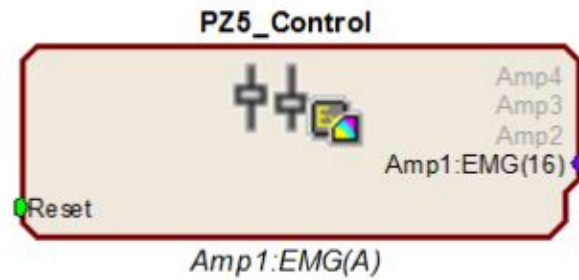


Figure 2.12: PZ5_Control block used to configure the PZ5 Amplifier

- Channel count: 16;
- Sampling rate: it is the same of the RZ5 processor, so 6KHz;
- Filter: 45% of sampling Rate. It is the down-sampling filter;
- Reference Mode: differential;
- No external ground, no Dc Couple.
- Target Impedance: $1K\Omega$ (default value).

Once signals have been recorded they are transmitted to the RZ5 base station. The macro reported in Figure 2.13 has been used at this purpose. In this case, only the number of channels has been specified, equal to 16: in fact in differential mode, channel 1 is obtained as Ch1 minus Ch2, channel 2 is equal to Ch2 minus Ch1 and so on. In subsequent processes only one of the two possible differential signals is used.



Figure 2.13: RZ5_PZ_input is the Macro block used to transmit signals from PZ5 Amplifier to the RZ5 processor

2.3 MATLAB® GUI functions and Interaction between MATLAB® and TDT's RP Visual Design Tool environments

This section describes the functionalities of the platform and the code used for their realization. User interacts with the system through a MATLAB® interface which has been reported in Figure 2.14. Three are the main functions implemented.

- Connection Matlab - TDT System;
- Stimulation;
- Data elaboration and Plotting of Results;

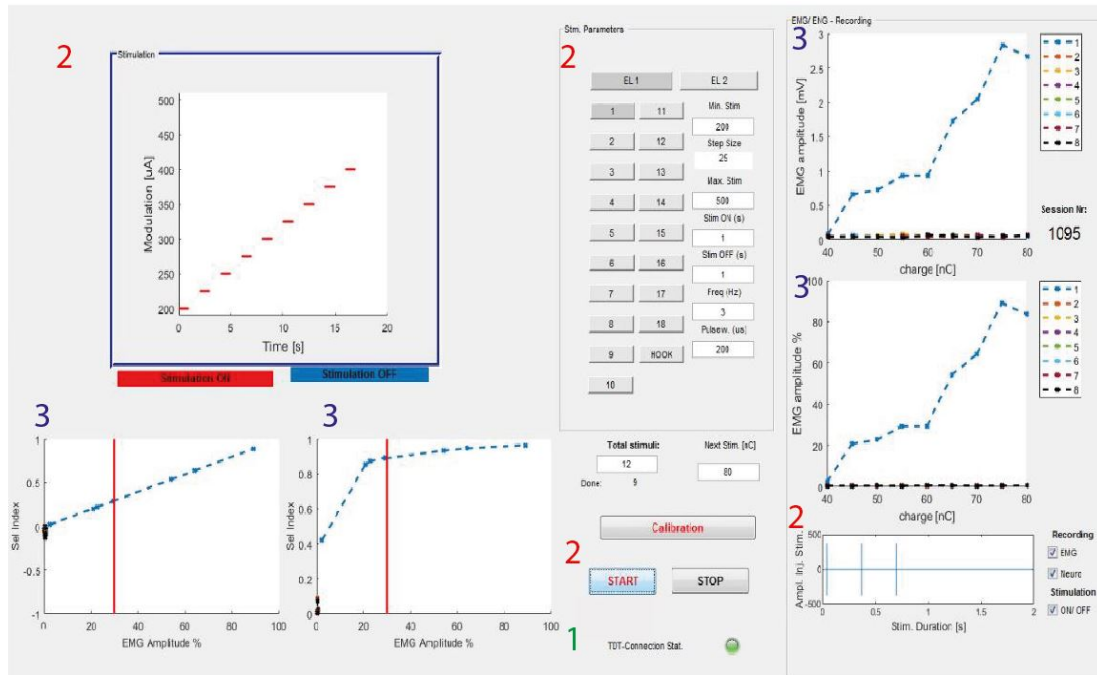


Figure 2.14: MATLAB GUI: in the figure functions have been highlighted with three numbers.

1) Connection Matlab-TDT System, 2) Stimulation, 3) Data elaboration and Results

2.3.1 Connection Matlab - TDT System

The connection of Matlab® with the TDT System is a preliminary fundamental step in order to achieve the real-time control of the TDT System. In order to do this, **ActiveX Controls** have been used, namely libraries of commands which can be used in different programming languages to directly interact with the hardware architecture of the system. As anticipated in section 2.1, circuits developed in **RPvdsEx** have

been implemented for the programming of the devices. The server used for the loading of these circuit files onto the hardware RZ5D processor is **Openworkbench**. In the processing chain also "parameter tags" have been included which are objects used for the real-time control. Among the family of ActiveX Controls in OpenDeveloper driver [23], **TDevAccX methods** have been used, as they allow the real-time access to RZ5D hardware, connected to OpenWorkbench server. In order to implement real-time control, TDevAccX methods use "targets" which include: the name of a hardware device and the parameter tags. The TDevAccX instructions to connect to the OpenWorkbench server are reported below.

Listing 2.1: Connection between Matlab and TDT System

```

1  handles.DA= actxcontrol ('TDevAcc.X');
   while 1 % while the if statment is true,so while
       CheckServerConnection is 0
3     handles.DA.ConnectServer('Local')
       % enables connection with OpenWorkbench server.
5     % 'Local' is most common ServerName

7     handles.rco =handles.DA.GetDeviceRCO('RZ2_1');
       handles.status =handles.DA.GetDeviceStatus('RZ2_1')
9
       if handles.DA.CheckServerConnection==0
11        warning('Client application not connect to server
           ')
           % CheckServerConnection verifies if OpenWorkbench
           server has loaded
13        % a circuit file to the OpenWorkbench application
           .
           % It returns 1 if OpenWorkbench is correctly
           loaded.
15        % It returns 0 if OpenWorkbench is not loaded, or
           in Idle mode.

17        disp('...connected')
           break
19     end
       pause(0.5)
21 end

```

In order to start an experimental session, therefore, the user has to do the steps summarised below:

- Open OpenEx platform;
- Access to the OpenWorkbench application;
- Load the specific RPvdsEx circuit containing the setup options for the system's device and the processing chain;
- Set, through the OpenWorkbench application, the system in Preview or Standby Mode;
- Run the Matlab® GUI and wait for the connection between Matlab® and TDT System: the successful connection is indicated by a green button in the GUI interface near the voice *TDT-Connection Stat.* (Figure 2.14);
- Begin the stimulation. It is described in the session 2.3.2

2.3.2 Control of the stimulation

From the Matlab® GUI the user can set the stimulation parameters. Stimuli are erogated in form of current pulses. Each stimulus is a rectangular symmetrical biphasic waveform with 50% duty cycle. The user can choose the following parameters:

- Minimum and maximum amplitude of stimulation in (μA);
- Period in which stimuli are erogated in (s) (StimOn);
- Period in which stimulation is turned off in (s)(StimOff);
- Frequency at which stimuli are erogated in (Hz);
- Pulsewidth of each phase of the rectangular waveform in (μs);
- The total number of stimuli or steps between the minimum and maximum amplitudes;
- The electrode's active site from which stimulation is erogated: from 1 to 18;
- If the Hook button is pressed, stimuli are erogated from the 32th channel of the stimulator.

From the parameters of maximum, minimum amplitude and the number of stimuli to erogate the value of incremental step to add at each iteration is calculated as:

$$Step = \frac{(Min - Max)}{Num_stimuli}$$

In this way an increasing ramp stimulation is obtained.

In Figure 2.15 is reported an example of two subsequent cycles of stimulation with growing amplitude.

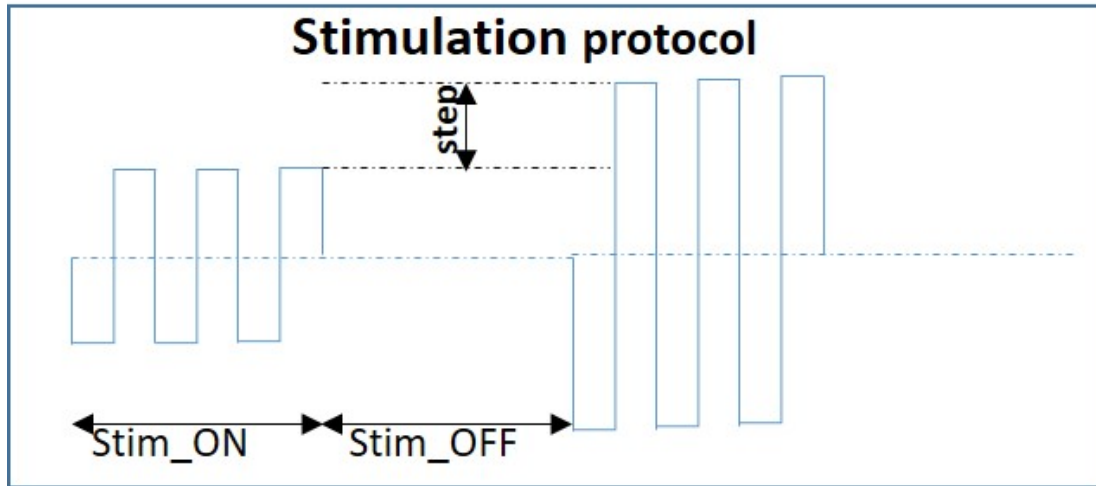


Figure 2.15: Stimulation protocol: biphasic rectangular pulses are defined during a stimulation period (Stim_ON). Between a Stim_ON period and the next one a step of amplitude is added in order to obtain an increasing ramp stimulation protocol

All stimulation parameters are passed to R PvdsEx environment through the instructions reported in the following Listing where *RZ2_1* is the name assigned to the RZ5D processor and the name which is after the dot is the name assigned to each "parameter tag" in R PvdsEx, while the variable after comma is the correspondent value assigned in Matlab®.

Listing 2.2: Transfer of stimulation parameters from Matlab® to R PvdsEx

```

2         handles.DA.SetTargetVal('RZ2_1.StartStim',0);
%         pause(0.01)
4         handles.DA.SetTargetVal('RZ2_1.pulseWidth1',Pw
%         );
%         pause(0.01)
6         handles.DA.SetTargetVal('RZ2_1.pulseWidth2',Pw
%         );
%         pause(0.01)
8         handles.DA.SetTargetVal('RZ2_1.PulseNum',Hz*
%         StimOn);
%         pause(0.01)
10        handles.DA.SetTargetVal('RZ2_1.Ratio',1);

```

```

12 %           pause(0.01)
           handles.DA.SetTargetVal('RZ2_1.stimAmp_1',Max)
           ;
14 %           pause(0.01)
           handles.DA.SetTargetVal('RZ2_1.stimAmp_2',Min)
           ;
16 %           pause(0.01)
           handles.DA.SetTargetVal('RZ2_1.CurrStep',Step)
           ;
18 %           pause(0.01)
           handles.DA.SetTargetVal('RZ2_1.BurstFreq',Hz);
20 %           pause(0.01)
           Period=1/Hz;
22 %           pause(0.01)
           handles.DA.SetTargetVal('RZ2_1.ISI',Period);
24 %           pause(0.01)
           handles.DA.SetTargetVal('RZ2_1.Stop',data.stop
           );

```

The acquired parameters are used as input of different blocks in the chain of the circuit which allows the definition of stimuli to send to the stimulator. A general parameter tag is represented by the symbol reported in Figure 2.16



Figure 2.16: Symbol of a parameter tag in RPvdsEx

Organization of Stimulation circuit in RPvdsEx

In the processing chain it is fundamental first of all the insertion of a block which defines the core timing. It is used as synchronization for the RZ5D processor and the devices controlled by it (stimulator and amplifier). It is also useful for the generation of timing pulses exploited for stimulation protocols.

The circuit has been organized in some functional blocks which communicate through the so called **HopIn** and **HopOut** objects which enable the data transfer. In the following the several circuit blocks are described:

1. **Definition of the total number of stimuli to erogate.** This block takes as

inputs: the parameters of minimum, maximum amplitude, the incremental step and the number of stimuli of same amplitude to erogate in one cycle (defined as stimulation frequency times StimOn and indicated as *PulseNum*). The number of step is obtained as:

$$number_Step = \frac{(Min - Max)}{Incremental_step}$$

So the total number of stimuli is:

$$tot_stimuli = PulseNum \times number_Step$$

2. **Generation of pulse trains.** This block takes as input period between two subsequent pulses (indicated as ISI and defined as $1/\text{stimulation_frequency}$). It also uses the *tot_stimuli* defined in the previous block and a trigger that enables the stimulation.

The ISI period is converted from seconds into samples. Outputs are two pulse trains with a number of pulses equal to *tot_stimuli* and period of ISI samples. Outputs are named *Trigger3* and *Trigger4*.

3. **Conversion of the pulsewidth parameter passed from Matlab® from time into samples;**

4. **Definition of stimuli to erogate in one cycle & verification if Stop Stimulation conditions are reached.** The inputs of this block are: *Trigger4*, *PulseNum*, actual amplitude of stimulation current (it is input from Matlab where it is updated at each cycle by adding the *incremental_step* at the previous value), minimum and maximum amplitude values.

Trigger4 enters into a counter which counts the number of pulses (one at each clock's cycle). When the number of counted pulses is equal to *PulseNum*, that is to say that the number of pulses to erogate during a single StimOn period has been reached, the exit of a particular object becomes 1. At this point two operations are activated:

- the counter is reset to zero, so at the next stimulation cycle it restarts the count of stimuli;
- verification if it is possible to erogate stimuli at the next cycle. This condition is plausible only if the increase of amplitude that would be at the subsequent cycle does not exceed the maximum of amplitude that has been declared. If this condition is not respected, a trigger which makes the stimulation to end is activated. This is activated also if the user has pressed the STOP button in the Matlab® GUI.

5. **Enabling of stimulation.** This block enables the stimulation only if the following conditions are respected:
 - no trigger of end stimulation has been activated;
 - button STOP has not been pressed;
 - START button has been pressed.
6. **Stimuli waveform definition.** Inputs are: minimum amplitude, *Trigger3*, pulsewidth (in samples), actual value of stimulation amplitude. When a rising edge of *Trigger3* is encountered, the output of a block goes high and remains constant for a number of samples determined by the pulsewidth parameter. This value is multiplied for the actual value of stimulation. In this way one phase of the waveform is defined. After this period has passed, when a falling edge of *Trigger3* occurs, it makes fixed to 1 the output of another block for a number of samples always determined by the pulsewidth value: this latter is multiplied for the negative of actual value of current, so the negative phase is obtained. The two phases are then concatenated in time in order to obtain the rectangular waveform.
7. **Sending of the stimulation waveform to stimulator's channel.** Only the parameter tag associated to the active site selectionated by the user on the GUI interface is set to 1, while the other ones are set to zero. In this way, when a multiplication between the waveform and the parameter assigned to each active site is executed (1 or 0), only the output of the desired active site contains the stimulation waveform. Remember that the association between electrode's pins and stimulator's channels is done as described in section 2.2.2.1.

2.3.3 Recording circuit: data elaboration

EMG signals are acquired with the PZ5 amplifier and transferred to the RZ5D processor as described in Section 2.2.3.1. From here several elaboration step have been performed:

1. **Filtering:** the filtering has been executed directly in RPvdsEx environment with appropriate blocks which allow the definition of filtering parameters. Then signals have been band-pass filtered (15 Hz -1000 Hz).
2. **Rectification:** one of the two available differential signals (ch1-ch2 or ch2-ch1, ch3-ch4 or ch4-ch3 and so on) has been selectioned for each couple of Emg electrodes. For each extracted signal the absolute value has been calculated. Indeed, the information to extrapolate is relative to the level of activation of each muscle.

The steps enumerated above are visible in the Figure 2.17. The image has been obtained by asking a subject to do a voluntary contraction in a synchronous way to the first stimulus visible on th TDT interface and to maintain the contraction during the stimulation period.

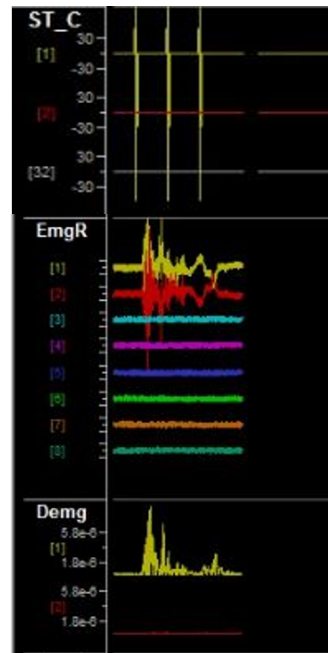


Figure 2.17: Elaboration steps on EMG signal executed on the RPvdsEx environment . From the top to the bottom: ST_C is the stimulus erogated (3Hz), EmgR is the signal directly acquired from PZ5 amplifier before filtering, Demg is the signal after filtering and rectification.

At this point both stimulu and EMG signal data are transferred to Matlab®

for further elaboration. The following steps are executed for each EMG channel acquired.

3. Calculation of Recruitment Curve:

- **Identification of starting stimulation instants.** Hence the stimuli have the negative phase first and then the positive one, the indexes correspondent to a negative value in the vector of the stimuli have been searched. The obtained binary vector has been multiplied for the vector containing the stimuli amplitude and the absolute value of the multiplication has been calculated.

The subtraction between the amplitude values of adjacent samples has been executed. The instant of start stimulation has been defined as that sample for which the result of subtraction is greater than the 50% of the maximum amplitude of the stimuli.

- **Definition of the analysis window.** From each pulse erogated during the stimulation period, the starting instants have been identified in the previous step. A window of 300 *ms* starting from each pulse's initial instant has been considered..
- **Research of maximum value of EMG.** In each defined window, the maximum of the EMG signal has been researched. Therefore, a mean of the maximum values has been calculated. This value represent the ordinate value of the Recruitment Curve, while the x-value is associated to the charge injected.

4. Calculation of Normalized Recruitment Curve.

The values obtained as described in the previous point are normalized respect to the maximum value of EMG obtained for each channel in the day. Values related to the maximum are overwritten only in the initial phase of characterization of electrodes and when the “**HOOK button**” on the GUI is used for the stimulation.

5. Calculation of Selectivity Index.

Selectivity in stimulation has been evaluated by means of two different definitions of Selectivity Index which allow to quantify the level of activation of a specific muscle compared with the activity of all considered muscles.

- the selectivity in stimulation of a *i* muscle respect to all *j* target muscles from a specific active site *k* has been calculated with the definition of Selectivity index reported in the following formula [26]. In it, **NEMG_i** indicates the value of normalized EMG value found for a specific muscle *i* and **NEMG_j** is the normalized EMG of the other muscles considered in the analysis. It is comprises between -1 correspondent to the total activation(100%) of all

j-th muscles and a zero value of NEMG for the i-th muscle, and 1 when only the wanted muscle i reaches its maximum activation and the activity of the other muscles is zero.

$$SI_{i,k} = NEMG_{i,k} - \frac{1}{n-1} \sum_{j=1, j \neq i}^n NEMG_{j,k}$$

where n is the number of considered muscles. In the following sections this index will be indicated as Sel Index 1.

- the other definition of Selectivity index is expressed as the ratio between the value of normalized EMG value found for a specific muscle i, **NEMG_i**, and the sum of normalized EMG values of all muscles **NEMG_j** ([24] and [25]).

$$SI_{i,k} = \frac{NEMG_{i,k}}{\sum_{j=1}^n NEMG_{j,k}}$$

It has values between 0 where the specific muscle i is not active at all (no selectivity) and 1 when only it is activated (maximum selectivity). In the following sections this index will be indicated as Sel Index 2.

Chapter 3

Testing of the system and results

3.1 Preliminary tests

Before using the system in vivo-applications, it has been tested in laboratory under known conditions in order to verify that all functionalities were performed in a proper manner.

3.1.1 Verification of stimulation waveform

The figure 3.1 shows an example of stimulation parameters imposed (on the left) and how stimuli are visible on TDT's interface (on the right). The parameters defined are: Min= 200 μA , Max= 500 μA , pulsewidth= 160 μs , frequency =3Hz during 1 second of stimulation (StimOn). Hook channel has been selected which is associate to IZ2-MH 32th stimulation channel. It is visible on the right of the figure that three symmetric pulses are erogated during 1 second of stimulation from 32th channel.

Once all stimulation parameters have been imposed, the correct generation of the waveform has been verified by means of a Sigilent SDS 1202X-E oscilloscope. The current erogated is passed through a resistance of 10 $K\Omega$ and the voltage is visualized.

In Figure 3.2 the voltage waveforms of a single stimulu are reported, they are associated to the minimum (200 μA) and maximum (500 μA) stimulation amplitudes, so the peak values of voltage V_p expected to visualize on oscilloscope are respectively of 2V and 5V. The visualization parameters imposed on the oscilloscope are 100 μ per division for the time scale and 2V per division for y-scale.

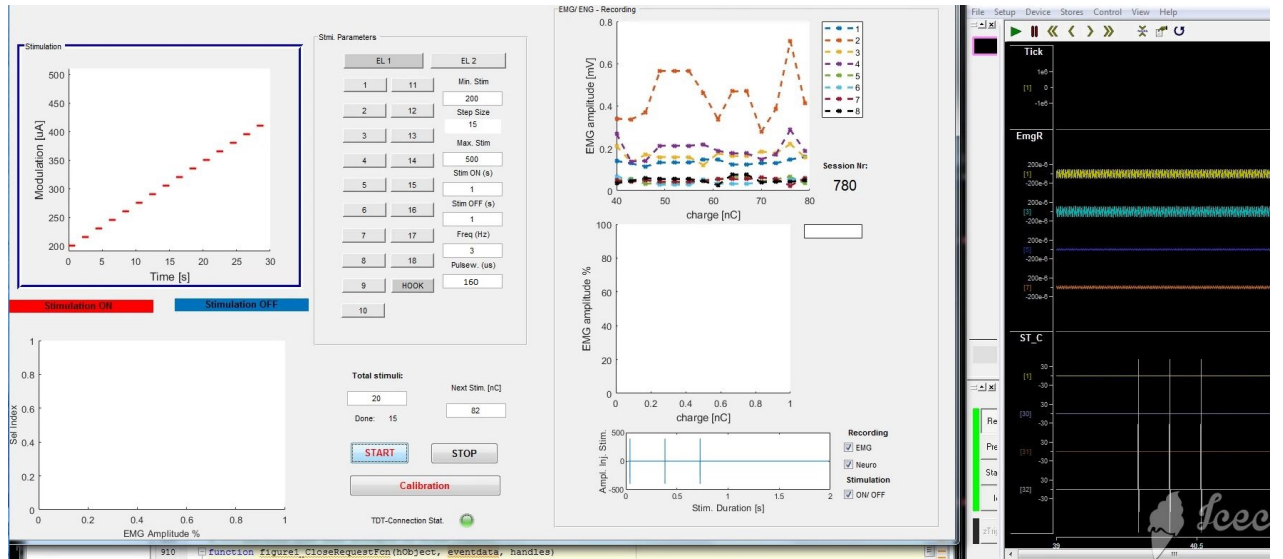


Figure 3.1: Stimulation parameters definition on MATLAB® GUI on the left and visualization of defined waveform on TDT's OpenEX Interface on the right. Biphasic symmetric stimuli at a frequency of 3Hz during 1 second of stimulation are erogated from the 32-th TDT IZ2-MH stimulator.

3.1.2 Verification of results calculation

In order to verify the correct calculation of Recruitment curve and selectivity indexes, test with subjects have been done. In figure 3.3 it is possible to observe the results returned from the system when eight differential emg channels have been acquired from arm muscles. The subject has been required to contract one single muscle (whose activity is registered from channel 1) with gradual growing contraction when stimuli were visible on the interface, while the other muscles have been maintained in a rest state. As aspected, the recruitment curves associated to the muscles not activated remain constant at around 0%, while channel 1 has a gradual monotonical increase. The selectivity indexes are correctly calculated, infact:

- Selectivity index 1 shows that channel 1 follows exactly the percentage activation of the contracting muscle, as the other muscles have approximately a constant null activity. At the end of stimulation, when channel 1 activity is at 80% and the recorded EMG for the other muscles is around 0, Selectivity index 1 has a value of -0,1 for muscles in rest activity. This is coherent with definition of that index given in section5.
- Selectivity index 2 also shows, as expected, a selectivity which is around zero for muscles not activated and a selectivity which rapidly goes to high value for channel 1 as it is the only active muscle. It is not exactly 1 because,



Figure 3.2: Visualization on oscilloscope of voltage waveform obtained over a $10\text{K}\Omega$ resistance associated to defined current pulses. On upper image the current amplitude is $200\mu\text{A}$, on the lower image the current amplitude is $500\mu\text{A}$. Oscilloscope visualization parameters: $100\mu\text{s}$ and 2V

however minimum, a basic activity is registered from other channels.

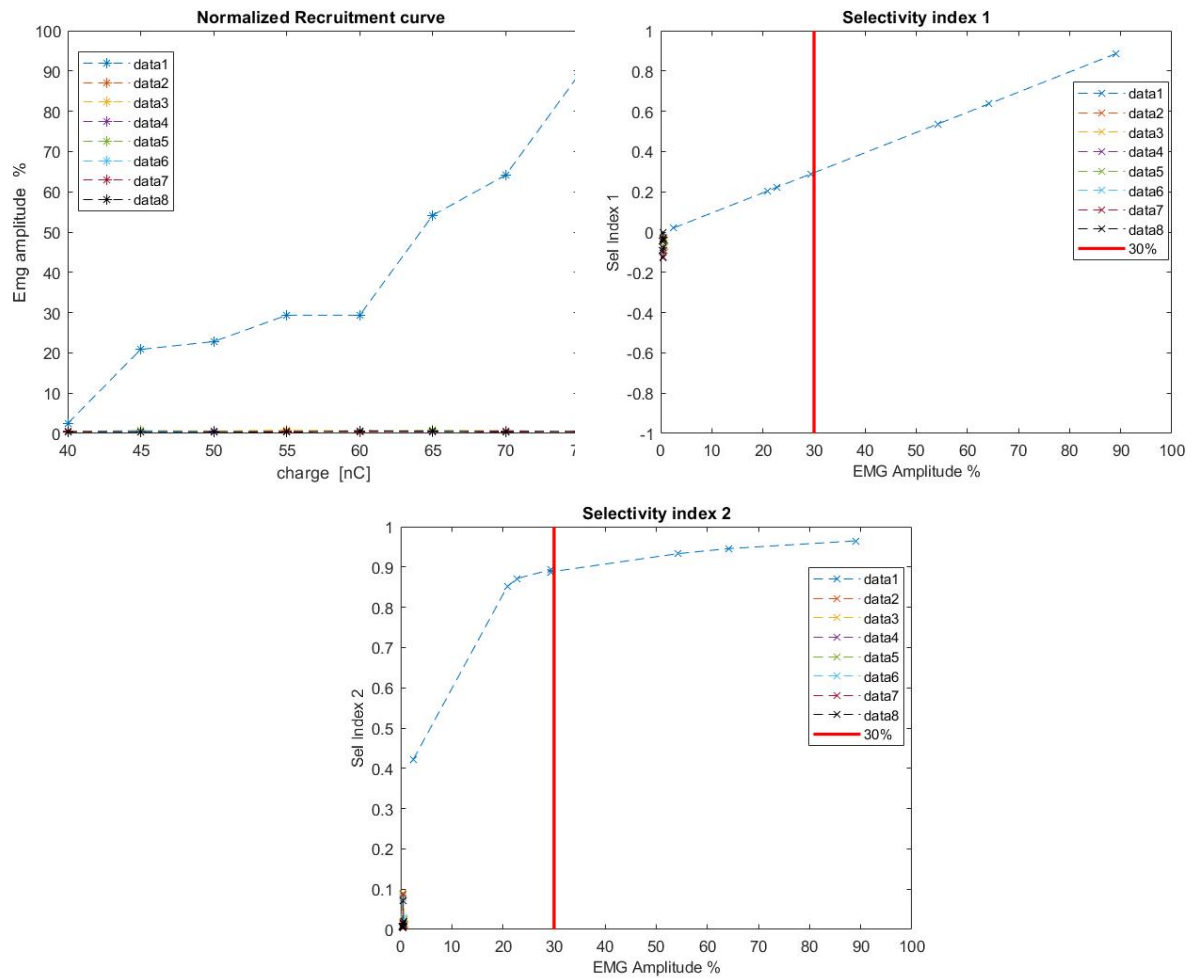


Figure 3.3: Test of output results of the system in a known condition of contraction: one muscle among 8 target muscles has been contracted with a growing force. Normalized recruitment curves and associated selectivity indexes are reported.

3.2 In vivo experiments on animal model

The system has been tested in experimental sessions held on pigs, where different types of electrodes (extraneural cuff and transversal, intrafascicular electrodes) have been utilized to stimulate the sciatic nerve. All experimental procedures have been executed in accordance with the European Parliament and Council Directive 2010/63 / EU. The experimental protocol is described in the following.

A. Animal preparation

Pigs selected for the tests were all around 30 Kg. Animals have been sedated with 2/3 ml of Zoletil, hypno-inducing, and 1 vial of Valium, all intramuscular. After which, 3 to 10 ml of propofol have been added intravenously to promote intubation, depending on the animal. Then artificial ventilation has been performed with isofluran, a general anesthetic of the alotane family, at variable concentrations of 1-2% of the total volume inspired at each act.

B. Identification of target muscles

Before performing the access to the sciatic nerve of the animal, in order to facilitate the identification of the different muscles, their position has been marked on the thigh skin. The selection of target muscles has been conducted on the basis of anatomical consideration about their innervation from the different branches of sciatic nerve.

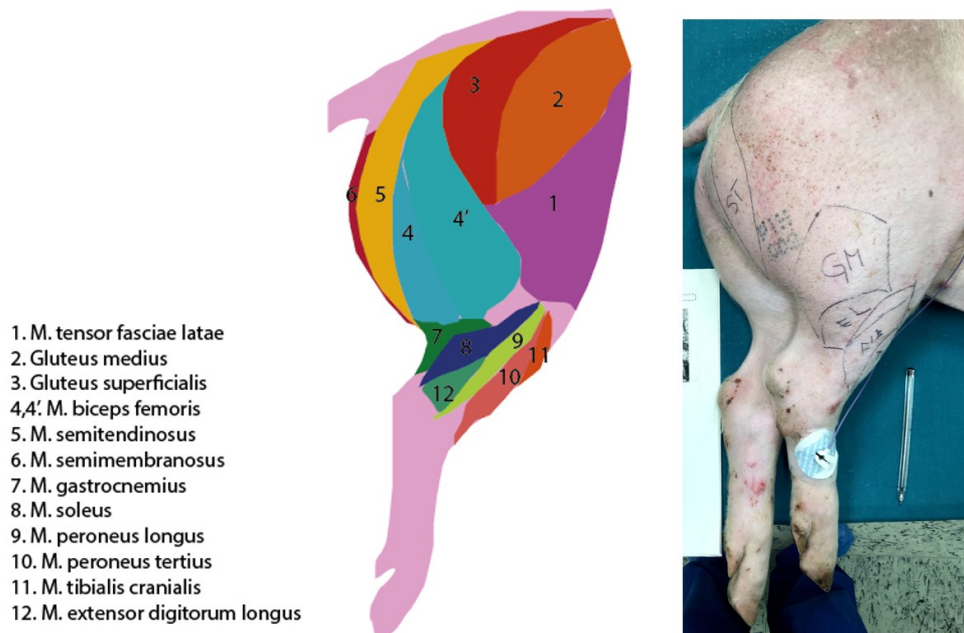


Figure 3.4: Identification of muscles on pig thigh.

C. Access of sciatic nerve

A long skin incision that goes from the zone near the tail towards the knee is made in the lateral part of the lower limb. The biceps femoris and gluteus muscles are separated to access the common trunk of the sciatic nerve. Then the two bellies of the gastrocnemius muscle are divided in order to make accessible the two major branches of the sciatic, the tibial and peroneal nerves. The different nerve branches are separated and kept detached by means of wires.

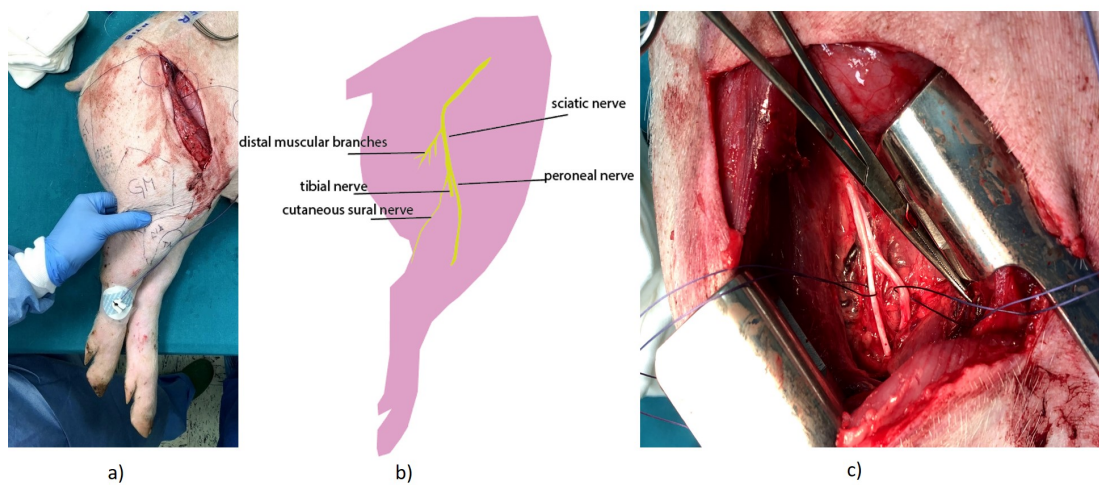


Figure 3.5: Sciatic nerve access. a) Incision praticated during experiments to access sciatic nerve. b) Representation of sciatic nerve structure with the major branches of sciatic nerve (distal muscle branch, tibial nerve, sural nerve, peroneal nerve) c) Division of peroneal and tibial branches of the sciatic nerve during experimental sessions

D. Implant of cuff electrodes

Two cuff electrodes of the type described in section 2.2.2.2 have been implanted. For the implantation each cuff has been opened and positioned around the nerve of interest. Then the two ends of the electrode have been joined together so that the cuff completely encircles the nerve without generating compression. One cuff has been implanted around the tibial nerve (cuff2) and the other one around the peroneal nerve (cuff1).

E. Positioning of emg recording electrodes

For the correct positioning of recording electrodes, one cuff at time has been connected to the stimulator and stimulation has been sent through it. While stimulating it has been observed the point for each muscle subjected to a greater contraction and in correspondence of it a couple of adhesive Ag/AgCl electrodes has been positioned on the muscle to allow a differential recording.

The ground electrode has been taken on the bone in the distal part of the leg.

F. Characterization: obtainment of maximum for all target muscles

The cuff1 electrode (around peroneal nerve) has been connected to the 32-th pin of Samtec connector on the PCB which is associated to the 32-th stimulation channel of the IZ2-MH stimulator. A ramp stimulation protocol has been applied, see section 2.3.2 for more details. The erogated current has been incremented up to have a plateau in the recruitment curves of muscles which are innervated from the peroneal branch. This is useful in order to obtain the maximum level of activation for each muscle to which normalize the subsequent values reached in the experiment day. The stimulation has been repeated three times and the maximum for each muscle has been overwritten if a greater value has been obtained. The cuff 1 has been disconnected and cuff2 has been linked to 32-th channel of the stimulator. The operations have been repeated for cuff2 electrode.

G. Verification of normalization

The ramp stimulation has been repeated with the same stimulation parameters used in the previous phase of characterization for both implanted electrodes, but this time they have been connected to a different channel. Indeed, the code which manages the data elaboration has been implmented in such a way that the maximum for each acquisition channel can be overwritten only when channel 32 is used for stimulation. Using a different channel to erogate stimulation allows to preserve the reference values obtained in the characterization phase.

H. Implant of cuff electrode on the common trunk of sciatic nerve

Ramp stimulation has been done also with cuff electrode around the sciatic nerve. When cuff is positioned in this configuration, it is expected to obtain the activation of muscles both innervated from the peroneal branch and of those ones innervated from the tibial nerve as the cuff is before the biforcation.

I. Implant of tf-life electrodes on the common trunk of sciatic nerve

The cuff electrode implanted in the previous step has been removed and tf-life electrodes of the type described in section 2.2.2.2 have been implanted at the same height of the cuff electrode in the sciatic nerve. In order to achieve a close contact with different nerve fascicles, tf-life electrodes have not been implanted longitudinally to the fiber nerves but in a transversely manner. In addition, different implantation angles have been chosen for each used tf-life electrode with the aim to increase the probability to contact different nerve fibers.

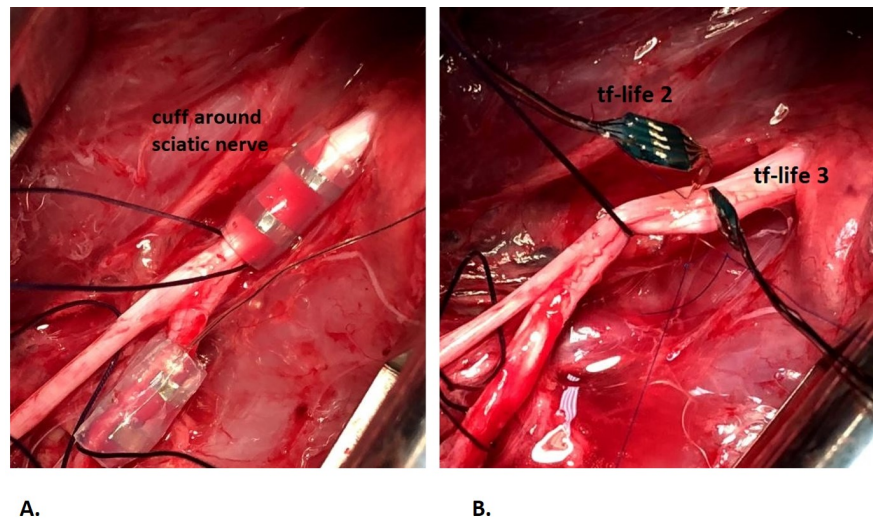


Figure 3.6: Sciatic nerve access. a) Incision b) Representation of sciatic nerve structure c) Division of peroneal and tibial branches of the sciatic nerve

J. Calibration of active sites of tf-life electrode

Each tf-life electrode has six active site from which erogate stimulation. A ramp stimulation protocol has been erogated from each of the electrrode's active sites. This procedure allows a rapid identification of active sites that during the implantation procedure have been effectively positioned within a nerve bundle.

K. Stimulation from tf-life electrode's active sites properly positioned

The stimulation has been reiterated three times for each active site which has produced the activation of one or more muscles in the calibration step. Steps J and K are effectuated for each implanted tf-life electrode.

3.3 Results

In the following sections results obtained during in vivo experiments are reported. The collected data are related to acute experimental sessions carried out in different days. The same cuff electrodes have been reused in the different experiment days. In addition a comparison between results obtained by stimulation of sciatic nerve with cuff and tf-life electrodes in the same experimental conditions has been made.

The stimulation through cuff electrodes implanted around tibial and peroneal nerves has activated clearly the same subset of muscles in the experimental days of which results are reported. The target muscles are: gastrocnemius (GM), soleus (SL), fibularis and tibialis anterior (TA).

For each protocol step, stimulation has been applied three times with the same parameters in order to allow a mean of data.

For an easier comparison, curves retrieved for each performed step in two different days are presented in parallel. The outputs returned from the implemented platform are: recruitment curves, selectivity index 1 (calculated with definition [26]) and selectivity index 2 (calculated with definition [24]). The obtainment of these curves has been described in section 2.3.3.

3.3.1 Stimulation of peroneal nerve with cuff electrode

In Figure 3.7 are reported the normalized recruitment curves obtained for target muscles during the stimulation of peroneal nerve with cuff electrode. Each curve is normalized respect to the maximum value which each muscle has reached in the day. It is possible to observe that, in these stimulation conditions, TA and fibularis muscles are very near to their 100% activation when plateau is reached. This result is coherent as we know that the peroneal branch provides innervation to the dorsal regions of the leg and foot, while gastrocnemius and soleous muscles are innervated by the tibial branch [27].

With regard to the other muscles, their activation is lower than 15% (SL) and 5% (GM).

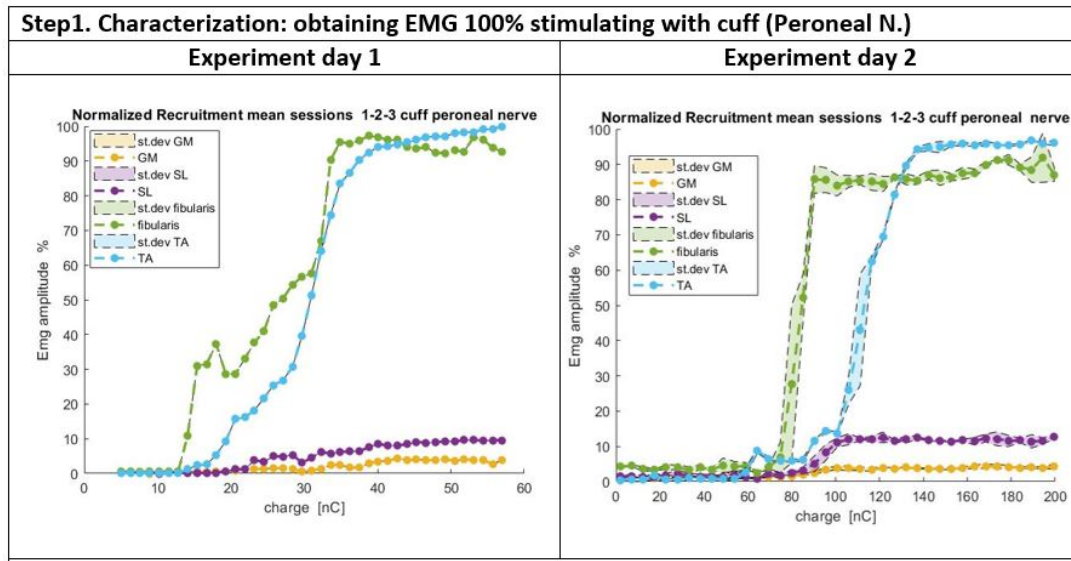


Figure 3.7: **Characterization of cuff electrodes implanted around the peroneal nerve.**

Recruitment curves obtained with a ramp stimulation in two different experiment days. The curves related to four different muscles are showed: gastrocnemius (GM) in yellow, soleus (SL) in purple, fibularis in green and tibialis anterior (TA) in blue. Each curve is represented as mean \pm standard deviation

The correctness of normalization has been evaluated by repeating the stimulation in the same conditions of the previous step. In the figure 3.8 are displayed the recruitment curves obtained in this phase of normalization check and their associated selectivity indexes. Two indexes are reported, indicated as Selectivity Index Raspopovic (Sel Index 1) and Selectivity Index Jensen (Sel Index 2) whose definitions have been given in (5).

By comparing the recruitment curves reported in Figure 3.8 and those ones showed in Figure 3.7 it is possible to observe that all muscles reach the same percentage activation registered in the first phase of calibration, so normalization is executed properly. In both experiment days, Sel Index 1 assumes, as expected, negative values for muscles which are not innervated from the peroneal nerve (gastrocnemius and soleus) and Sel Index 2 is in the low portion of its range for these muscles. This aspect indicates that these muscles are not solleccitated when stimulation is applied to the peroneal branch of the sciatic nerve.

Sel Index 1 never exceeds for each muscle the real percentage of its activation. At the beginning of ramp stimulation, it is zero for each acquired channel as all muscles have approximately a null activity. For fibularis and tibial muscles it follows coherently their respective levels of activation, allowing the identification of the most solleccitated muscle during the stimulation. In experiment day 1, selectivity index 1 assumes near values for the two muscles innervated by the

peroneal nerve. In experiment day 2 it has been possible stimulating with a greater selectivity one of the muscles (fibularis) until both them have reached their maximum activation and again their selectivity indexes are confrontable and near to 0.6.

Sel Index 2 assumes greater values respect to Sel Index 1 at the beginning of stimulation, as it gives greater weight to the number of activated muscles among the total, independently of the real value of activation reached. At the end of stimulation, it has a value of 0.5 as 2/4 muscles are activated with the same percentage.

Step2. Normalization Check. Cuff electrode implanted around Peroneal Nerve

Experiment day 1

Experiment day 2

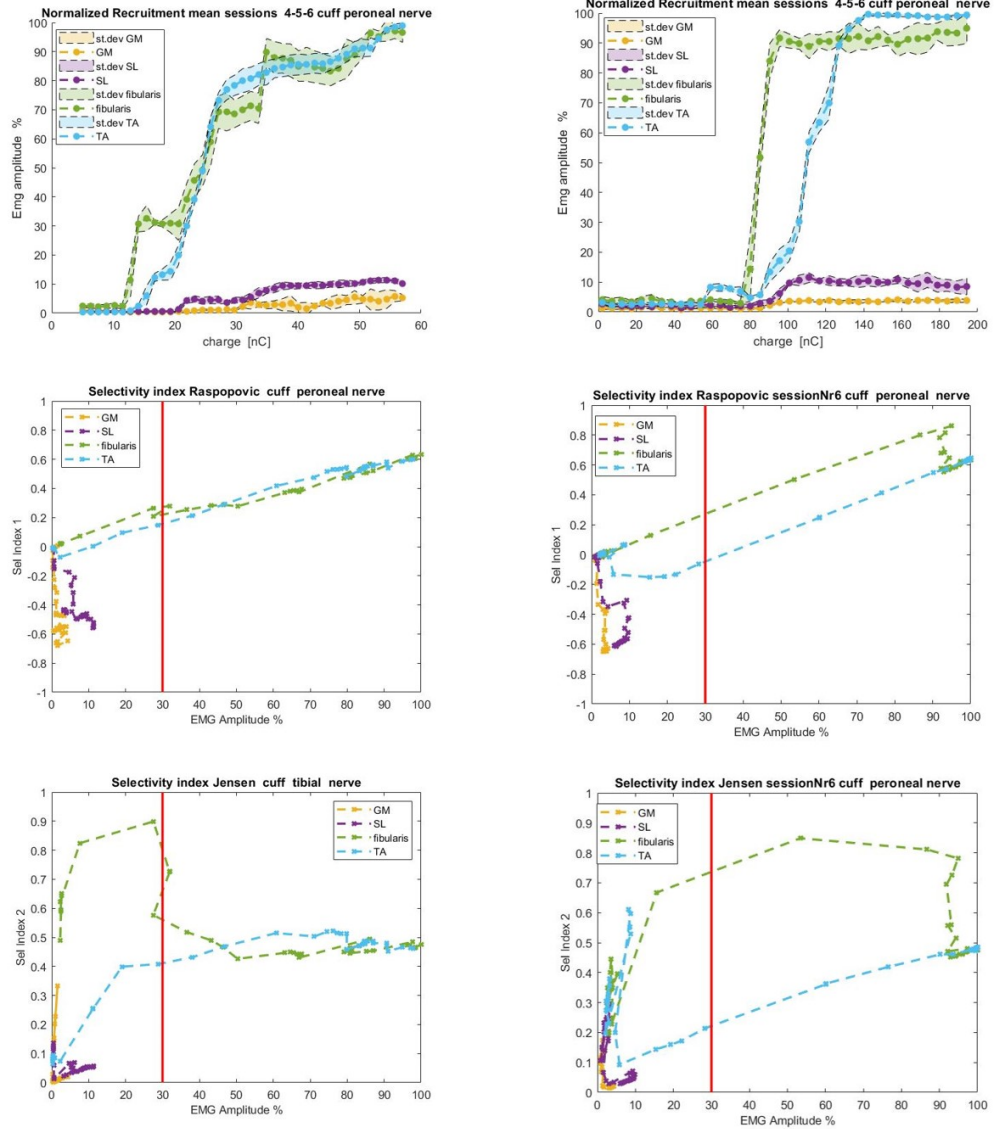


Figure 3.8: **Normalization check, cuff electrode implanted around peroneal nerve.**

Left column: normalized recruitment curve, Selectivity Index Raspovic and Selectivity Index Jensen for experiment day 1. Right column: normalized recruitment curve, Selectivity Index Raspovic and Selectivity Index Jensen for for experiment day 1. The value obtained for four target muscles are reported: gastrocnemius (GM) in yellow, soleus (SL) in purple, fibularis in green and tibialis anterior (TA) in blue.

3.3.2 Stimulation of tibial nerve with cuff electrode

The same steps reported before for the stimulation of peroneal nerve have been repeated by stimulating the tibial nerve with cuff electrode.

In Figure 3.9 are reported the normalized recruitment curves obtained for target muscles during the stimulation of tibial nerve with cuff electrode. Each curve is normalized respect to the maximum value which each muscle has reached in the day. It is possible to observe that, in these stimulation conditions, gastrocnemius (GM) and soleus muscles are very near to their 100% activation when plateau is reached. This result is coherent with the functional innervation [27]. With regard to the other muscles, their activation is lower of 10% during experiment day 1. A higher percentage activity is registered for fibularis and tibialis anterior muscles during experiment day 2, however it is justifiable by lower maximum values of CMAP obtained for these muscles in day 2. The maximum values obtained for each muscle in experiment day 1 are:

- gastrocnemius: 4,233 mV;
- soleus: 14,646 mV;
- fibularis: 17,849 mV;
- tibialis anterior: 49,355 mV;

The maximum values obtained for each muscle in experiment day 2 are:

- gastrocnemius: 24,631 mV;
- soleus: 9,762 mV;
- fibularis: 6,426 mV;
- tibialis anterior: 9,176 mV;

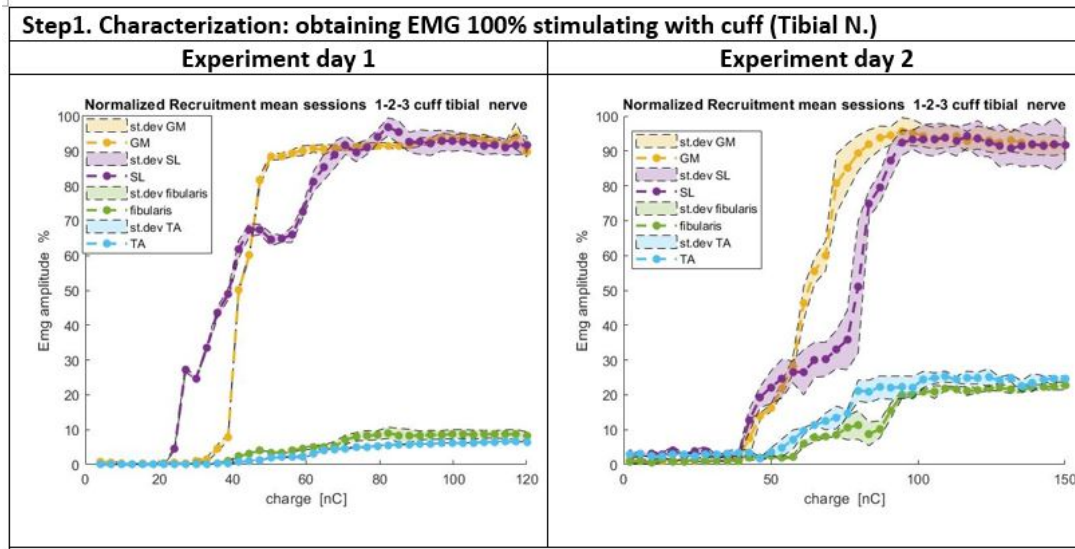


Figure 3.9: **Characterization of cuff electrodes implanted around the tibial nerve.** Recruitment curves obtained with a ramp stimulation in two different experiment days. The curves related to four different muscles are showed: gastrocnemius (GM) in yellow, soleus (SL) in purple, fibularis in green and tibialis anterior (TA). Each curve is represented as mean \pm standard deviation

The correctness of normalization has been evaluated by repeating the stimulation of tibial nerve in the same conditions of the previous step. In the figure 3.10 are displayed the recruitment curves obtained in this phase of normalization check and their associated selectivity indexes. Two indexes are reported, indicated as Selectivity Index Raspopovic (Sel Index 1) and Selectivity Index Jensen (Sel Index 2) whose definitions have been given in (5).

By comparing the recruitment curves reported in Figure 3.10 and those ones showed in Figure 3.9 it is possible to observe that all muscles reach the same percentage activation registered in the first phase of calibration, so normalization is executed properly.

In both experiment days, Sel Index 1 assumes, as expected, negative values for muscles which are not innervated from the tibial nerve (fibularis and tibialis anterior) and Sel Index 2 is in the low portion of its range for these muscles. This aspect indicates that the system gives correct information about the recruited muscles after stimulation is applied to nerves.

Sel Index 1 never exceeds for each muscle the real percentage of its activation. At the beginning of ramp stimulation, it is zero for each acquired channel as all muscles have approximately a null activity. Then, for gastrocnemius and soleus muscles it follows coherently their respective levels of activation, allowing the identification of the most solleccitated muscle during the stimulation.

Sel Index 2 assumes greater values respect to Sel Index 1 at the beginning of stimulation, as it gives greater weight to the number of activated muscles among the total, independently of the real value of activation reached. This is evident particularly in experiment day 1, where selectivity index reaches a value near to 1 for soleus muscle when it is at its 70% of activation, while the other muscles have not yet been contracted. At the end of stimulation, it has a value of 0.5 as 2/4 muscles are activated with the same percentage.

Step2. Normalization Check. Cuff electrode implanted around Tibial Nerve

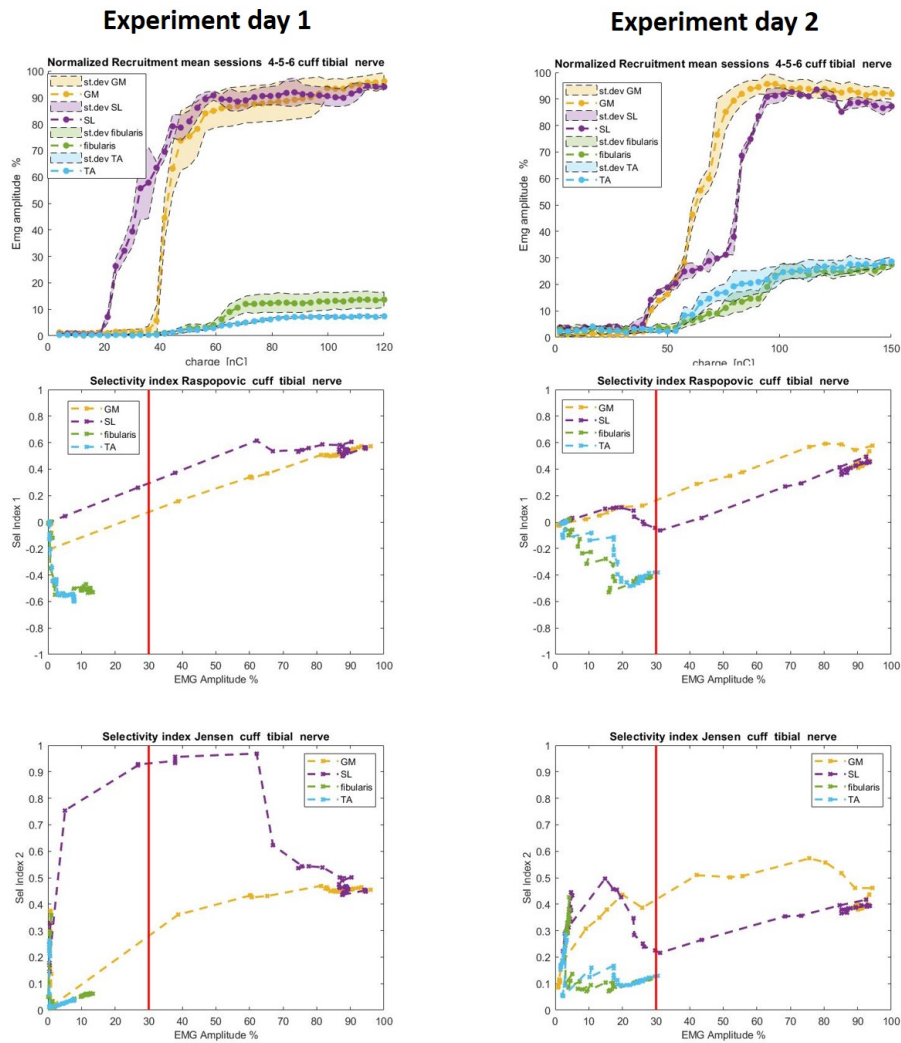


Figure 3.10: **Normalization check, cuff electrode implanted around tibial nerve.** Left column: normalized recruitment curve, Selectivity Index Raspopovic and Selectivity Index Jensen for experiment day 1. Right column: normalized recruitment curve, Selectivity Index Raspopovic and Selectivity Index Jensen for for experiment day 1. The value obtained for four target muscles are reported: gastrocnemius (GM) in yellow, soleus (SL) in purple, fibularis in green and tibialis anterior (TA) in blue.

3.3.3 Comparison between cuff and tf-life electrode while stimulating common trunk of sciatic nerve

With the aim to make a comparison between different types of electrodes, both cuff and thin-film life electrodes (tf-life) have been implanted at approximately the same height of common trunk of sciatic nerve, before the bifurcation in tibial and peroneal nerves, see Figure 3.6. In this way, all target muscles can be equally activated. The values of compound muscle action potential (CMAP) of target muscles have been normalized respect to maximum reached by each muscle while stimulation has been applied to sciatic nerve.

The tf-life electrodes have been implanted transversally to nerve. This implantation has been done in attempt to touch different nervous bundles and to verify the system's capability to discriminate between active sites properly positioned by those ones which are not within the nerve fascicles. Infact, because the used tf-life electrodes had a length greater than sciatic nerve diameter, not all the electrodes' active sites have been resulted within the nerve. So, firstly a fast calibration has been done, that is to say that a ramp stimulation with few steps has been sent through all the six active sites of each tf-life electrode to identify those ones correctly positioned in the nerve. Active sites have been considered properly positioned if an activation of at least 30% of maximum CMAP has been elicited in one muscle. Only for these active sites the stimulation has been repeated to compare results and make a comparison with cuff electrode.

In figure 3.11 the active sites which have respected this condition are resumed for each tf-life electrode.

AS	tf-life 2	tf-life 3
	Refa	1a
	Refb	1b

Figure 3.11: **Indication of active sites of tf-life electrodes which have caused an activation of at least 30% of maximum CMAP in at least one muscle**

In Figure 3.12 results for tf-life2 electrode are showed. It is possible to observe that trend of recruitment curves and values of selectivity are very similar with the two used active sites, Refa and Refb. This is a reasonable result as active sites Refa and Refb are located at the same height on the mirrored halves of the tf-life electrode, so the probability to contact the same sub-population of nervous fibers is high.

The gastrocnemius is the most solleccitated muscle. The percentage values of EMG for the other target muscles don't exceed the value of 30% also when gastrocnemius

is at its 100% of activity. Observating the values of selectivity indexes it is possible to affirm that tf-life2 electrode is able to selectivity stimulate one single muscle, the gastrocnemius, with values up to 0.8 for Sel index 1 and 0.7 for Sel Index 2. The selectivity for this muscle increases with the rise of injected charge because its percentage activation grows up without the occurrence of a significative increase in the activity of the other target muscles. The activation current wich produces a percentage EMG $\geq 30\%$ is $\sim 6,5$ nC.

Analogous considerations can be made for electrode tf-life3 whose results after stimulations are reported in Figure 3.13. The two electrodes have been implanted with at different inclination. In this case a selective stimulation of gastrocnemius muscle has been obtained through the active sites As1a and As1b. Values of recruitment curves and selective indexes are comparable for the two implanted tf-life electrodes.

Figure 3.14 reports the recruitment curves and the relative selectivity indexes when stimulation of sciatic nerve is done by means of a cuff electrode and therefore by an extraneural electrode. It is possible to observe that, all target muscles involved in the analysis have an activation threshold of about 20 nC and they reach their respective 30% of activity in a range of charge comprised between 25-30 nC. So it is not possible the attuation of of a selective recruitment of muscles. Thereby, as Raspopovic Selindex shows, the selectivity for each muscle remains around the zero level both at the beginning of the ramp stimulation, when muscles are inactivated and at the end, when their level of activation is around 90% for fibularis and tibialis anterior muscles and about 70% for gastrocnemius and soleus. The maximum value of Sel Index 1 is 0,3 and it is reached by tibialis anterior and soleus muscles. Also values of Sel index 2 are in the lower range ($<0,5$) indicating that with cuff electrode applied around the sciatic nerve it is not possible to have an interfascicular selectivity and activating separately muscles innervated from the different branches of the sciatic nerve.

tf-LIFE 2 electrode implanted in sciatic nerve

Characterization: tfLIFE 2 – AS Refa

Characterization: tfLIFE 2 – AS Refb

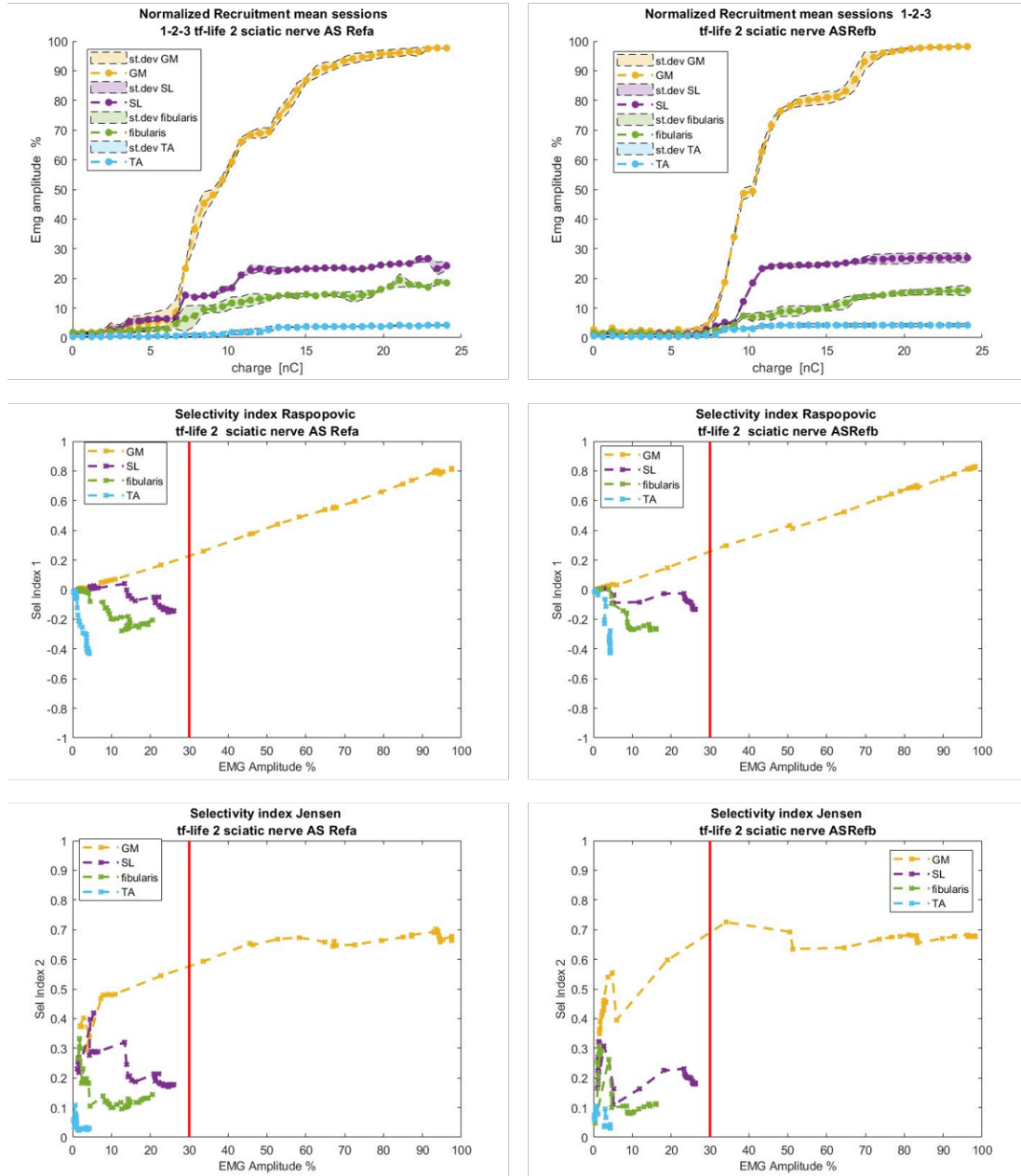


Figure 3.12: Normalized Recruitment curves and Selectivity Indexes for tf-life2 electrode implanted in sciatic nerve. Left column: normalized recruitment curve, Selectivity Index Raspovic and Selectivity Index Jensen for active site As Refa of tf-life2 electrode. Right column: normalized recruitment curve, Selectivity Index Raspovic and Selectivity Index Jensen for active site As Refb of tf-life2 electrode. The value obtained for four target muscles are reported :gastrocnemius (GM) in yellow, soleus (SL) in purple, fibularis in green and tibialis anterior (TA) in blue.

tf-LIFE 3 electrode implanted in sciatic nerve

Characterization: tfLIFE 3 – AS 1a

Characterization: tfLIFE 3 – AS 1b

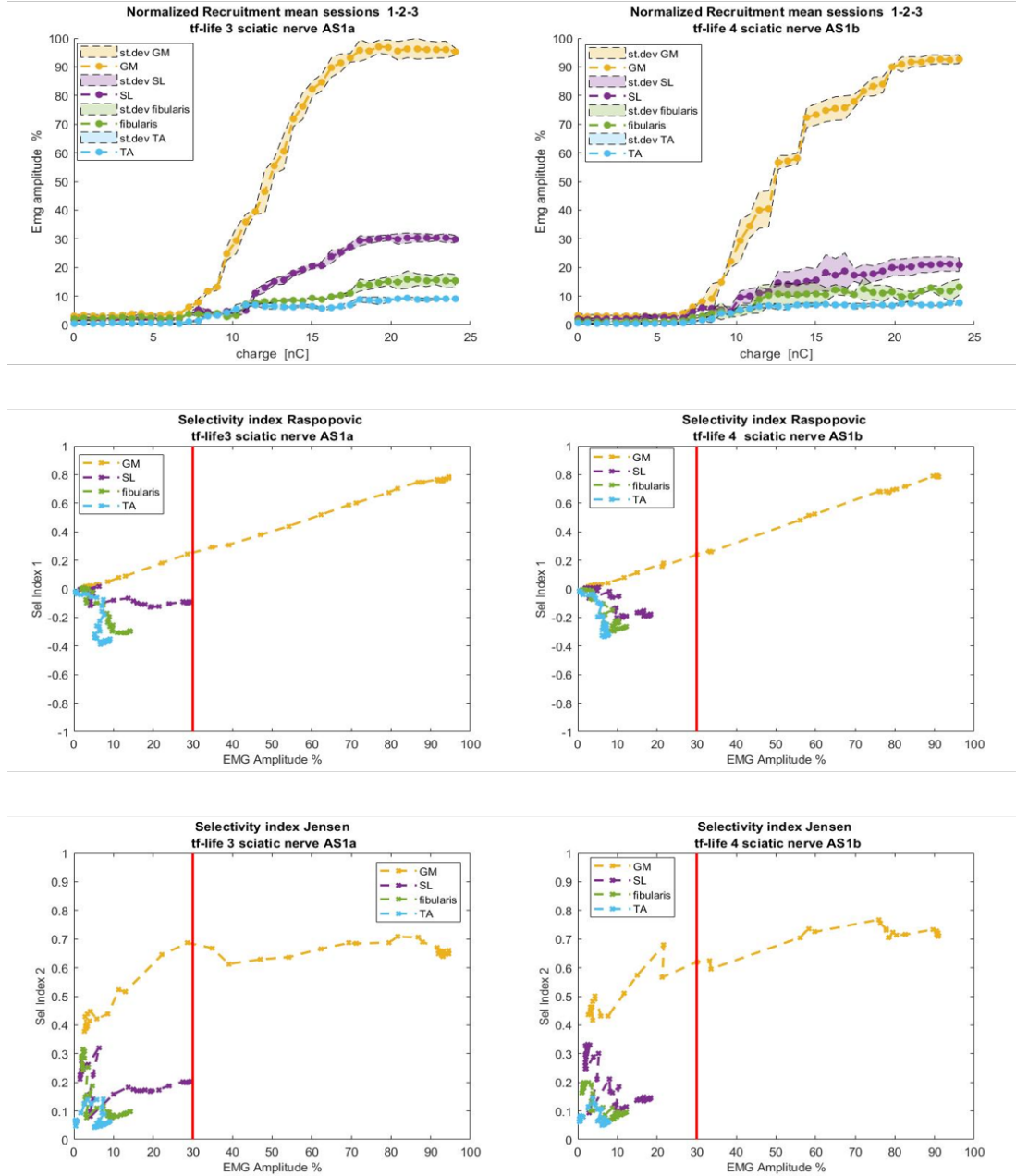


Figure 3.13: **Normalized Recruitment curves and Selectivity Indexes for tf-life3 electrode implanted in sciatic nerve.** Left column: normalized recruitment curve, Selectivity Index Raspopovic and Selectivity Index Jensen for active site As 1a of tf-life3 electrode. Right column: normalized recruitment curve, Selectivity Index Raspopovic and Selectivity Index Jensen for active site As 1b of tf-life3 electrode. The value obtained for four target muscles are reported :gastrocnemius (GM) in yellow, soleus (SL) in purple, fibularis in green and tibialis anterior (TA) in blue.

Cuff electrode implanted around sciatic nerve

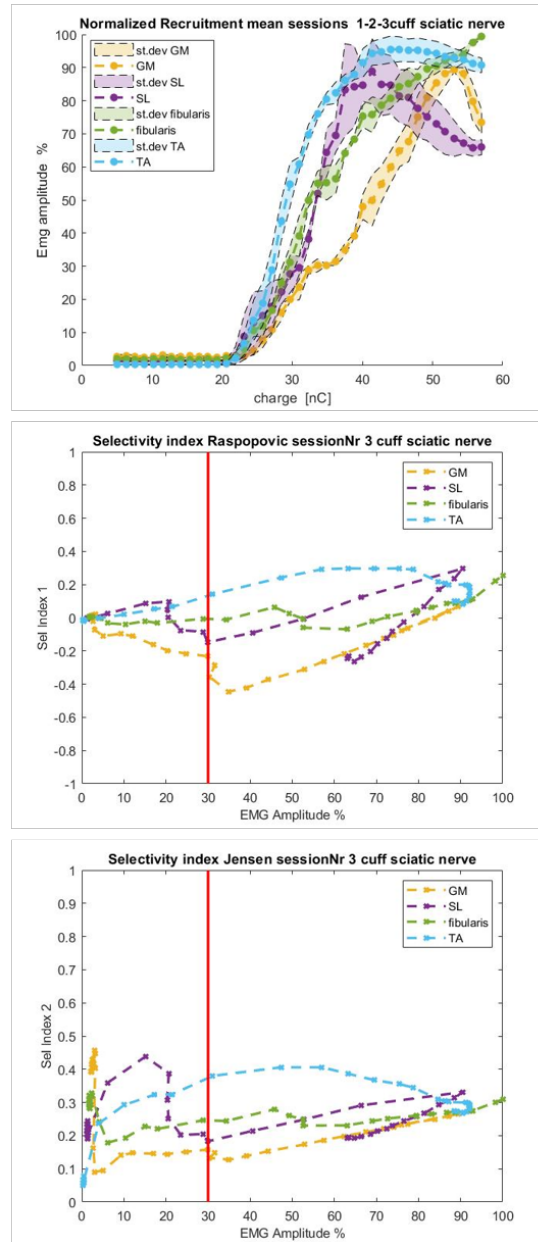


Figure 3.14: **Normalized Recruitment curves and Selectivity Indexes for cuff electrode implanted around sciatic nerve.** From top to bottom: normalized recruitment curve, Selectivity Index Raspopovic and Selectivity Index Jensen. The value obtained for four target muscles are reported :gastrocnemius (GM) in yellow, soleus (SL) in purple, fibularis in green and tibialis anterior (TA) in blue.

Chapter 4

Discussions

The work of this thesis is part of a project whose aim is the realization of a new peripheral nerve interface to be able to provide sensory feedback to amputees using myoelectric prosthesis.

The thesis focused on the development of a system which makes it possible to verify the performance (selectivity) of different types of neural electrodes. To this end, in the realized platform, stimulation, recording and data elaboration functionalities have been implemented. The system communicates with a Graphical User Interface developed in Matlab® through which the user can control the stimulation parameters and visualize results in real-time.

The stimulation and data elaboration functions have been verified in preliminary tests, under known simulated conditions. In particular, the stimulation waveform at the output of the stimulator has been visualized on oscilloscope and it has been verified that it respects the stimulation parameters imposed by the user.

The system has been tested during in vivo experimental sessions held on pigs, where stimulation of sciatic nerve has been effectuated through cuff and tf-life electrodes and EMG signal has been recorded from muscles of the lower limb. The properties of stimulation of the target muscles have been evaluated in terms of Recruitment curves and selectivity, employing two different definition of selectivity index.

Firstly the correctness of data elaboration circuit has been verified. The selected muscle were: gastrocnemius (GM), soleus (SL), tibialis anterior (TA) and fibularis muscles. The first two muscles are innervated from tibial nerve, the two latter ones from the peroneal branch. As reported in section 3.3 the separated stimulation of tibial and peroneal nerves has led to symmetric recruitment situations of the four target muscles involved in the analysis. When stimulation is applied to the tibial nerve, gastrocnemius (GM) and soleus (SL) muscles reach their 100% of activation, while tibialis anterior (TA) and fibularis muscles remain to a percentage value

lower than their respective 25% of maximum activation for the entire duration of the stimulation. By stimulation of the peroneal nerve, the 100% of activity is achieved for TA and fibularis muscles, while GM and soleus don't exceed a percentage value of 15%.

In the second phase of experimental sessions the recruitment properties and the selectivity in stimulation obtained with cuff and tf-life electrodes have been compared. Both types of electrodes have been implanted at approximately the same height of common trunk of sciatic nerve.

Stimulation with the cuff extraneural electrode has led to the almost simultaneous activation of all four target muscles and with comparable percentage levels. This result highlights that, when stimulation is applied by an extraneural electrode, the current rapidly spreads among all nerve fascicles enclosed in the surrounding cuff electrode, so it is not possible to selectively activate muscles which are innervated by different nervous bundles. Consequently, the selectivity indexes obtained in this stimulation conditions are:

- Sel Index 1, whose range is between $[-1 \ 1]$ with 1= maximum selectivity and -1= total absence of selectivity, assumes values around the zero level both at the beginning and at the end of the stimulation. A value of -1 for a muscle would indicate that it is not activated at all, while a zero level implies that all muscle are activated at the same manner.
- Sel index 2, whose range is between $[0 \ 1]$ with 0 = absence of selectivity and 1= maximum selectivity, assumes values $<0,5$.

Results of the stimulation with tf-life electrodes show that it has been possible to selective stimulate one among four target muscles, the gastrocnemius, which has reached its maximum percentage of recruitment and maximum values of selectivity indexes of 0.8 for SelIndex 1 and 0,7 for Sel Index 2. A lower activation ($<30\%$) has been obtained for soleus muscle and even lower activity for muscles innervated by peroneal branch. These results indicate that with an intrafascicular electrode, as tf-life electrode, it is possible to obtain not only a interfascicular selectivity, discriminating by muscles which are innervated from peroneal nerve (TA and fibularis) and tibial nerve (GM and soleus), but also an intrafascicular selectivity, between GM and soleus.

Consideration about the activation threshold can be made. It is possible to note how a low value of charge is necessary to activate the gastrocnemius muscle when a tf-life electrode ($\sim 6 \text{ nC}$) against $\sim 22 \text{ nC}$ required when stimulation is done by cuff electrodes.

Results returned from the system are coherent with studies reported in literature which compare the performances of different electrodes by stimulation of peripheral

nerves [11], [25].

It is possible to state that, the implemented platform allows: the real-time control and variation of protocol stimulation, provides real-time evidence about the level of activation of each of target muscle in respect of the charge injected and about the selectivity of stimulation of intraneural electrodes. It can be used with different types of electrodes with a variable number of active sites and a fast calibration of the positioning of active sites within the nerve is possible with a dedicated button. All functionalities are accessible by means of a GUI realized in Matlab environment.

Chapter 5

Future developments

The implemented system it will be utilized with new typologies of peripheral nerve interfaces allowing a real-time characterization of selectivity performances. The system, infact, has been implemented to be utilized in a project whose aim is the development of a new peripheral nerve interface which should make it possible for lower limb amputees to receive sensory feedback from their prosthetic devices. The intraneural electrode, already developed, will be optimized in terms of mechanical and electrical properties and its performances will be tested in alive pigs. If successful, the electrode will be implanted in cadavers, and later on in humans. In addition, the great advantage of the platform is the possibility to be customizable according to the applications by simply varying the hardware configuration and the signal processing chain. In this work of thesis, the system has been utilized to stimulate through neural electrodes and to record the EMG signals from muscles. However, the system can be used also to record the signal coming from nerve fibers, the electroneurographic signal (ENG). The idea is to add also the ENG recording and to manage all functionalities by a user-friendly and intuitive interface. In this perspective, the system will be configured in such a way that dedicated banks of the PZ5 amplifiers can be allocated for EMG recording and other ones for ENG by maintaining different hardware configuration of the two subgroups of signals. The recording of ENG signal is exploitable not only in neuroprosthetics applications, where the signal can be used to promote a finer control of the prosthesis, but also for bioelectronic medicine purposes. The system will be utilized for the recording of ENG activity from vagus nerve with the aim to understand its answer to different external stimuli, as the somministration of drugs, the heart rate variation and so on.

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