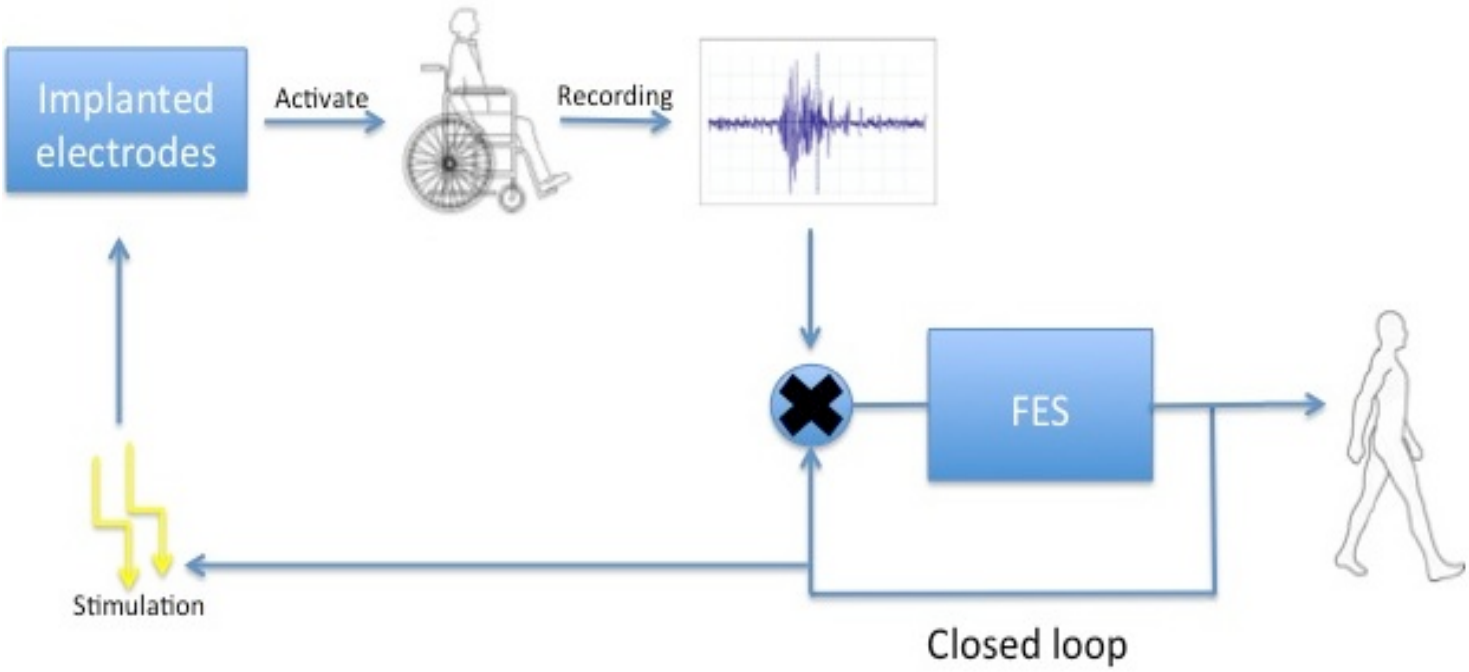
Improvements of Functional Electrical Stimulation Parameters



Master's Thesis 2014

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Abstract:

The objectives of this study was to examine the effect of stimulation pulse shape on the recruitment selectivity of a novel combined interfascicular and cuff electrode (CICE) in an in vitro pig nerve model and secondly to examine if information extracted from intramuscular EMG (iEMG) recordings can provide reliable information on a functional movement and during development of muscle fatigue in a rat model. To investigate this, two experiments with a pig nerve model and a rat model was conducted. The first investigation was performed in vitro on a pig nerve model. The CICE was placed around and in the nerve, to give the most possible selectivity during Functional electrical stimulation. The results showed that a combination of the five pulses increased the selectivity also the CICE showed higher device selectivity compared to the cuff electrode alone. Besides this it was also observed that pulse 4 showed the highest selective activation of the fascicles. For the second part a rat was stimulated with longitudinal intrafascicular electrodes in the hindlimb. The results showed that the ankle angle and the iEMG amplitude decreased when the muscles fatigued also a higher correlation between the ankle angle and iEMG was present when stimulation frequencies above 40 Hz were applied. It also indicated that TA might be a more reliable source of feedback than the GM since the TA had a higher correlation.

PREFACE

The project is completed by group 973 during the master's thesis in the education of Biomedical Engineering and Informatics at Aalborg University with the supervisor Kristian Rauhe Harreby and co-supervisor Winnie Jensen.
The aim for this project was to improve the application of the current functional electrical stimulation technique used for spinal cord injured people. One half of the project was conducted at the Adaptive Neural Systems Laboratory at Florida International University with the supervisors Ranu Jung and Anil Thota. The group participated in the International Conference on Neurorehabilitation 2014 in Aalborg with a paper on the findings from this half of the project.
The other half of the project was conducted at the Center for Sensory-Motor Interaction Department at Aalborg University. This part is documented in a paper aimed for submission and publication in IEEE Transactions on Neural Systems and Rehabilitation Engineering.
The project was written in the period September 2nd 2013 to August 15th 2014. This project is aimed for fellow students and researchers with interest in the area of improvements for functional electrical stimulation.

Acknowledgment
We would like to thank the Adaptive Neural Systems Laboratory at Florida International University for the opportunity to conduct our project at their Laboratory. We want to thank Dr. Ranu Jung and Anil Thota for the supervision and guidance during this half of the project. We also want to thank Anil Thota for conducting the experiments with us and PhD student Ricardo Siu for making the longitudinal intrafascicular electrodes for the experiments.
We would also like to thank the staff at the pathological institute at Aalborg University Hospital for assistance during the explant of pig nerves.

Reading Guidelines
The two articles written for the given project serves as the main documentation along with a common introduction. The rest of the documentation is supplementary chapters, where chapter 2 and 3 relates to the first paper and chapter 4 and 5 relates to the second paper.
Abbreviations will be made for terms the first time it is used and following only the abbreviation will be used. The citations used in the articles are according to the specified standards of the journal, whereas the citations in the project are noted according to the Harvard method where the authors surname and the year of the publication will be noted in square brackets. Is there more than one author, the first authors surname will be mentioned and the rest will be mentioned as et al. followed by the publication year. If the citation is before the dot, the citation belongs only to that sentence. If the citation is after the dot the citation belongs to the last part. The figures and tables found in this project are numbered sequentially according to when they appear in the project. An example could be a figure numbered 2.1, which is the first figure in chapter 2. The description of the figure or table is placed below or together with a reference of its origin. If there are no reference the figure or table belongs to the project group.

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CHAPTER 1

INTRODUCTION

Globally, every year there are more than 16 million new cases were people are bound to wheelchairs due to paralysis in hands, legs or both caused by stroke or neurotrauma to the brain or the spinal cord [Kaye et al., 2002], [ICCP, 2014],[Davis & Norrving, 2013]. These individuals have limited or no capability to perform activities of daily living such as getting out of bed, eating, and transportation. Because of these limitations, they are constantly dependent on family or other caretakers and live with reduced quality of life [Buchanan & Nawoczenski, 1987].
The severity of the paralysis depends on the extent of neural damage (Figure 1.1). Paralysis of limbs in a spinal cord injury (SCI) is due to injured neural connections from the brain to the muscles. The severity of paralysis depends on if it is an incomplete or complete SCI and the spinal level where the injury occurred. In incomplete SCI, some motor and sensory functions are preserved below the level of injury. A complete SCI occurs when the injury is causing all the motor functions, reflexes and sensory functions to be lost in the trunk and the extremities. This can lead to difficulties in walking and control over body functions such as the bladder and bowel control. [Buchanan & Nawoczenski, 1987], [Somers, 2001]

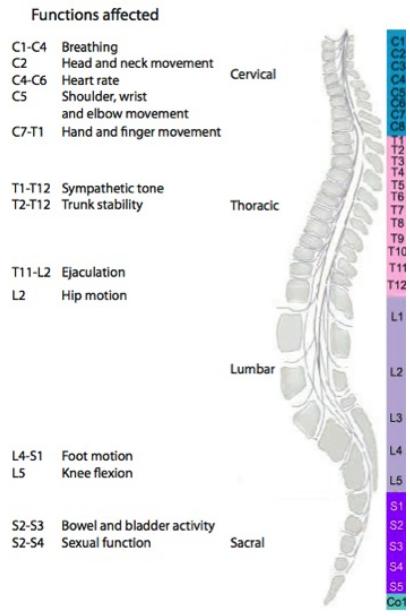

Figure 1.1: *Illustrates the different vertebrates of the spinal cord. Furthermore, the figure shows how the level of injury will affect the different functions. Modified from [Neuroscience, 2014].*

Humans who suffer from severe SCI are not capable of performing their activities of daily living because of the limitations in their functionalities, which takes away their independency. All of the lost functionality that influence the activities of daily living often gives the injured person a diminished quality of life, which can affect the recovery and rehabilitation after a SCI [Dijkers, 2005], [Buchanan & Nawoczenski, 1987]. Trough research it has been shown that improvement can be made by development and use of better rehabilitation methods and technologies [Dijkers, 2005].

In case of partial paralysis due to incomplete SCI, treatment includes physiotherapeutic exercises to increase muscle strength and to improve coordination that helps in restoring the lost motor functions [Buchanan & Nawoczenski, 1987], [Somers, 2001]. However, in a complete SCI, the treatment options are very limited. One promising option is to use functional electrical stimulation (FES) as a replacement to the lost motor commands from the brain to activate the muscles [Peckham, 1987], [Granat et al., 1993]. FES is a technology that potentially can restore the ability to walk by achieving excitation of the nerve fibers that innervates the paralyzed muscle. This is a way of mimicking the activation that would normally come from the central nervous system [Peckham, 1987], [Granat et al., 1993]. The benefit of the FES system is that it can assist in standing and walking and may help increase the level of independency.
Commonly FES system works in an open-loop manner. This system is relatively simple and do not provide any feedback to the system to secure the system output is as desired [Landau et al., 2011], [VMware, 2013]. An idea to solve this problem has been through a closed-loop FES system. This should be able to adjust the output of the system to correct the current movement (Figure 1.2).

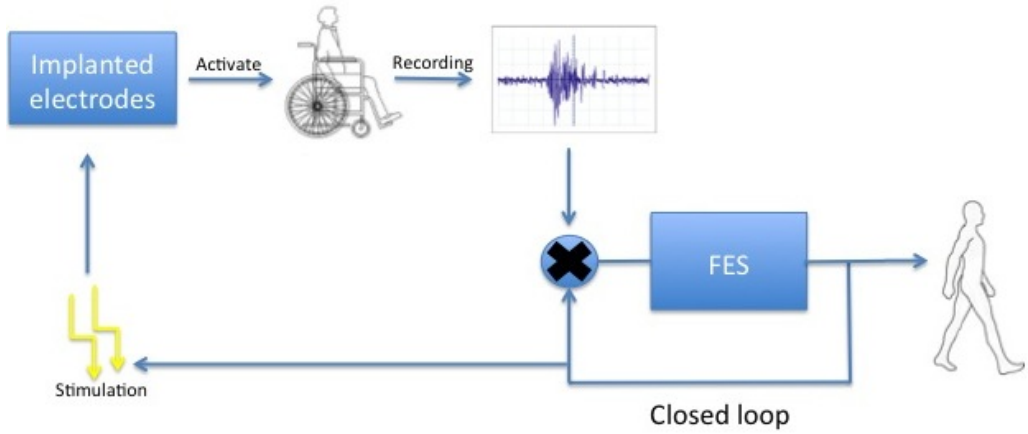

Figure 1.2: *Illustrates the functionality of a closed-loop system, for the restoration of walking with a person that has a spinal cord injury, based on implanted electrodes. Recordings are made from the stimulated muscle to assess if the desired activation is present. If the desired activation is not reached the functional electrical stimulation (FES) system will give an error, which will be the difference between the measured values and the desired output. From this information the feedback controller will adjust the input values, with purpose to get closer to the desired outcome.*

The only current FDA approved system for standing and walking, is a non-implantable open-loop FES system called Parastep [Graupe, 2005]. Since the available FES system is non-invasive, the user has to place surface electrodes every time before use. Wrong placements can cause activation of unnecessary muscles. This will not give the user the full benefit of the system [Day, 2002], [Luca, 2002]. Since the system is not implanted, everything is outside of the body and

noticeable. This can make the user feel stigmatized because of the appearance the system will give the user. [Sundhedstyrelsen, 2008]

The stimulation with the FES system is given trough electrodes designed specifically for this purpose. As mentioned the electrodes used for the current FES system is surface electrodes. These are typically placed on the skin at the belly of the target muscle. These electrodes require high electric charge to activate the muscle. The electric charge may diffuse to adjacent muscles, activating non-targeted muscles and will not be able to activate muscles that are deep inside the limb [Türker, 1993].
When working with electrodes it is important to choose an electrode that stimulates a target muscle without activating non-target areas and has a high selectivity and utilizes minimum amount of charge [Jensen & Harreby, 2013], [Navarro et al., 2005]. This is mainly important because it is preferred not to create fatigue in the muscles, which indicates that the use of FES will be shortened with many pauses. This will decrease the value of the effectiveness that the FES system will have on the rehabilitation. Using intramuscular electrodes in the target muscle or electrodes placed near the neuromuscular junction, the selective stimulation of the target is more likely to be achieved. [Türker, 1993]

The importance regarding how the FES is working is how selective the stimulation is during the use, which could lead to an implantable system for a more permanent use for the activities of daily living. Implanted systems require no daily don and doff of electrodes. But the user would have to undergo surgery to get the system implanted, which is a drawback compared to non-implantable FES systems. [Graupe, 2005]
However, the FES technologies reported in the literature require large amount of electric charge and induce excessive neuromuscular fatigue. This can be used only for short period of times that lasts several minutes and have an increased risk of fall injuries. These factors are a crucial part of the rehabilitation. In addition, most FES technologies implemented do not automatically adjust the electric charge levels that are needed for the function and hence need to fine-tune the electrical parameters very often. Due to these reasons more research in closed-loop FES system is still needed. [Hamid & Hayek, 2008]

In the study by Yoshida and Horch they had a goal of determining if a simple artificial closed-loop control scheme could be used to control biological systems [Yoshida & Horch, 1996]. This was investigated by only using natural afferent activity as feedback to the controller. They looked at the FES system as an implanted system in the nerve fascicles. Here they used intrafascicular electrodes for the stimulation. Another factor that is noticeable in this study was the fact that they used a closed-loop system for the stimulation. This makes the difference from the clinics, which still uses an open-loop stimulation procedure [Fairchild et al., 2010], [Yoshida & Horch, 1996]. From this study they concluded that the FES system may be useful in functional control of the limbs during different positions in the gate cycle, and besides this it might provide an alternative to artificial goniometers. [Yoshida & Horch, 1996]

Objective

The current study is looking at the FES system, to see how improvements can be made and obtain the outcome seen in figure 1.2. Here the main focus is to investigate the highest selectivity in relation to an ideal neural interface, which can achieve the desired goal. Besides this it is also important to have a system that postpones fatigue during stimulation to provide a longer

lasting treatment with the FES system for SCI people. This leads to two specific objectives:

The first objective of this study was to examine the effect of stimulation pulse shape on the recruitment selectivity of a novel combined interfascicular and cuff electrode (CICE) in an in vitro pig nerve model.

The second objective of this study was to examine if information extracted from intramuscular EMG (iEMG) recordings can provide reliable information on a functional movement and during development of muscle fatigue in a rat model.

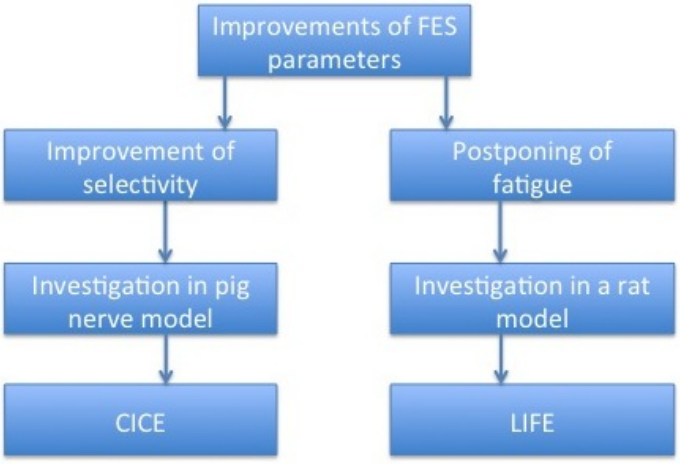

Figure 1.3: *Gives an overview of the structure of the two studies. The general objective of improvements of FES parameters leads down to two specific objectives, with two different approaches. For the first paper it was preferred to work with a pig model, and then use a combined interfascicular and cuff electrode (CICE). This was to reach the highest possible selectivity with the least invasive method. The other paper focused on a rat model. Here longitudinal intrafascicular electrodes (LIFEs) were used for the stimulation and the gastrocnemius medialis (GM) and tibialis anterior (TA) was observed in relation to fatigue.*

The desired goals that have to be performed for the improvements of the FES system are too invasive to conduct on humans at this stage. It was therefore selected to work with animal models. For the given investigation of the two studies, there are different models that are best suited for the particular purpose. The used models were a pig model and a rat model. The pig model has a great resemblance to human structures, which makes it easier to project the results to a human model (Section. 3.1 on page 49). The investigation was performed in vitro on a pig nerve model. For this study a specific designed CICE was used for the experiments (Section. 3.2 on page 50).
For the second part a rat model was easier to work with and was more suited for the given tasks that had to be performed during the stimulation (Section 5.1 on page 85). Since the postponing of fatigue was the goal for this study, it indicates that movement was the main factor observed regarding how the muscles reacted. For the stimulation longitudinal intrafascicular electrodes (LIFEs) were placed in the sciatic nerve in the hindlimb of the rat for the stimulation with FES (Section 5.2 on page 85).

Part I

Paper I - Effect of Pulse Shape on the Recruitment Selectivity of a Combined Interfascicular and Cuff Electrode (CICE) in an *in vitro* Pig Nerve Model

Effect of Pulse Shape on the Recruitment Selectivity of a Combined Interfascicular and Cuff Electrode (CICE) in an *in vitro* Pig Nerve Model

Sahana Ganeswarathas*, Line E. Lykholt*, Winnie Jensen*, and Kristian R. Harreby*

***Abstract*— The development of fully implantable functional electrical stimulation applications is dependent on the availability of a highly selective, minimal invasive and biocompatible neural interface. The objective of this study was to examine the effect of stimulation pulse shape on the recruitment selectivity of a novel combined interfascicular and cuff electrode (CICE) in an *in vitro* pig nerve model.**

The CICE consisted of a multi-contact cuff electrode with 18 contact sites (6 contacts placed in three rings, 60^{o} spacing between contacts) and an interfascicular electrode. The electrode was implanted on 10 median nerves explanted from seven Danish Landrace pigs. Five different pulse shapes and 12 different configurations were applied during stimulation, which was randomized. Also after every five stimulations a supra maximal stimulation was performed.

The results showed that combining all five pulses increased the selectivity. If only one pulse should be used it should be either pulse three or four that had the highest contribution to activating fascicles selectively or the highest contribution to the optimal stimulation.

The results also showed that the CICE increased the selectivity in comparison to the multi-contact cuff electrode configuration alone.

I. INTRODUCTION

Functional electrical stimulation (FES) has proven effective in restoration of motor function and provides coordinated control over muscles [1]. For some applications it may be preferable to use an implantable FES system due to visual appearance and to avoid regularly replacing of surface electrodes, and risk of skin irritation.

The electrode constitutes the interface between the hardware of stimulator and the biological tissue of the body. In some applications, it may be necessary to activate specific nerve fibers to perform a desired task, without activating other fibers in the same nerve simultaneously (typically referred to as selectivity of the electrode [1]). The activation of other nerve fibers could induce an antagonistic muscle contraction, which would hinder the desired movement.

One advantage of implantable nerve based electrodes over surface electrodes are the amount of electrodes. A small amount placed at the same place is enough to potentially allow control of a higher number of muscle groups. Also less activation current is typically needed than for the surface electrodes. A clear drawback of implanted electrodes is the added invasiveness. Earlier studies have shown promising results with selective nerve-based electrodes and been able to overcome the drawbacks of surface electrodes, which mostly helps in case of daily living use [1-3].

Nerve fibers located close to the active sites of the electrode will tend to be recruited at lower stimulation intensities, and as such, unwanted muscle fatigue is likely postponed [1]. With a more selective stimulation the antagonistic muscles will not be activated, which will postpone the occurring fatigue since less muscles are activated and with a lower stimulation intensity. To minimize the needed activation current, various nerve electrode designs have therefore been suggested in the past while also having the invasiveness, long-term stability and biocompatibility in mind.

One example of a nerve-based electrode is the Longitudinal intrafascicular electrode (LIFE) that consists of a thin wire, that is implanted inside the nerve fascicles by penetrating the epineurium. The LIFE has proven to be able to activate fascicles with a high selectivity in comparison to other electrodes. The disadvantage with the LIFE is that it requires implantation of several of these electrodes to interface all fascicles inside a nerve [1].

The cuff electrode takes on different approach to achieve selective stimulation of nerves. The cuff electrode consists of an isolating tube with contact sites mounted on the inside, which encloses the nerve. The simplest cuff electrode has only two contact sites that will allow whole nerve activation [2] and can be used when there is little need for selectivity (e.g. limited adverse effect of activating non-target fibers). A way to enable selectivity during stimulation with a cuff electrode is to use a multi-contact cuff electrode, which gives the opportunity to stimulate with different contact sites and configurations. However, the multi-contact cuff electrode cannot typically reach the same level of selectivity during stimulation as the LIFE [3]. A disadvantage of the cuff electrode is the risk of nerve compression and potentially nerve damage following implant due to swelling of the nerve [4]. However, the cuff electrode has proven to provide a safe and stable design to the nerve and is in use in clinical applications [4], [5].

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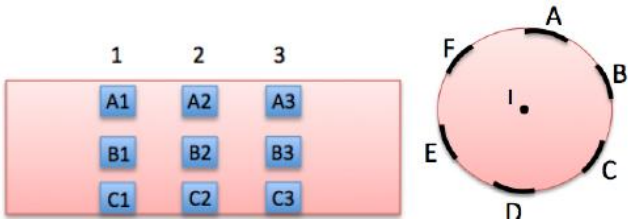

Fig 1: The combined interfascicular and cuff electrode (CICE). The cuff part consists of 18 contact sites placed in three parallel rings (contact size = 1 mm x 1.5 mm, 60^o spacing, distance between contacts = 1.5 mm). The interfascicular electrode was inserted in the middle of the median nerve while the cuff surrounded the nerve.

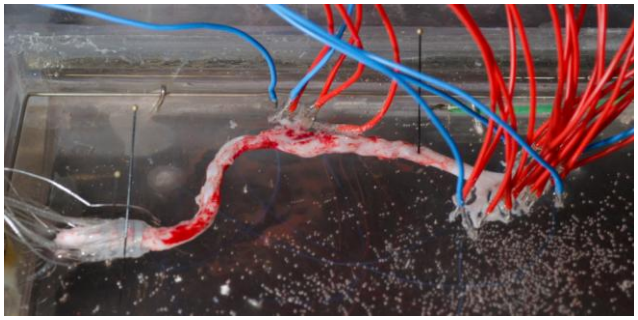

Fig 2: The median nerve with the CICE (left) placed in the proximal end of the nerve and tungsten electrodes placed in each of the individual fascicles at the distal end (middle and right).

Another cuff electrode design is the flat interface nerve electrode (FINE), that forces the nerve into a rectangular shape [6]. This interface has shown to provide a good stimulation selectivity and it is safe to use (i.e. it does not induce nerve damage) [7]. However, the investigation of the FINE has mainly been carried out on nerves with relatively few fascicles [8].

In a study by Riso. et al. the selectivity of an interface that combined a multi-contact cuff electrode and an intrafascicular electrode was tested [9], and compared with results from stimulation with the multi-contact cuff electrode alone. The combined electrode was able to give modest improvement in achieving selective activation in muscles. However the interface was not able to reach absolute selectivity of the monitored muscle, and the stimulation pulse waveform will also affect the selectivity and the safety of the tissue during stimulation [10].

The typically applied pulses are mono- and biphasic rectangular pulses [11]. Biphasic charged balanced pulses prevent corrosion of the electrode and tissue damage [11], [10]. However, the drawback charge balancing pulse may tend to block some of the fibers just activated (if there is no intrapulse delay) thus a higher amount of charge will be needed than when applying a single pulse.

The combined interfascicular and cuff electrode (CICE) design may contribute to a higher selectivity than obtained with the LIFE or multi-contact cuff electrode alone. However, there is limited knowledge in the literature that directly compares the effect of different pulse shapes on the recruitment selectivity. In addition, the majority of previous work has been carried out in nerves or animal models with few fascicles that cannot be directly compared with humans.

The objective of this study was to examine the effect of stimulation pulse shape on the recruitment selectivity of a novel combined interfascicular and cuff electrode (CICE) in an *in vitro* pig nerve model.

II. METHODS

A. *Design of CICE*

The multi-contact cuff electrode part of the CICE consisted of a silicone tube ring (size of 1.5 x 1 mm, inner diameter = 3.4 mm) with 18 platinum contact sites each connected to a teflon coated wire (0.3 mm) (Fig. 1). Each contact was placed in one of three parallel rings (contact size = 1 mm x 1.5 mm, 60^o spacing, distance between contacts = 1.5 mm). The fabrication method of the cuff electrode has previously been described in [2].

The interfascicular part of the CICE was constructed using a thin teflon coated wire (0.3 mm) that was inserted into a curved needle (27 G x 1 1/2) and was secured by clamping the end of the needle. The wire was de-isolated for approximately 2 mm at a distance of 2 cm from the end of the needle to create a contact site.

To marks were made on the wire of the interfascicular electrode to ensure correct placement of the contact side in relation to the cuff electrode contact side.

B. *In-vitro Nerve Preparation*

In total 10 median nerves from seven Danish Landrace pigs (male and female, weight ranging 35-80 kg) were included in the study. The pigs were supplied by a local farmer and euthanized in accordance with the Danish law. Following confirmed death the median nerves were harvested from the left and the right forelimbs with incision placed from the axilla to the middle of the lower front limb of the pig. Immediately after explant the nerves were immersed in a Na-Krebs solution [12]. During transport from the farmer to the laboratory the temperature was kept constant at 12 ^{O}C – 16 ^{O}C. The duration of the transport time was approximately 30 min. The nerves were stored for 1 h before use. From some of the pigs the median nerves from both forelimbs were used. Here the second nerve would be stored for approximately 5 h.

In the laboratory the experiment was performed in a

TABLE I: Stimulation combinations

Configuration	Anode	Cathode
1	A1 and A3	A2
2	B1 and B3	B2
3	C1 and C3	C2
4	D1 and D3	D2
5	E1 and E3	E2
6	F1 and F3	F2
7	A1 and A3	I
8	B1 and B3	I
9	C1 and C3	I
10	D1 and D3	I
11	E1 and E3	I
12	F1 and F3	I

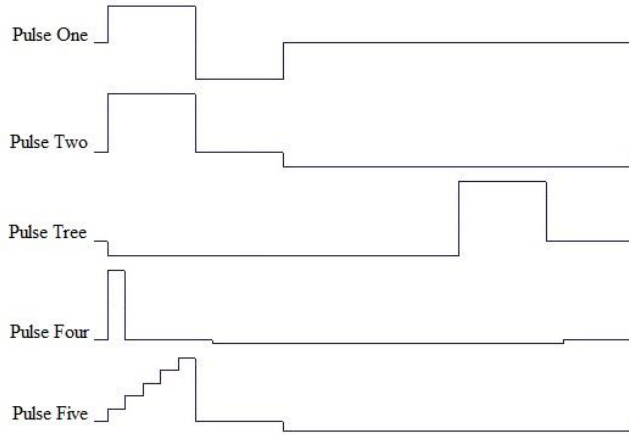

Fig 3: Illustrates the five different pulses used for the stimulation, which are examples. Pulse one is the charge balanced biphasic pulse, pulse two is the charge balanced asymmetrical biphasic, pulse three is the reversed charge balanced biphasic pulse, pulse four is the short charge balanced asymmetrical biphasic pulse, and pulse five is the increasing step function pulse.

custom-made organ bath that was circulated and oxygenated with Carbox (95 % O_2 and 5 % CO_2) at room temperature (21 ^{O}C).

The epineurium was opened at the distal end (i.e. corresponding to the pig anatomy) where individual fascicles were isolated (up to 31 fascicles). The cuff electrode part of the CICE was placed at the proximal end of the nerve, i.e. the interfascicular part of the CICE was implanted first and then the multi-contact cuff electrode was placed to enclose the nerve.

C. Recording and Stimulation

To record the evoked responses a custom-made tungsten rod electrode was placed in each fascicle (Fig. 2) (a 12 mm long tungsten rod attached to a 130 mm copper wire, covered by Plexiglas. This was removed for 4 mm at the tip of the tungsten rod). The tungsten rod electrodes also served to secure the position of each fascicle to avoid micro-movement of both the fascicle and the electrodes. One tungsten rod electrode was placed in the bath outside the neural tissue to be used as a reference for recording (Fig. 2). Each tungsten electrode was connected to an amplifier (band-pass filter 80-5000 Hz, gain ranging between 500-5000, CyberAmp 380, Axon Instruments Inc. Union City, USA) and sampled (sampling frequency = 20 kHz, PCI-NI-6221, National Instruments, Austin, USA).

The stimulation was performed trough the CICE with a stimulator (STG4008, Multichannel systems, Reutlingen, Germany). The stimulation configuration was controlled by a custom-made manually controlled switchbox.

For the stimulation 12 different configurations were used (Table 1). In the case of longitudinal stimulation the contact sites from ring one and three were used as anode and the contact site from ring two as the cathode. In the case of transverse stimulation the contact sites from ring one and three worked as anodes and the interfascicular electrode as cathode.

To investigate the effect of the stimulation pulse shape on the selectivity, five different stimulation pulses were selected: Charge balanced biphasic, charge balanced asymmetrical biphasic, reversed charge balanced biphasic, narrow charge balanced asymmetric biphasic with intrapulse delay, and increasing step pulse was used.

The *charge balanced biphasic pulse* has the same magnitude of the cathodic and anodic pulse, whereas the *charge balanced asymmetrical biphasic* pulse has a four times longer anodic pulse and the intensity is accordingly four times less. This pulse also has an intrapulse delay to avoid the blocking of nerve fibers. Another type of biphasic pulse used is the *reversed charge balanced biphasic pulse*, which uses a hyperpolarizing pulse before the cathodic pulse to facilitate activation and activate nerve fibers at a greater distance. A fourth type of biphasic pulse is the *short charge balanced asymmetrical biphasic pulse*, used to investigate the possibility of activating nerve fibers at different distances from the contact site and obtaining a higher selectivity by adjusting the pulse width. A fifth type of waveform is the *increasing step function* (Fig. 3), which was designed for this specific experiment by inspiration from [13]. It was tended to investigate if it may achieve a higher selectivity by activating structures deeper in the tissue than what can be activated with rectangular pulses.

For all types of pulse shapes (Fig. 3), the frequency of the stimulation was 5 Hz with a charge balance except for pulse five that had half the charge as the rest. The range of amplitude for pulse one, two, three, and five was from 0-4000 µA (for nerve 1-6) and from 0-2000 µA (for nerve 7-10) and was for pulse four from 0-20.000 µA (for nerve 1-6) and from 0-10.000 µA (for nerve 7-10). A ratio of 1:4 between the cathode and anode were used for the time and amplitude. The stimulation pulses were designed with different parameters (Table 2 and 3). There were 20 repetitions for nerve 1-6 and 10 repetitions for nerve 7-10.

To avoid experimental bias, the delivery of the 12 different stimulation configurations and five pulses were

TABLE II: Stimulation parameters

	Pulse One	Pulse Two	Pulse Three	Pulse Four	Pulse Five
Primary pulse width	100 µs	100 µs	100 µs	20 µs	100 µs
Secondary pulse width	100 µs	400 µs	400 µs	400 µs	400 µs
Primary pulse	Cathode	Cathode	Anode	Cathode	Cathode
Intrapulse delay	None	100 µs	None	100 µs	100 µs

TABLE III: Stimulation combinations

Parameter	Selected Value Nerve 1-6	Selected Value Nerve 7-10
Frequency	5 Hz	5 Hz
Amplitude range	0-4000 µA	0-2000 µA
Amplitude step size	250 µA	50 µA
Repetitions	20	10
Electrode configuration	Varies*	Varies*
Charge balanced	Yes	Yes

* See table I

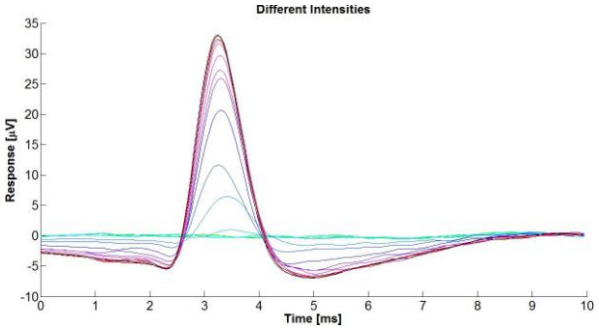

Fig 4: Illustrates response of the nerve from the different intensities used. The more red the line is, the higher the intensity was used. It is seen that the response maintain the same shape for all intensities.

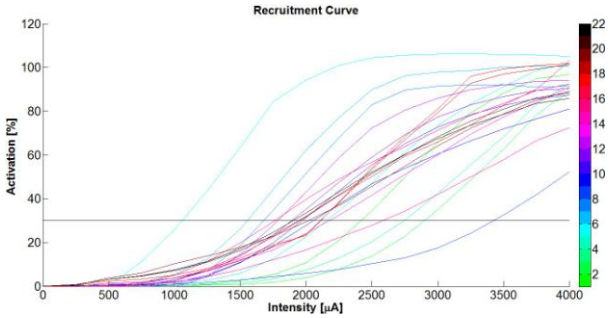

Fig 5: Illustrates the recruitment curves for nerve 1, with the configuration 11 and pulse 5. It is seen how much the individual fascicles have been activated according to the prior supra maximal stimulation. The black line at 30 % indicates when the activation threshold is reached.

randomized during the experiment.

In addition to the combinations described above, supra maximal stimulation (pulse two was used) was applied to enable estimation of the 100 % activation level for each fascicle. In this case, all the contact sites of ring one and three were used as cathodes and all the contacts in ring two as anodes. This was performed at the beginning and end of each experiment and after every fifth stimulation pulse.

D. Offline Data Analysis

First the gain was corrected followed by removal of the stimulation artifacts, which was implemented by forcing the points during the stimulation (0.5 ms (10 samples) before until 1 ms (20 samples) after the stimulation pulse) to assume the same activity (noise) level as prior to the stimulation (corresponding to the average of the last 0.5 ms (10 samples)).

Then the evoked response was filtered (2nd order Butterworth band-pass filter with cut-off frequencies of 80-1500 Hz) and averaged across the stimulation intensities.

Recruitment curves were calculated for the 12 configurations and five pulses to be able to investigate when 30 % activation of the fascicle was reached (Fig. 4). For the calculation of the recruitment curves the RMS method was used for a period of 10 ms, were the data was normalized according to the prior supra maximal stimulation. For this calculation there was a smoothing (MatLab® function *smooth*, 5-point moving average) and an interpolation (MatLab® function *interp1*) of the signals. This was done to improve the slope of the curve along with obtaining a better resolution (one sample pr. 1 µA).

To quantify and compare the recruitment performance a selectivity index (SI) was defined, inspired from [14]:

$$SI_F = t_j - \frac{\sum_{i=1, j\neq i}^{N} t_i}{N-1} \quad (1)$$

For eq.1 the target activity is given where the mean activation of all the non-targets is subtracted. The results range between 0 and 100, where a SI score of 100 correspond to the most selective (i.e. one fascicle is recruited 100 %) and a score of 0 is not selective at all.

An activation threshold was set to 30 % for the activation of the individual fascicle during the stimulation. The threshold was set to 30 % since this is sufficient for work task performance [15]. For the calculation of the activation threshold there was a calculation of the activation for each data point (1 µA) according to the prior maximal stimulation. This was calculated as a percentage. The SI was calculated for each 1 µA where the highest SI was used for the further analysis.

A calculation of how many fascicles that were selectively activated for each of the five pulses were made. A selective activation was set to when the SI for a fascicle was higher than $5 \cdot (1/N)$. This was calculated to investigate how selectively the five different pulses could activate the different fascicles.

To give an overview of the optimal stimulation that should be used for each of the individual fascicles for each nerve an optimal stimulation table was constructed. This gives information on the specific configuration, pulse and intensity that should be used for the individual nerve. Furthermore it was investigated how many times each pulse was used for the optimal stimulation for each nerve. This was plotted, to give a better overview of the used optimal stimulation pulses.

Also the mean charge for each of the pulses to reach 30 % activation according to the prior maximal stimulation was calculated. This was calculated to investigate how the different pulses used different charge for the activation and to be able to compare if this was as expected from the theory.

To assess if the added interfascicular electrode increased the device selectivity, the device SI (DSI) was calculated for both the CICE (configuration 1-12) and for the multi-contact cuff electrode part (configuration 1-6) of the electrode alone. For the calculation of the DSI the best SI across the five pulses were used. As such, the results from eq. 1 were used to calculate the device selectivity (eq. 2). This equation takes the average of the maximum SI for each channel derived from eq.1.

$$DSI = \frac{\sum_{i=1}^{N} \max(SI_F)}{N} \quad (2)$$

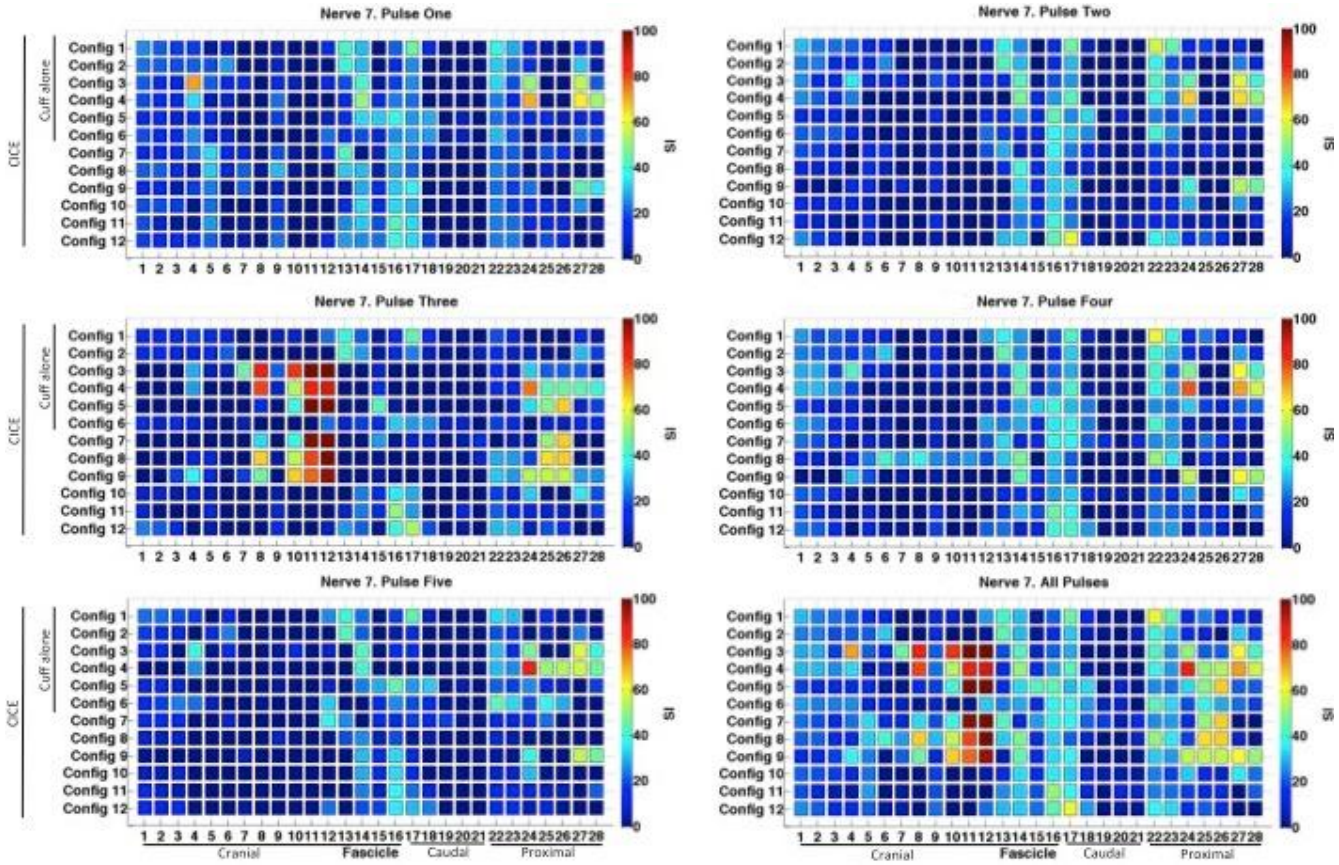

Fig 6: Illustrates a color plot of SI for nerve 7, with all five pulses used during stimulation, also a combination of all the five pulses is illustrated in the last bar plot. On the x-axis the fascicles is shown, with corresponding branches. On the y-axis the 12 configurations were listed, the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. Red color indicates high selectivity, while blue color indicates lower selectivity.

III. RESULTS

During stimulation with increasing intensities an increase in the response was observed. Some of the responses were monophasic whereas others where biphasic, but they maintained the same shape during the increase of the intensity (Fig. 4).

The recruitments curves show the activation of the individual fascicles compared to the prior supra maximal stimulation. The shape of the curves was sigmoidal. It is seen for the recruitment curves that there was a great difference in them, for the different nerves, configurations, and pulses. It was seen that in some nerves that one fascicle was increasing in activation prior to the other fascicles (Fig. 5), whereas in other nerves all fascicles had the same tendency and no one stood out.

The tendency of the SI level for all the 10 nerves (20-28 fascicles) was that pulse three seemed to have the highest SI in the part of the proximal branch of the nerve compared to the other pulses. This can also be seen in the color plot for nerve 7, where the difference from pulse three and the other pulses was clearly observed (Fig. 6). There was a difference in the number of fascicles and arranging of these. There was seen a clear relation between the selectivity and anatomy. The area with the highest SI was seen in the proximal branch for all nerves except for nerve 10. In some nerves a high SI was also

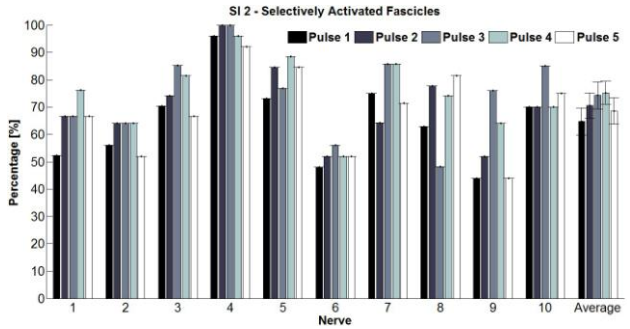

Fig 7: Illustrates the selectively activated fascicles for all 10 nerves for SI. It is seen that there is a relatively high selectively activation as the percentage-wise activation is between 55 % and 100 %.

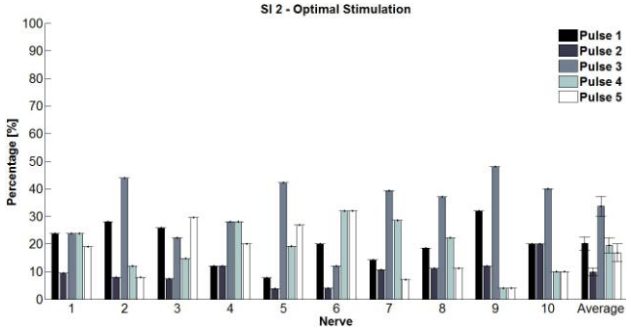

*Fig **8**: Illustrates how many times percentage-wise each pulse was used to obtain the optimal stimulation of each fascicle for each nerve. It is seen that pulse three is the pulse most frequently used.*

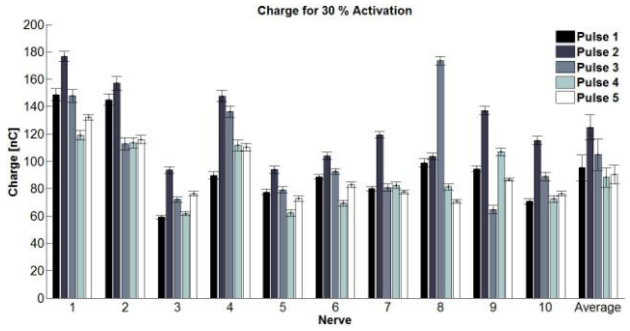

*Fig **9**: Illustrates the charge induced for activation over 30 % during stimulation in the 10 nerves, with each five pulses and for an average of all the five pulses.*

seen in some fascicles in the distal branches. For example a higher selectivity in nerve 7 was observed for four fascicles in the distal end. It was here seen that configuration 3-5 and 7-9 reached the fascicles during stimulation more than the other configurations. Furthermore it was observed that pulse one, two, four, and five had a high degree of resemblance in the activation patterns throughout the nerves. The last plot is a combination of all the five pulses. Here it was observed that combining all the different pulses the SI would increase, which was seen throughout all the 10 nerves. When observing the results when stimulating with (configuration 7-12) and without (configuration 1-6) the interfascicular part of the CICE it was seen that the fascicles activated during stimulation are different throughout the 10 nerves.

For the investigation of how many fascicles that was selectively activated by the five pulses it was seen that there was an activation of 55-100 % for all 10 nerves (Fig. 7). The average for all 10 nerves activation of fascicles was between 60 % and 80 %, which shows that there was a high selective activation of fascicles with all five pulses.

To give a more specific overview of the most optimal stimulation configuration, pulses and intensities during stimulation with the CICE a table was constructed (Table IV). It is observed from the table that the most used pulse is pulse three followed by pulse one and four respectively. This optimal stimulation tables can be used as a reference for which parameters to be used for an optimal stimulation of a fascicle (e.g. muscle in the clinics) (Fig. 8). Pulse three (45 %) was the pulse mostly used for obtaining the optimal stimulation for all fascicles. Pulse one (20 %) and three (20 %) was used approximately the same times, followed by pulse five (18 %). Pulse two (10 %) was used the least amount of times for the optimal stimulation.

The DSI compares the CICE and the multi-contact cuff electrode alone to give a more clear knowledge on the effect of the CICE (table V). It is here observed that the average DSI for the CICE was 56.8 and for the multi-contact cuff electrode the average DSI was 53.5. It is also noticeable that the DSI's found for all the 10 nerves was higher for the CICE compared with the multi-contact cuff electrode configurations alone.

TABLE IV: Optimal stimulation

Fascicle	Selectivity Index	Configu-ration	Pulse	Intensity [µA]
1	30.5	4	4	10000
2	30.4	4	4	10000
3	25.3	4	4	10000
4	70.7	3	1	601
5	33.0	7	1	1101
6	42.0	8	4	9255
7	45.6	3	3	1751
8	85.8	3	3	651
9	30.5	8	1	1151
10	81.4	3	3	2000
11	104	5	3	1301
12	155	3	3	951
13	44.7	2	4	3204
14	51.0	4	1	601
15	42.4	5	3	1351
16	50.5	11	3	2000
17	59.9	12	2	2000
18	33.8	5	2	951
19	18.3	7	5	2000
20	10.7	11	3	2000
21	14.4	7	3	1001
22	59.6	1	4	10000
23	44.3	1	2	2000
24	86.3	4	5	1001
25	64.6	8	3	1101
26	67.2	7	3	1001
27	70.4	4	4	2255
28	57.8	4	4	2005

IV. DISCUSSION

In the current study the effect of five stimulation pulse shapes on the recruitment selectivity of a CICE was investigated and compared to the recruitment selectivity of the multi-contact cuff electrode configurations alone.

A. Effect of Pulse-type on Stimulation Selectivity

With the use of five different pulses there was not observed a pulse that reached a higher selectivity compared to the others. On the other hand it was observed that a combination of the five pulses gave a higher selectivity (Fig. 6), and thereby the FES can be improved with an implementation of these. From this an increased activation of fascicles could also be reached. All five pulses selectively activated a relatively high amount of fascicles in average for all 10 nerves (60-80 %). This indicates that all five pulses can be used individually, although the stimulation would selectively activate more fascicles with all five of them. Some of the pulse can be redundant in the results, whereas only a couple of pulses can be chosen for the stimulation. If only two or three pulses were to be chosen it should be pulse three, four and one, respectively, since these contributed the most to the optimal stimulation (Table IV and Fig. 7) and has the highest SI's (Fig. 8).

The charge used for the individual pulses to activate the fascicles to 30 % was not as expected. It was expected that pulse one had to have a higher charge delivery than pulse two, since pulse one has no intrapulse delay and is thereby theoretically blocking the fibers [16]. This was not the case for the results of this study (Fig. 9), where pulse one is using a lower charge delivery than pulse two. It was also

expected that pulse three would need a lower charge delivery than both pulse one and two, since it has a hyperpolarizing pulse, which should make it easier to depolarize the nerve for an action potential to be generated [10]. This was also not the case since it only uses a lower charge than pulse two and higher than pulse one (Fig. 9). It was expected that pulse four would be the pulse using the lowest charge delivery, because it has a five times higher intensity than the other pulses. This was the case for the current study, where it used a lower charge than the four other pulses (Fig. 9). There was no specific expectation of the charge needed for pulse five, since this is new and inspired from believed [13].

For some of the optimal stimulations (Table IV) it was observed that the SI was higher than 100 %. This was due to the fact that the supra maximal stimulation did not reach maximum. In the current study pulse two was used for the supra maximal stimulation, which can be why some of the SI's is above 100 because these pulses are using a lower charge to obtain the same output as pulse two. Also the root mean square feature is not an absolute measure of the number of fibers activated.

B. Effect of Device on Stimulation Selectivity

The comparison of the CICE and multi-contact cuff electrode configurations alone is an important factor to establish the effect of the CICE. It was clearly observed that a higher overall selectivity was reached with the use of the CICE throughout the experiments of the 10 nerves (Table. V). This was also observed by comparing the device performances during stimulation on the individual nerves alone and during stimulation on average for all the 10 nerves (Table. V). The DSI will always be higher for the CICE than for the cuff, since the cuff configurations were a subset of the CICE configurations.

C. Performance of CICE in Relation to other Nerve Based Electrodes

The design of the CICE in the present work is based on previous work with a similar multi-contact cuff electrode with 18 contact sites [9]. The middle electrode used in the current paper was an interfascicular electrode where Riso et al. used an intrafascicular electrode. There is no evidence that verifies the electrode was placed as an intrafascicular electrode, but it is expect that this was the case. This variation in the design could contribute to a difference in the activation of fascicles. With the use of an intrafascicular electrode the electrode would be placed inside the fascicle where this fascicle will always be activated, along with the nearby fascicles. With the use of an interfascicular electrode it will be avoided to activate one specific fascicle each time but only the surrounding fascicles. In the previous work from Riso et al. it was suggested that higher selectivity could be reached if there was a higher density of the tripole channels in the cuff electrode. From the current study it was clearly observed that increasing the number of contact sides would not help increasing the selectivity. It was seen that contacts near each other tended to activate the same fascicles. On the other hand increasing the amount on interfascicular electrodes might increase the selectivity, since this will decrease the distance between the fascicles and the contact sites of the CICE. The study by Rio et al. showed that there was a difference in the activation pattern of the muscles depending on whether only there was a use of the multi-contact cuff electrode alone or of the intrafascicular electrode in combination with the multi-contact cuff electrode. The difference seen from the work by Riso et al. and the current work is the choice of stimulation parameters and number of stimulation pulses used during the stimulation, which might contribute to an even higher selectivity to the activation of muscles. In the current work it was interesting to observe how great an impact each stimulation pulse had on the selectivity. Even though there are many similarities between the study from Riso et el. and the current study it can be difficult to compare these due to the choice of different models and success criteria. The study from Riso et el. used and in vivo model and recorded EMG signals whereas an in vitro model and ENG recordings were used for the current study. The study from Riso et al. used muscle activation as a success criterion whereas the current study uses a SI as a success criterion. To get a better understanding of the used parameters in the current study, it should be tested in a chronic in vivo model.

D. Methodological Considerations

Two different SI's was investigated to see which one was appropriate for the specific experiment [11]. From this eq.1 was applied for the current results. This specific SI was chosen because it has the advantages that it only encounter the mean noise into consideration whereas the other SI takes the cumulative noise into consideration. A drawback for this SI can be that several fascicles can be activated at a time but can still reach a good SI. A clinical relevance might be that it is desirable to only activate one muscle at a time, but several fascicles can innervate the same muscle at once. It can also sometimes be of clinical relevance to innervate several muscles at a time, where activation of several fascicles is needed. For this clinical relevance the optimal stimulation table can be used, to most selectively activate a specific fascicle with a given configuration, pulse and intensity (table IV).

The time used to prepare the nerve before the stimulation did not seem to have any influence on the experiment. It was observed if the nerve could survive to the next day for stimulation, to investigate the viability of the nerve. Here it was seen, that after it had been kept at a constant temperature, the nerve was still viable. Another influence on the viability is the pH value of the Na-Krebs solution,

TABLE V: Device and Cuff Selectivity

Nerve	CICE Selectivity	Cuff Selectivity
1	53.0	50.3
2	40.2	36.8
3	40.0	39.0
4	63.0	57.6
5	42.2	39.2
6	32.8	31.1
7	54.0	51.0
8	99.8	92.2
9	43.3	39.9
10	100	98.0

which was kept at a pH of 7.4. It was observed that when the pH dropped or increased from a value of 7.4 the nerve response decreased.

The structure of the nerve had an influence on the separation of the fascicles. When a cable like structure was present it was relatively easy to identify and separate the individual fascicles. Whereas when a plexiform structure was seen it could be difficult to identify the individual fascicles due to the fascicles branched and joined multiple places. This could make the process of separating the fascicles difficult, which could cause damage to the individual fascicles. The plexiform structure of the nerve could also have an influence on the obtained selectivity. This is due to if only one fascicle is stimulated perfectly and is branching out into two fascicles, before the point where the tungsten rod electrode measures, the two fascicles will be activated, and thereby give a lower SI.

When the tungsten electrode was placed in a fascicle the recording electrode performance was observed on the screen. It was clearly observed that when the electrode was placed more distal on the fascicle a weaker response appeared, than when moving the electrode more proximal of the separated part of the fascicles. The fact that the CICE was placed in the proximal end might have an influence on the response that was observed.

The placement of the interfascicular electrode might have had an influence on the selectivity. It was believed that the most optimal placement, for the most selective outcome, the interfascicular electrode had to be placed in the middle of the nerve. With an interfascicular electrode placed in the middle of the nerve, it ensures that the activated fascicles will be equal no matter what configuration there is used, when stimulating with the CICE. During the insertion of the interfascicular electrode it is difficult to make sure that the electrode is placed in the middle of the nerve due to a very little visual guidance.

With the CICE the possibilities of having only one electrode implanted to control several muscles could be a future perspective with this electrode. With this possibility there would be a reduced need for surgery compared to using intrafascicular electrodes.

V. CONCLUSION

In the present study the effect of pulse shapes on the stimulation selectivity of a CICE was investigated. It was observed that a combination of the five different stimulation pulses increased the selectivity compared to using only one pulse (Fig. 6). Although there was not observed a pulse that had a higher selective activation of fascicles it was observed that pulse four showed a slightly higher selective activation of the fascicles.

A higher DSI was observed for the CICE than the multi-contact cuff electrode configurations alone, which showed that the combination of an interfascicular and a multi-contact cuff electrode improves the selectivity (Table IV).

Future research is needed to evaluate if the effect of the CICE, stimulation configurations and stimulation pulses increases the selectivity during the use of FES in a clinical application.

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Part II

Paper II - Information on Ankle Angle from Intramuscular EMG Signals during development of Muscle Fatigue in an Open-Loop Functional Electrical Stimulation system in Rats

Information on Ankle Angle from Intramuscular EMG Signals during Development of Muscle Fatigue in an Open-Loop Functional Electrical Stimulation System in Rats

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Abstract. Functional Electrical Stimulation (FES) is one method available for rehabilitation of spinal cord injured subjects. Although FES is used in the clinic today, reliable and robust feedback for a closed-loop system is limited.

The objective was to examine if intramuscular electromyographic (iEMG) recordings (of tibialis anterior and gastrocnemius medialis) can provide reliable information of functional movement (i.e. ankle angle) during development of fatigue.

Four longitudinal intrafascicular electrodes (LIFEs) were implanted in two fascicles of the sciatic nerve in three adult Sprague-Dawley rats. Open-loop FES was applied to produce rhythmic ankle movement. The FES stimulation pulse widths and amplitudes were determined for the individual rats based on the strength duration curve. Each frequency (30, 40, 50, 60 and 70 Hz) was applied to perform 100 step cycles followed by a 15 min rest period. Kinematic information on the ankle angle and iEMG were recorded simultaneously.

The results showed that the ankle angle and the iEMG amplitude decreased when the muscles fatigued. A correlation between the ankle angle and iEMG was present, which indicates that iEMG information can be used as feedback for a closed-loop system. The correlation was higher at higher stimulation frequencies (>0.76 at stimulation frequencies above 40 Hz).

1 Introduction

Injury to the spinal cord may cause permanent loss of voluntary motor function and sensation below the level of the lesion. Functional electrical stimulation (FES)

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is a technique that has been used for many years for the rehabilitation of subjects with spinal cord injury. [1], [2], [3]

The aim of the FES is to electrically activate the paralyzed muscles in a controlled way to restore motor function. FES can be applied to the subject using an open-loop (feedforward) or a closed-loop (feedback) control strategy. [1], [2], [3]

FES is commonly applied in the clinic in an open-loop mode that operates with fixed stimulation parameters. A clear advantage is that the paradigm is simple and easy to use. However, prolonged stimulation leads to muscle fatigue. An appropriate closed-loop stimulation strategy could alleviate this problem. However, closed-loop FES systems are dependent on feedback from the part of the body that is controlled, and the availability of sensors and signals to provide a reliable feedback signal from the controlled limb or organ is therefore essential. [1], [2], [3]

One source of feedback can be achieved by recording kinematic data that provides information on the joint angles. Kinematics would reveal when fatigue occurs. [1], [4] However, the recording of kinematic information requires specialized equipment and is a highly time consuming procedure, which is not suitable for daily use in the clinic or at home. Therefore it will be important to have access to another source of information. Information on movement and muscle fatigue can also be obtained through electromyography (EMG) recordings. The use of EMG recordings would be relatively easy to implement in the clinic or in a portable system since it is cheap, quick to setup and is used routinely today. Surface EMG has the advantage of being easy to record but suffers from cross talk and the daily need to don and doff the electrodes. An alternative signal source is the use of intramuscular EMG (iEMG), which is a more invasive technique. This may help overcome some of the drawback associated with surface EMG. Previous studies show that it is possible to extract information from the iEMG related to the force during movement [5].

There is today limited knowledge on whether information on joint angles may be extracted from iEMG during normal movement and how muscle fatigue may influence this.

The objective of this study was therefore to examine if information extracted from iEMG recordings can provide reliable information on a functional movement and during development of muscle fatigue in a rat model.

To investigate the objective, FES using longitudinal intrafascicular electrodes (LIFEs) was used to produce a cyclic movement of the hindlimb of the rat while recording iEMG. The FES was applied in an open-loop mode to induce muscle fatigue over time. Kinematic data was also recorded as a reliable measure of the movement.

2 Methods

Data was obtained from three adult healthy male Sprague-Dawley rats (294-615 g). The experimental procedures were approved by Florida International University Institutional Animal Care and Use Committee.

2.1 Animal Preparation

After induction of anesthesia with isoflurane gas (5 %), a single injection of Sodium Pentobarbital (40 mg/kg ip) was given. Anesthesia was maintained with isoflurane (0.5-2.0 %), throughout the experiment. The level of anesthesia was assessed with toe pinch, and observation of eye blink and the respiration rate. To prevent dehydration regular subcutaneous injections of isotonic saline in the dorsal cavity were administered.

The left sciatic nerve was exposed and four single channel LIFEs were inserted into the fascicles innervating the Tibialis Anterior (TA) and Gastrocnemius Medialis (GM) muscles. The TA and GM are the main muscles involved in the movement of the ankle and can be activated from one nerve. To verify that the electrodes were placed correctly inside the fascicles, electrical stimulation was applied while observing TA and GM muscle twitch. The LIFEs were sutured to the epineurium and the incision was closed.

Table 1 Applied LIFE stimulation parameters

	Amplitude (μA)	Pulse width (μs)
Rat 1: GM	50	30
Rat 1: TA	30	30
Rat 2: GM	50	60
Rat 2: TA	250	100
Rat 3: GM	30	40
Rat 3: TA	20	30

2.2 Experimental Setup and Data Acquisition

The rat was placed in a prone position on an elevated platform so that the hindlimbs were hanging freely.

3-D kinematic data was recorded by a Peak Motus System (Peak Performance Technologies, Inc. Centennial, CO) by placing cone shaped three-dimensional reflective markers on the hip, knee, ankle and toe. The system included two infrared cameras focused on the rat at an oblique angle of approximately 45° each. The kinematic data was sampled at 60 Hz.

To record differential iEMG, two stainless steel fine wire electrodes were inserted with an average interelectrode distance of 3.5 mm in the TA and GM muscles. The reference electrode was placed under the skin at the back of the rat. The iEMG data was amplified (A-M systems Model 1700, gain = 100) filtered (band pass: filter 100 Hz -10 kHz, notch filter at 60 Hz), sampled (10 kHz, NI USB-6259, National Instruments, USA) and saved in a PC using a custom LabView routine.

To determine the stimulation pulse width and amplitude a strength duration curve was first established by consecutively stimulating the four implanted LIFEs with different pulse widths (30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 and 300 μs)

while increasing the stimulation pulse amplitudes until a muscle twitch was seen. A pulse width and 1.5 times the amplitude at rheobase were selected for stimulating the fascicles (see Table 1).

To determine the stimulation frequencies the muscle contraction was visually observed. The stimulation frequency was chosen such that it provided a fused contraction, which was later confirmed from kinematic data.

The open-loop stimulation was applied to the fascicles innervating TA and GM muscles alternately to produce a rhythmic movement. One hundred step cycles were performed at each stimulation frequency (30, 40, 50, 60 and 70 Hz) followed by a 15 min period of rest. Kinematic data from ankle movement and corresponding iEMG were recorded simultaneously.

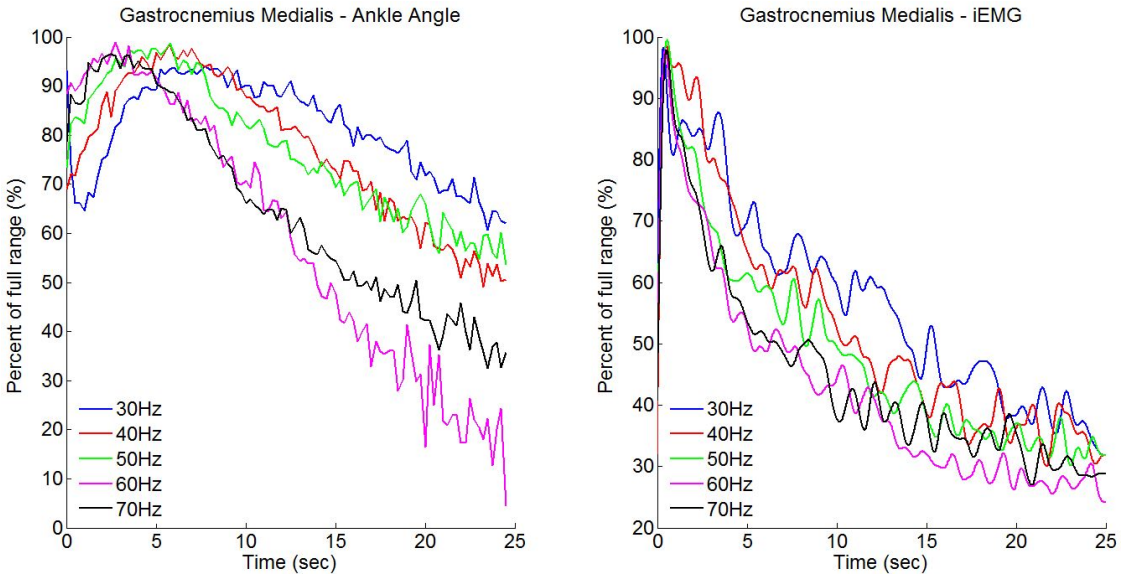

Fig. 1 Shows the range of movement of the ankle angle and iEMG amplitude envelope over time for the GM muscle. The maximal range of ankle angle and iEMG amplitude envelope was normalized to 100 %. A gradual decrease in the ankle angle and iEMG was observed for the GM over time.

2.3 Data Analysis

The kinematic markers were identified using the Peak Motus software. The data were digitized and filtered with a fourth-order band-pass Butterworth filter [6]. To find the range of movement for both the GM and TA individually the maximum range of the movement (maximal extension to maximal flexion) was calculated for each cycle.

To obtain the iEMG amplitude envelope to investigate when the amplitude decreased, the iEMG amplitude was full wave rectified and low-pass filtered (3rd order low-pass Butterworth filter with 0.5 Hz cut off frequency). The maximal range of ankle angle and the iEMG amplitude envelope were normalized to 100 % and an average of the normalized data obtained for the three rats. These averaged data were used for the rest of the data analysis. Fatigue was defined as a decrease

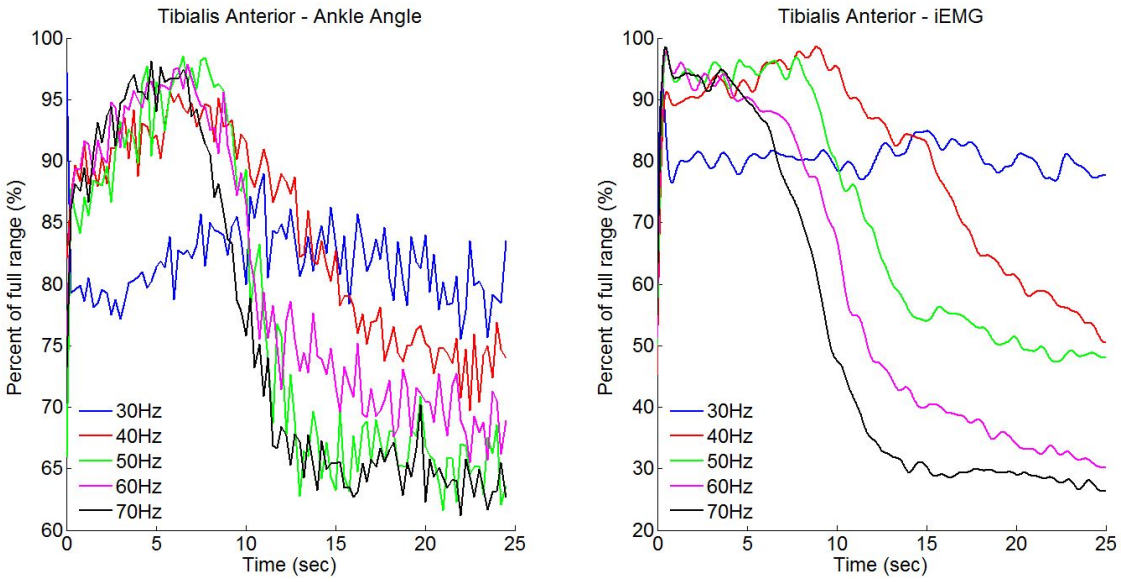

Fig. 2 Shows the range of movement of the ankle angle and the iEMG amplitude envelope over time for the TA. The maximum range for ankle angle and the iEMG amplitude envelope were normalized to 100 %. A correlation between the two signals can be observed. The decrease in the iEMG and ankle angle can be observed for the TA after 10-15 s indicating presence of fatigue.

in the ankle angle and the iEMG amplitude envelope. A correlation coefficient was calculated between the iEMG and ankle angle for both muscles for each frequency.

3 Results

The movement produced by the GM muscle for all the stimulation frequencies was in the range of 5-100 % of the maximum ankle angle, see Fig 1. The ankle angle increased during the first step cycles (approximately 10 s). After this it decreased during the rest of the step cycles. The response after 60 Hz decreased more rapidly than the other frequencies. The iEMG amplitude decreased continuously and rapidly for approximately the first 25 s (40-60 %). After this the rate of the decrease was less for the rest of stimulation (30-40 %).

Table 2 Correlations coefficients between ankle angle and iEMG for GM and TA for the different LIFE stimulations

	GM	TA
30 Hz	0.36	0.22
40 Hz	0.63	0.95
50 Hz	0.77	0.96
60 Hz	0.85	0.96
70 Hz	0.85	0.96

The movement produced by the TA muscle for all the stimulation frequencies was in the range of 65-100 % of the maximum ankle angle, see Fig 2. The ankle angle increased for the first 10 s. After this there was a decrease from 10-25 s thereafter a plateau was reached for the rest of the step cycles (65-85 %). The iEMG amplitude was found to be stable for the first 10 s. After this the 50, 60 and 70 Hz response rapidly decreased from 10-30 s (30-60 %), while the 30 Hz response produced no changes for the rest of the step cycles (80 %). The 40 Hz response also had a different tendency where it decreased less compared to 50, 60 and 70 Hz response (85 %). After this a plateau was reached for the 50, 60 and 70 Hz response (30-50 %). Here the 40 Hz response still decreased and did not reach a plateau (60 %). The ankle angle and the iEMG amplitude demonstrated similar response.

To quantify the degree of correlation between the ankle angle and the iEMG, the correlation coefficient between the ankle angle and the iEMG data were calculated (see Table 2). In the case of the GM muscle, it was observed that the higher the stimulation frequency that was applied, the higher the correlation observed (mean and standard deviation of 0.76 +/- 0.1 for 40 HZ – 70 Hz frequencies). In the case of the TA muscle the same tendency was observed, i.e. coefficients for the TA was high for the 40, 50, 60 and 70 Hz (0.95 +/- 0.1). This indicated that there was a good correlation between the ankle angle and the iEMG amplitude except when applying 30 Hz stimulation.

4 Discussion

In the current study the ankle angle was measured with kinematic data. This was compared to the iEMG amplitude to examine the correlation between these. The results showed that there was a correlation between the ankle angle and the iEMG. The correlation was higher for the TA than the GM.

4.1 Comparison of results with Other Studies

Previous studies from E. A. T. De Laat et al., S. G. Boe et al., J. R. Potvin et al. revealed that the relation between muscle force and amplitude is present and that the variable used for this was root mean square amplitude. Here they were looking at the linear force and root mean square amplitude using surface electrodes. [7], [8], [9]

4.2 Methodological Considerations

With the use of an animal model instead of a human model the physiological influences are not the same. When a human walks normally there is a force applied to the leg due to maintaining balance and standing upright. In this experiment, this was not taken into account since the leg of the rats was hanging freely and no external force was applied.

The markers were placed by visual inspection of the animal's anatomical structure. Placement of the markers may therefore have varied slightly from animal to animal. During the offline digitization of the kinematic video data it was possible that some degree of error was present in the marker identification because of indistinct images. Especially the toe marker was difficult to distinguish in the video during the extension phase of the movement.

During the stimulation the frequency was changed from 30-70 Hz. The stimulation was done in that same order during all of the experiments. It is not possible to judge if a particular stimulation frequency caused some cumulative influence on the next stimulation sequence. This could be solved by randomization.

A factor that may have had an influence on the results is potentiation. This occurs during continuous stimulation and also has a tendency to happen in fast twitch fibers, and causes a positive staircase phenomenon [10]. This could likely explain that some of the kinematic data had a tendency to not reach a maximum of 100 % just after the stimulation onset. Here the maximum movement range was not reached until approximately 10 s after the onset of the stimulation.

5 Conclusion

In the present study it was investigated if information extracted from iEMG could provide reliable information on a functional movement (i.e. the ankle angle) during development of muscle fatigue. A higher degree of correlation was found between iEMG and ankle angle when stimulation frequencies above 40 Hz were applied to produce muscle contractions. Also, the results indicate that TA may be a more reliable source of feedback than the GM since the TA had higher correlation coefficient than the GM (GM: average of 0.76 +/- 0.01, TA: 0.96 +/- 0.01).

Further research should focus on developing an animal model where the correlation coefficient would be higher. In a future perspective the improvement would be beneficial for the clinical rehabilitation with the FES for subjects with spinal cord injury.

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Part III

Worksheets

CHAPTER 2

FUNCTIONAL ELECTRICAL STIMULATION

FES is the application of electrical stimulation to obtain movement by responses in the nervous system. This can be achieved by excitation of the nerve fibers that innervates the desired muscle. This is a way of mimicking the nerve response for activation of muscles that would normally come from the central nervous system. With this fatigue might be postponed and reduced compared to direct electrical stimulation of the muscles. This is due to the reversal of the recruitment of the fast fatigable fibers. It is shown that the FES builds fatigue resistance and increases the strength-force in the muscles. Therefore the FES has proven to be effective for rehabilitation of people with SCI. [Peckham, 1987], [Venkatasubramanian et al., 2010], [Granat et al., 1993]

It is known that the FES has a high potential use for incomplete SCI because this group of people has some preserved sensory and motor function. Granat et al. investigated the beneficial use of FES on six people with an incomplete SCI [Granat et al., 1993]. This was evaluated by measuring the spasticity, muscle strength, and postural stability for standing and gait, and physiological cost for gait along with independence in daily living. They observed improvement of voluntary muscle strength. Also one of the subjects had an improvement from before being able to move the leg against gravity with less than full range of movement, to be able to move the leg against applied resistance and in full range of movement. This indicated that the FES might be useful in the rehabilitation of people with a SCI. [Granat et al., 1993]

Typically used FES has an open-loop system incorporated (Figure 2.1) for control. The way the open-loop system is used is by placing electrodes on the preferred muscles that needs activation by stimulation. From the stimulation the open-loop system will generate an output. This is the most common type of stimulation used in the clinics today for FES rehabilitation of SCI people. The open-loop system is working as a feed-forward system, which only takes a model and the current state of the system to compute the input for the system (Figure 2.1). This means that the open-loop system does not take the actual performed movement into account. By using this system in an optimal scenario it would make a SCI person independent of a wheelchair. Unfortunately this is not the case with the currently available rehabilitation options. [VMware, 2013], [Landau et al., 2011]

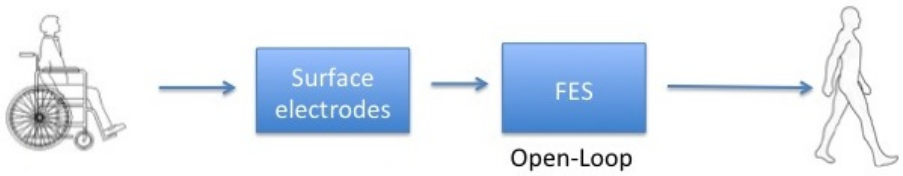

Figure 2.1: *Illustrates the way an open-loop control system works. There is a wheelchair dependent person. To help the person regain motor function the FES system is used. To be able to do this there have to be electrodes placed on the person to stimulate with the open-loop FES. From the open-loop FES there is an output, the desired output would be that the person can walk again.*

Even though the open-loop system is used in the clinics, it still has some disadvantages. It is well known that fatigue occurs when a muscle is being stimulated, which the system do not counteract for. An open-loop system cannot compensate for any disturbances in the system. Even though the open-loop system has some drawbacks, it is still easy to implement. An approach to improve the system is by incorporating a feedback system to determine if the output is achieved as desired. [Landau et al., 2011] [VMware, 2013], [Fairchild et al., 2010]

The research regarding improvements of the FES system has focused on development of a closed-loop FES system. The closed-loop controller has shown to be more successful because of the incorporated feedback mechanism. The principle is shown in figure 2.2 where the controller works by calculating an error, which will be the difference between the measured values and the desired output. With this feedback the controller will attempt to minimize the error by adjusting the input values for the actual performed movement. [Landau et al., 2011]

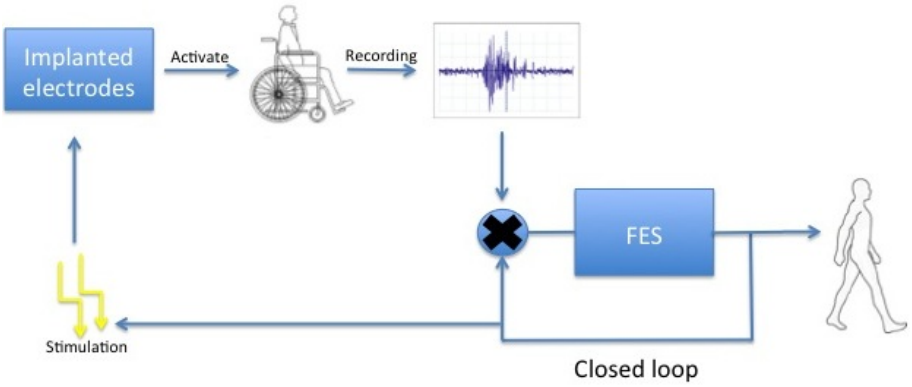

Figure 2.2: *Illustrates the functionality of a closed-loop system for the restoration of walking with a person that has a spinal cord injury based on implanted electrodes. Recordings are made from the stimulated muscle to assess if the desired activation is present. If the desired activation is not reached the system will give an error, which will be the difference between the measured values and the desired output. From this information the feedback controller will adjust the input values. This is to get closer to the desired outcome.*

Fairchild et al. studied how electrical stimulation in movement therapies may harness neural plasticity during enhanced sensorimotor recovery after an incomplete SCI [Fairchild et al., 2010]. In this study FES was applied to generate repeatable hip flexion/extension movements. This was done in a rat hindlimb, which should mimic patterns observed during locomotion. The results from this paper showed that the FES controller had the ability to produce the de-

sired hip movement pattern in rodents. This was applied to the flexor and extensor muscles of each hindlimb. The performance of the controller was seen as being consistent throughout several sessions, which was conducted over a number of days. When stimulation is applied to a muscle they tend to fatigue very quickly. This is due to the larger and more fatigable fibers, which has a lower stimulation threshold and are thereby activated first. Due to a lower stimulation threshold the fibers have to be stimulated with a higher frequency, than the slow fatigable fibers, to obtain the desired activation of the muscle. [Fairchild et al., 2010]

2.1 Electrical Stimulation

To be able to design FES that is both safe and efficient the characteristics and parameters of the stimulation have to be taken into account. For the safety the stimulated tissue may not be damaged from the stimulation as well as the electrode may not be damaged from e.g. corrosion. For the stimulation to be efficient it should be able to elicit the desired response e.g. movement of the hand. [Merrill et al., 2005], [Grill & Mortimer, 1995]

For artificial recruitment of an action potential, electrical stimulation is applied. With electrical stimulation the stimuli that are applied, in or around the nerve to activate it, are electrical currents from electrodes and waveforms specifically designed for this purpose. To mimic the physiological condition that applies when an action potential is generated, electrical stimulation can be used to lower the potential of the surroundings of the membrane. This will increase the potential in the membrane relatively to the surroundings, which causes an action potential to be generated. [Grill & Mortimer, 1995], [Merrill et al., 2005], [Cogan, 2008]

2.1.1 Generation of action potentials

When there is an excitation of a neuron an action potential occurs. This excitation can occur if the membrane potential changes from the resting potential that is approximately -70mV. The membrane potential has to change the resting potential to the threshold potential which typically is -60mV to -55mV. It does not matter if the stimulus that excites the neuron is applied suddenly or gradually. An action potential is always generated if the threshold is reached, where the action potential is independent of the strength of the stimulus as long as it exceeds the threshold. If the excitation does not depolarize the neuron to the threshold an action potential will not occur. This is the all-or-none principle. After the depolarization phase there is a repolarization phase of the membrane potential (Figure 2.3). This is the phase where the membrane potential is returning to the resting potential. The duration of an action potential including the depolarization and repolarization is approximately 1 ms. [Martini & Nath, 2009], [Silbernagl & Despopulos, 2009]

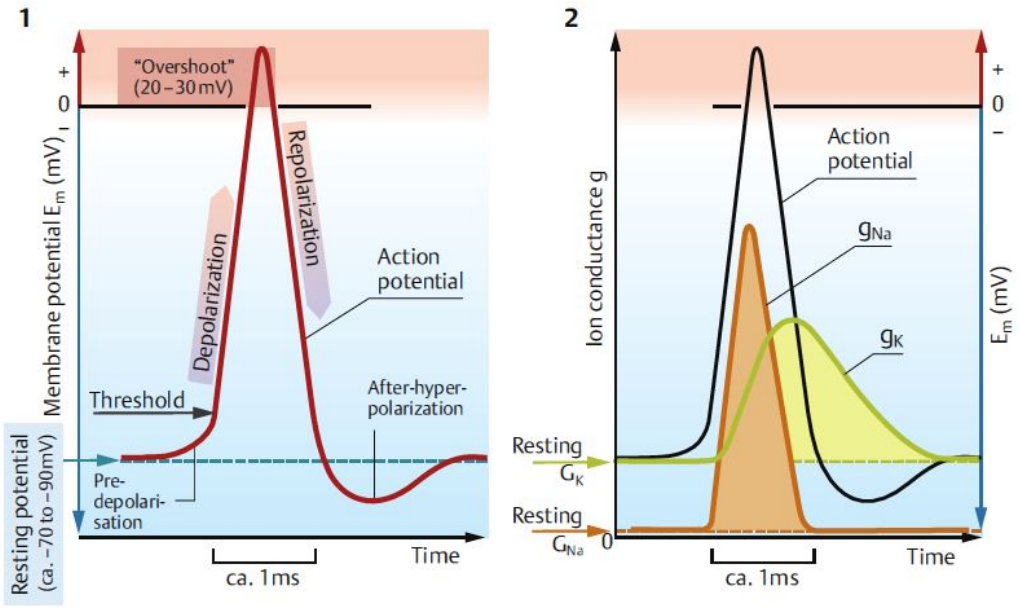

Figure 2.3: *1) Illustrates how the membrane potential depolarize and repolarize which generates and action potential. 2) Illustrates the generation of an action potential that is described from the ion conductance of the Sodium and Potassium gradient. [Silbernagl & Despopulos, 2009]*

2.1.2 Stimulation characteristics and parameters

To be able to stimulate, a charge have to be delivery to the nervous tissue, where the most common method applied is current controlled. With the current control a defined current is running between the cathode and the anode. This charge delivery is most often applied with square pulses, which both can be mono and biphasic pulses (Figure 2.4). Also considering the waveform used for the pulses has a factor in the phase of the design, to reach the best solution for the given purpose the stimulation is used for.

Mono and biphasic pulses

With monophasic stimulation a current is given for a specific period and then there is no stimulation until the next pulse. For biphasic pulses a current is given for a specific period in one direction, which is afterwards reversed followed by no stimulation until the next biphasic pulse. The most used pulse for neural stimulation are the biphasic pulses, where the first pulse initiates the activation and the second pulse are reversing the electrochemical processes to prevent damages of the electrolyte. For biphasic stimulation it is common to use a cathodic pulse first since this is negatively driven and the second pulse to be anodic since this is positively driven. [Merrill et al., 2005], [Grill & Mortimer, 1995], [Gorman & Mortimer, 1983]

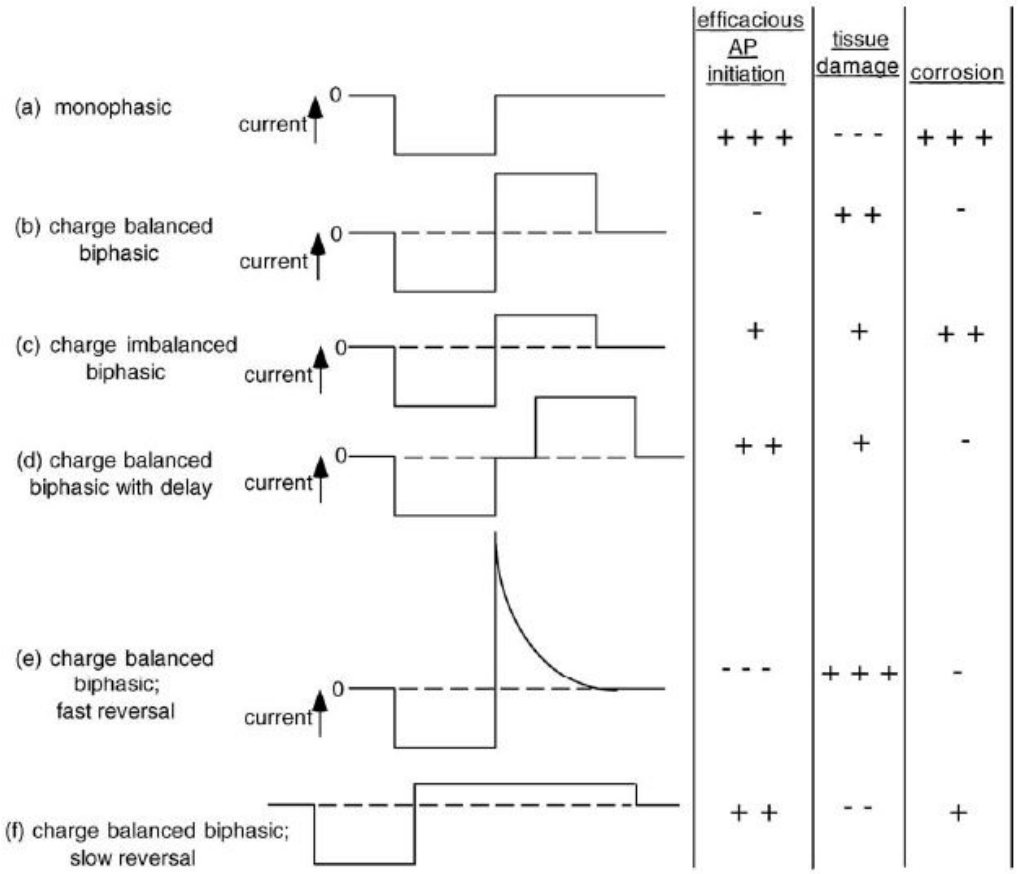

Figure 2.4: *Illustrates some different types of stimulation pulses including the monophasic and biphasic stimulation pulses. Along with this it illustrates the efficiency in initiating an action potential, the tissue damage, and the corrosion with the different stimulation pulses. [Merrill et al., 2005]*

For the use of biphasic pulses the pulses can be either charge balanced or imbalanced (Figure 2.4). The charge-balanced pulses have the same charge in both the first and second pulse whereas in the charge-imbalanced the charge is less for the second pulse. These biphasic pulses are used for the purpose of discharging the electrode faster than if a monophasic pulse was used. This is desirable so that when the next stimulation pulse arrives the electrode has completely discharged otherwise the next pulse would be more negatively driven than the one before. Therefore the use of biphasic pulses that changes the electrode potential directly after the first pulse, whereas the monophasic pulse potential remains negative where Faradaic reactions can occur. [Merrill et al., 2005], [Grill & Mortimer, 1995], [Gorman & Mortimer, 1983]

Waveform

The waveform that is used for the stimulation should be able to activate the nerve fascicles innervating the desired tissue. The most used waveform for stimulation including peripheral nerve stimulation are rectangular pulses (Figure 2.4). [Grill & Mortimer, 1995], [Gorman & Mortimer, 1983]

The rectangular biphasic pulses can vary with different parameters. Some of the pulses that could be of interest for the investigation of selectivity could be: charge balanced biphasic sym-

metrical and asymmetrical, reversed charge balanced and short charge balanced pulses.
When the charge balanced biphasic symmetrical pulse is used for stimulation a high frequency is required to obtain the desired results. A drawback can be that the pulse blocks the fibers that is desirable to activate since the secondary pulse is directly after the primary pulse and that they have the same magnitude.
The charge balanced asymmetrical biphasic pulse should reduce the blocking of the desired fibers during the reversal period. This pulse is also relatively commonly used for electrical stimulation due to a reduction of tissue damage [Peclin & Rozman, 2014], [Schuettler et al., 2002]. This kind of pulse innervates muscles which are more fatigue resistant. [Gorman & Mortimer, 1983]
The reversed charge balanced biphasic pulse with the use of a hyperpolarizing pulse before the cathodic pulse are expected to better facilitate the activation of nerve fibers than without this hyperpolarization. This kind of pulse should according to the study from Grill et al. be able to activate the nerve fibers at a distance than nerve fibers nearby (Figure 2.5) [Grill & Mortimer, 1995].
A short charge balanced asymmetrical biphasic pulse can be used to investigate if it is possible to activate nerve fibers at a distance to the contact site of the electrode. If this is achieved a better selectivity can be obtained by adjusting the pulse width. [Grill & Mortimer, 1995]

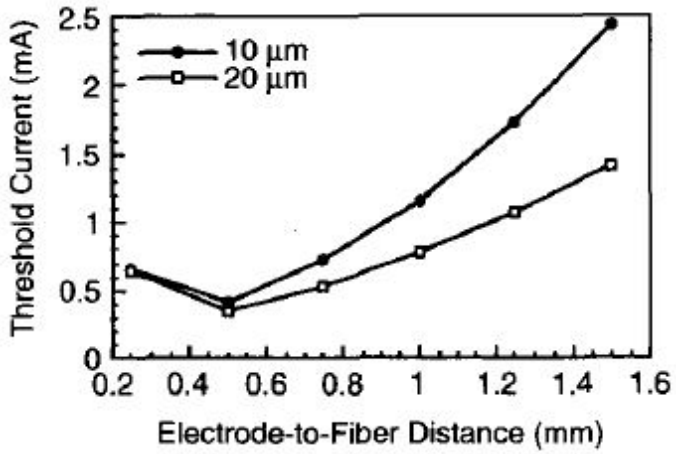

Figure 2.5: *Illustrates the electrode-to-fiber distance threshold currents for a reversed charge balanced pulse. It is seen that the fiber with a distance of 0.5 mm are activated at lower currents than the fiber closer to or further away than this distance.*

Amplitude, pulse width and intrapulse delay

The amplitude, pulse width and intrapulse delay of the stimulation pulse influence the outcome of the stimulation. The amplitude determines if the stimulation is sufficient to induce an action potential by changing the membrane potential. Therefore if low amplitudes are used there will be no response since it did not induce a significant change in the membrane potential. On the other hand if the amplitude is too high it could possibly damage the nerve. To induce selective stimulation appropriate amplitudes in the normal biological area of the specific nerve has to be used for the stimulation. [Grill & Mortimer, 1995], [Gorman & Mortimer, 1983]

The pulse width can influence the selectivity of the stimulation. This can be due to the total charge that is delivered to the tissue. To improve the selectivity different pulse widths, both

short and longer pulses, should be investigated. The use of short pulses will increase the stimulation threshold between the nerve fibers of different diameters and activate the large diameter fibers first. The use of longer pulses will activate the small diameter fibers first. This will allow for a more selective stimulation of the nerve fibers with different diameters by adjusting the pulse width and amplitude of the stimulation pulses. It was investigated in the paper from Gorman & Mortimer that the appropriate pulse width for a stimulation pulse would be 100 μs or more [Gorman & Mortimer, 1983]. This was due to the stimulation efficiency would increase with pulse widths of 100 μs or more, and if the pulse width was below 100 μs the stimulation efficiency would decrease. [Grill & Mortimer, 1995], [Gorman & Mortimer, 1983]

Furthermore an intrapulse delay between the primary and secondary pulse can have an influence on the recruitment of nerve fascicles. If the secondary pulse is of the same magnitude as the primary pulse and that there are no delay the secondary pulse might block the nerve fibers that was initially activated. The influence of an intrapulse delay between the primary and secondary pulse was investigated in the paper from Gorman & Mortimer. It was found that in animal experiments with recruitment of a nerve trunk that there was a decrease in the recruitment slope when no intrapulse delay was used. This indicates that an intrapulse delay should be present. However this intrapulse delay should be of a short duration. [Grill & Mortimer, 1995], [Gorman & Mortimer, 1983]

2.2 Neural Interfaces

A neural interface is used for the stimulation from a FES system to the human nervous system. This stimulation is usually obtained with microelectrodes that electrically stimulate the peripheral nerves or muscles. Since there are drawbacks with the use of surface electrodes, e.g. don and doff daily, the focus will be on implantable peripheral neural electrodes (PNE). [Navarro et al., 2005]

2.2.1 Peripheral neural electrodes

The ideal situation for rehabilitation with a FES system is to be able to activate each fascicle innervating a muscle. Hence this would allow the FES to stimulate each muscle or function desired. Therefore the electrodes used for the purpose of rehabilitation with a FES system are PNE. For a PNE to be successful desired subregions or fascicles should be able to be activated individually. The activation is to be able to activate multiple muscles individually. This is necessary in the application of a FES system for rehabilitation since this would reduce the need of implantation of multiple PNE's. The PNE have to be designed to selectively recruit the desired muscle or movement for several functions in one nerve trunk. [Badia et al., 2011], [Nielsen et al., 2012], [Tyler & Durand, 2002]

The selectivity of the different kind of PNE's have been investigated (Figure 2.6). The more selective the electrodes are the more invasive they are. The intraneural electrodes that penetrate the perineurium of the nerve fascicles have a high selectivity but it also have a high invasiveness. The less selective and invasive electrodes are the extraneural electrode e.g. cuff electrodes that are placed around the nerve. Therefore there is a trade of between the invasiveness and selectivity. The most used electrodes in research and rehabilitation is the extraneural cuff electrode, but interest has also been given to the intrafascicular electrode. [Nielsen et al., 2012], [Tyler & Durand, 2002]

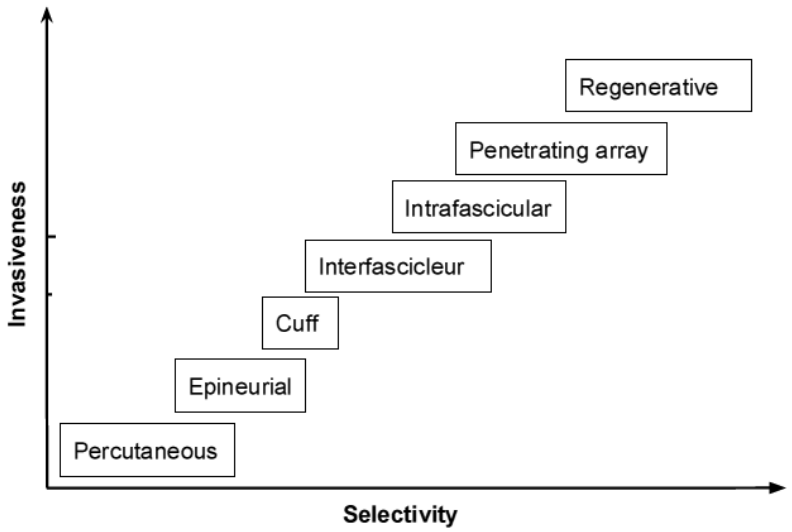

Figure 2.6: *Illustrates the selectivity vs. invasiveness of PNE's. The more invasive the PNE is the more selective is also is. It is seen that the percutaneous electrode is the least selective and invasive whereas the regenerative electrode is the most selective and invasive electrode. Modified from [Navarro et al., 2005].*

2.2.2 Comparison of intrafascicular and extraneural electrodes

There are advantages and disadvantages for all types of electrodes. To be able to implant an extraneural cuff electrode the nerve has to be completely freed for a small part. This can be difficult due to blood vessels and muscles surrounding the nerve. Whereas the intrafascicular electrode also needs to have the nerve freed completely, but it is also more invasive and laborious to implant. For the cuff electrodes it is important to carefully fit the electrode to the nerve to avoid reduced blood supply to the nerve. This is also important for the post implementation where swelling can occur. The swelling can compress the nerve because the cuff electrode is placed around the nerve. Whereas for the intrafascicular electrode there is one size that fits all fascicles and no fitting is needed. The intrafascicular electrode is not influenced by post implementation swelling but the implementation is more delicate. [Nielsen et al., 2012], [Badia et al., 2011]

For a selective activation of different muscles and to be able to stimulate more than one muscle only one cuff electrode is needed. This is achieved when the cuff electrode is placed around the nerve at a place that holds the fascicles that innervates the desired muscles. On the other hand for the intrafascicular electrode to be able to stimulate equally as many muscles, several electrodes have to be implanted. This is due to the intrafascicular electrode only stimulates the muscle that is innervated by the fascicle it is implanted in. Therefore to obtain the same selectivity more intrafascicular electrodes have to be implanted to be in contact with the same number of fascicles as a cuff electrode. [Navarro et al., 2005]

2.2.3 Safety considerations

The implanted electrodes have to have biostability and biofunctionality both if they are extraneural and intrafascicular. The material, size, and the surface of the electrodes have to be chosen in regard to these demands. Material that fulfills this could be gold, tungsten, and platinum for the conducting part e.g. polyetrafluoroethylene and silicone elastomer are used for

the insulating part. For the choice of the size of electrodes several things have to be taken into account. The size of the electrodes is e.g. affecting the selectivity. [Navarro et al., 2005]

2.3 Selectivity

The aim with selectivity is to activate a specific target without activating nearby non-targets, hence non-target activation can have the opposite effect than desired. This refers to that selective stimulation of a specific target e.g. nerve fascicle would give a more accurate control of the contraction of a specific muscle. This is important in e.g FES systems, where neural interface application need an increased selectivity. In relation to the overall goal a higher selectivity would enhance the rehabilitation opportunities for people with a SCI. [Jensen & Harreby, 2013], [Vuckovic, 2004], [Deurloo et al., 2000], [Raspopovic et al., 2011]

When the nerve is stimulated different kinds of selectivity can be obtained in the nerve; Spatial selective nerve stimulation which activates a set of axons in one of the regions of the nerve trunk. This selectivity refers to stimulation in a discrete group of nerve fibers, which in a localized region without stimulating neighboring nerve fibers. The other selectivity is the diameter selective nerve stimulation, which activates a group of nerve fibers with similar diameters in the nerve trunk. This selectivity is preferred if the specific nerve fibers are controlling a specific organ, where the other nerve fibers with different diameters control other organs. [Grill & Mortimer, 1995], [Vuckovic, 2004]

When an electrode is implemented an evaluation is done during the FES by recording one or more different parameters. A method to evaluate could be measurements of the neural activation with EMG. From this a difference between the selectivity of the electrode and the activation of other nearby non-targets can be done. By quantifying these parameters to something measurable a more valid estimation can be done towards the selectivity of the current electrode. For this purpose a selectivity index (SI) is used to convert the difference into a number. The most common one used is: [Jensen & Harreby, 2013], [Veraart et al., 1993], [Deurloo et al., 2000]

$$SI1_F = \frac{t_j}{\sum_{i=1}^{N} t_i} \tag{2.1}$$

The equation (2.1) represents the target activation compared with the activation of all non-targets. So the target activation t_j level is divided by the non-targets total activation t_i. The equation works with a range between 0 and 1. Each number corresponds to different outcomes. 0 is equal to that the specific target is not selective where 1 is equal to that the specific target is the only one active.
Equation (2.1) functions good in specific experimental situations, but nothing is unblemished. When the used target number is small the index will get higher, which makes it biased. So if it were assumed that the number of target and non-target are equal the expected index value would be 0. So when this is calculated, the result is 1/(1+1) = 0.5. If then there is one target and four non-targets activated this would result in 1/(1+1+1+1) = 0.25 for the SI. [Jensen & Harreby, 2013], [Raspopovic et al., 2011]

Since there are different elements to take into account when an experiment is performed, the SI can also be designed differently. Another SI equation (2.2) gives the target activity where the mean activation of all the non-targets is subtracted. The SI (2.2) with the value of 100 is an indicator that the target is selectively avtivated. [Jensen & Harreby, 2013], [Raspopovic et al., 2011]

$$SI2_F = t_j - \frac{\sum_{i=1, j\neq i}^{N} t_i}{N-1} \tag{2.2}$$

Equation (2.1) and (2.2) gives a description on how selective targets are activated. It is also important to observe how the neural interfaces deal with the selectivity. The found results from the two previous indexes can be used to calculate the selectivity of the device used for the specific experiment. [Jensen & Harreby, 2013]

$$DSI = \frac{\sum_{i=1}^{N} max(SI_i)}{N} \tag{2.3}$$

A way of calculating this is observed in equation (2.3) by taking the average of the maximum outcome from the SI of the targets and from that a result is given on how the performance of the neural interface was. [Jensen & Harreby, 2013]

In the current study it is preferred to explore different approaches regarding the selectivity. Therefore it would be interesting to compare equation (2.1) and (2.2).

2.4 Animal Models

When conducting an experiment with the purpose to mimic a disease or injury in humans the best model would be a human. The use of humans is not always feasible since there are some ethical considerations that have to be taken into account. Therefore the use of animal models to mimic these diseases and injuries has become valuable for the investigation of new experimental treatments for humans. For different diseases and injuries different kind of animals has their pros and cons. [LeDoux, 2005], [Poidron & Piguet, 2008], [Chen, 2009]

For the investigation of the effect of FES as a rehabilitation option for SCI people, in vivo models are of interest. This is due to need of a complete animal model that can show the outcomes of the FES for restoration of movement of e.g. an arm or a leg. Therefore it is useful to have an animal that can show resemblance to a SCI e.g. by contusion of the spinal cord. With an animal model movement can be induced in e.g. the legs. This can also be useful in the investigation in the study of when fatigue occurs in the muscles. [LeDoux, 2005]

Another model type is the in vitro experiments where either a cell culture or a part of the animal have been explanted and is investigated isolated from the body. This type of model has some positive features that cannot be obtained in an in vivo study. In the in vitro studies the isolated tissue are completely controlled and everything that are being investigated in the tissue can be directly observed. The tissue is not influenced by other parameters like other organs or stress of the animal. Unfortunately this can also be a drawback when it is desirable to investigate the found methods in an in vivo study. This does not always bring success because of the excluded factors during an in vitro study which is a more simplified way of investigation. [LeDoux, 2005]

CHAPTER 3

EXPERIMENTAL METHODS FOR ASSESMENT OF SELECTIVITY

3.1 Pig Nerve Model

To investigate the selectivity of FES an in vitro model was used. With an in vitro model and the used methods during the experiment it was possible to record from almost every fascicle of the nerve.

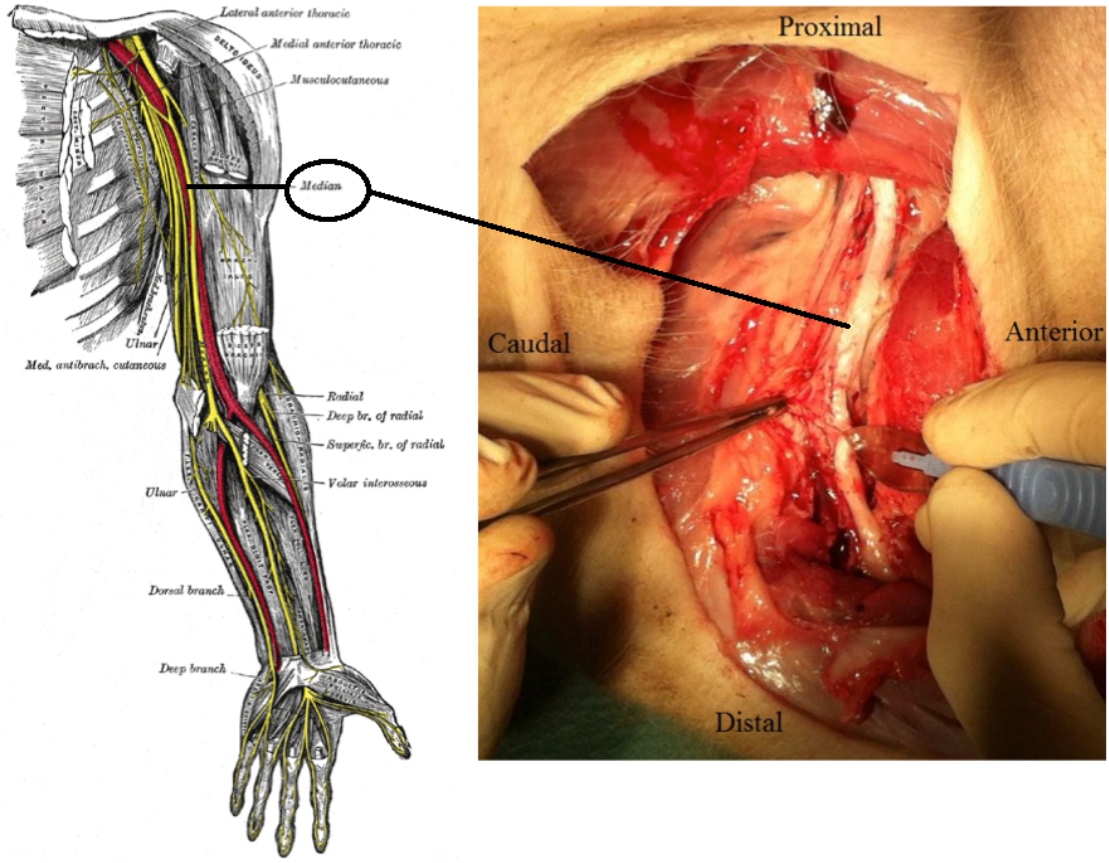

Figure 3.1: *The picture on left illustrates the human anatomy of an arm and the right picture is the pig anatomy of the forelimb during explant of the nerve. The median nerve is exposed from the point of axilla to the middle of the lower forelimb of the pig where the nerve branches out. Furthermore the median nerve is anterior to the ulnar nerve.*

The median nerve of the pig was used for the in vitro model. The median nerve was used since it contains nerve fascicles supplying control of the lower arm and hand functions in humans. The pig median nerve was used since the pig resembles humans both anatomically and physiologically (i.e. these resemblances makes a pig a good model of humans). The number of fascicles in the human median nerve (37 fascicles) and in the pig median nerve (34 ± 5 fascicles) are approximately the same [Kundu et al., 2012]. [Pond & Houpt, 1978]

The access to the median nerve of the pig is relatively easy and has approximately the same course in the forelimb as it has in the human forearm (Figure 3.1). Here the median nerve is branching along with the ulnar nerve at the axilla, where the median nerve lies anterior to the ulnar nerve. The median nerve of the pig has four branches: One above the elbow joint and three below the elbow joint, one slightly more proximal than the two most distal branches.

3.2 Electrode Design

The selectivity of the neural interfaces, that are available and used today for FES, is not high enough to obtain the desired functions. The reason is the inability to activate individual or groups of fascicles that innervates a given function. Therefore a new design of the neural interface is needed.
For this purpose a CICE was designed to achieve a higher degree of selectivity than what is offered with neural interfaces without being to invasive.

The multi-contact cuff electrode is less invasive than the interfascicular electrode and proved to have selective activations on fascicles. However it has some drawbacks as mentioned in chapter 2.2 on page 45. Since the traditional cuff electrode is surrounding the nerve it has trouble selectively activating deeper lying fascicle, therefore it is more likely to recruit only the more superficial fascicles. To achieve a higher selectivity the CICE was designed (Figure 3.2). With this combination it might be possible to reach fascicles that lies deeper in the nerve. The CICE is a relatively new approach, which has a limited knowledge in the literature and was therefore selected in regards to the background of the two types of electrodes, [Riso et al., 2001]. The multi-contact cuff electrode has the ability to stimulate more of the nerve with a lower current with only one electrode. Whereas the interfascicular electrode needs more implanted electrodes to reach the same level of selectivity of the whole nerve. On the other hand the interfascicular electrodes are more selective, which makes this combination interesting, further understanding on the topic can be reached in chapter 2.2 on page 45.

Three multi-contact cuff electrodes with an inner diameter of 2.6, 3.4 and 4.2 mm was constructed (Section 3.6 on page 57). These diameters was chosen in regards to the size of the median nerve found by Kundu et al. in landrace pigs [Kundu et al., 2012].
The multi-contact cuff electrode was designed with 18 platinum contact sites (size of 1.5 x 1 mm) distributed in three rings each with six contacts (Figure 3.2 and 3.3) each placed with 60 degrees spacing. The contact sites of the three rings are all aligned in parallel to the nerve. Each contact is connected to a teflon coated wire (0.3 mm). The contact sites at the two end rings are going to be used as the anodes where the contact sites of the middle ring and the interfascicular electrode are going to be used as cathodes respectively to the stimulation configuration. The interfascicular electrode was constructed using a thin teflon coated wire (0.3 mm) that was inserted into a curved needle (27 G x 1 1/2) and was secured by clamping the end of the needle. The wire was de-isolated for approximately 2 mm at a distance of 2 cm from the end of the needle to create a contact site (Section 3.6 on page 57).

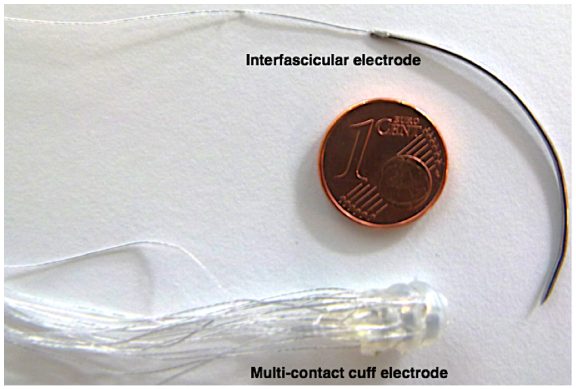

Figure 3.2: *Illustrates the multi-contact cuff electrode and the interfascicular electrode, which is placed near a 1 euro cent. The multi-contact cuff electrode is comprised of 18 contact sites aligned in three rings each with six contact sites. The interfascicular electrode is constructed with a wire attached to a needle for insertion. The wire was de-isolated for approximate 0.2 mm.*

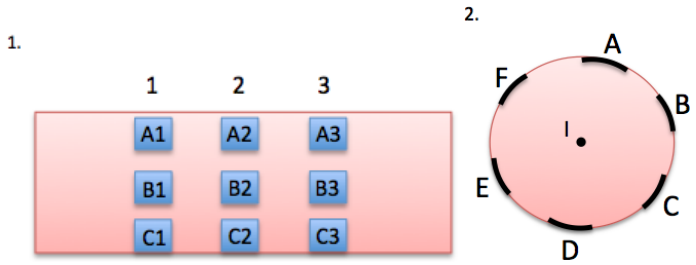

Figure 3.3: *Illustrates the contact sites of the multi-contact cuff electrode. It is seen that the contacts A-F of the tree rings were aligned in parallel with the nerve. It is also seen that the six contact sites A-F were arranged with equal distance (approximately 60°) in each ring.*

3.3 Stimulation Design

Five different stimulation pulses were used for the investigation of the further selectivity during stimulation with the CICE. The stimulation design was provided in regards to the considerations in chapter 2.1 on page 41. The rectangular biphasic stimulation pulses are the most commonly used why four different versions of these were selected. Increasing step pulse is a pulse designed for this experiment, with inspiration from [Grill et al., 1997]. The investigation regarding this pulse was to see if it has potential for selective stimulation according to fascicles with a different distances to the electrode than the rectangular pulses. It was therefore a possibility with the use of these rectangular and increasing step pulses to innervate both the fascicles that lies close to the contact sites and the ones that lies further away from the active contact sites (Figure 3.4). With this hopefully all of the nerve fascicles can be innervated individually in the subregions in which they belong.

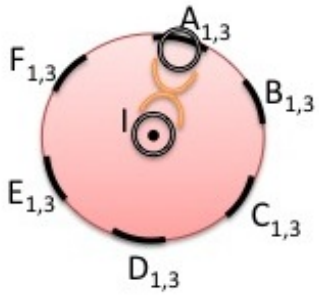

Figure 3.4: *Illustrates how the different rectangular and the increasing step pulses is intended to activate different parts of the nerve.*

For all the five pulse shapes the stimulation frequency was set to 5 Hz. This frequency was used to avoid fatigue in the nerve and have a proper repolarization of the nerve after the action potentials. The range of amplitude was set to 0-4000 μA with a step size of 250 μA for nerve 1-6 and was set to 0-2000 μA with a step size of 50 μA for nerve 7-10, to get an indication on the stimulation threshold with the different pulses (Table 3.1). The electrode configuration was the same for all five pulses for comparison of the different pulses (Table 3.3 on page 55). The primary and secondary pulses are charge balanced with a longer reversing pulse. This means that the same charge was delivered in both the primary and secondary pulse, but that the secondary pulse last longer with a lower amplitude than the primary pulse. This was used to avoid that the electrode potential to become to negative and preventing activation during the charge balancing pulse which could lead to tissue damage and electrode corrosion [Merrill et al., 2005].

Parameter	Selected Value/Unit, Nerve 1-6	Selected Value/Unit, Nerve 7-10
Frequency	5 Hz	5 Hz
Amplitude range	0-4000 μA	0-2000 μA
Amplitude step size	250 μA	50 μA
Repetitions	20	10
Electrode Configuration	Varies*	Varies*
Charge Balanced	Yes	Yes

* See table 3.3

Table 3.1: *Shows the different stimulation parameters that are common for all five stimulation pulses.*

3.3.1 Selected stimulation pulses

All other parameters were individually selected for the five different stimulation pulses. These parameters along with the benefits and the reason for using the different pulses are described in the following 3.2.

	Pulse One	Pulse Two	Pulse Tree	Pulse Four	Pulse Five
Pulse Width					
- Primary	100μs	100μs	100μs	20μs	100μs
- Secondary	100μs	400μs	400μs	400μs	400μs
Primary Pulse	Cathode	Cathode	Anode	Cathode	Cathode
Intrapulse Delay	None	100μs	None	100μs	100μs

Table 3.2: *Shows the different parameters used for the five different stimulation pulses. The primary pulse is the first pulse delivered whereas the secondary pulse is the second pulse.*

Pulse one - Charge balanced biphasic

The charge balanced biphasic symmetrical pulse (Figure 3.5) was used for the stimulation since this are the most commonly used stimulation pulse [Scheiner & Mortimer, 1990]. Since this pulse is already preprogrammed in most stimulator's it makes it easy to use.

Pulse two - Charge balanced asymmetrical biphasic with Intrapulse delay

The charge balanced asymmetrical biphasic pulse with an intrapulse delay (Figure 3.5) was used to investigate if this could overcome the drawbacks of the first pulse. Here there was an intrapulse delay and the magnitude of the primary and secondary pulse had a ratio of 4.

Pulse three - Reversed charge balanced biphasic

The reversed charge balanced biphasic pulse (Figure 3.5) was used to investigate the benefits of a hyperpolarizing pulse for selective stimulation.

Pulse four - Short charge balanced asymmetrical biphasic with intrapulse delay

A short charge balanced asymmetrical biphasic pulse (Figure 3.5) was used to activate the nerve fibers with different distance to the electrode. This pulse was also used with an intrapulse delay and a slow reversal.

Pulse five - Increasing step

The increasing step pulse (Figure 3.5) was used with a slow reversing anodic rectangular pulse with a ratio of 4 and had an intrapulse delay. This pulse shape was inspired by [Grill et al., 1997].

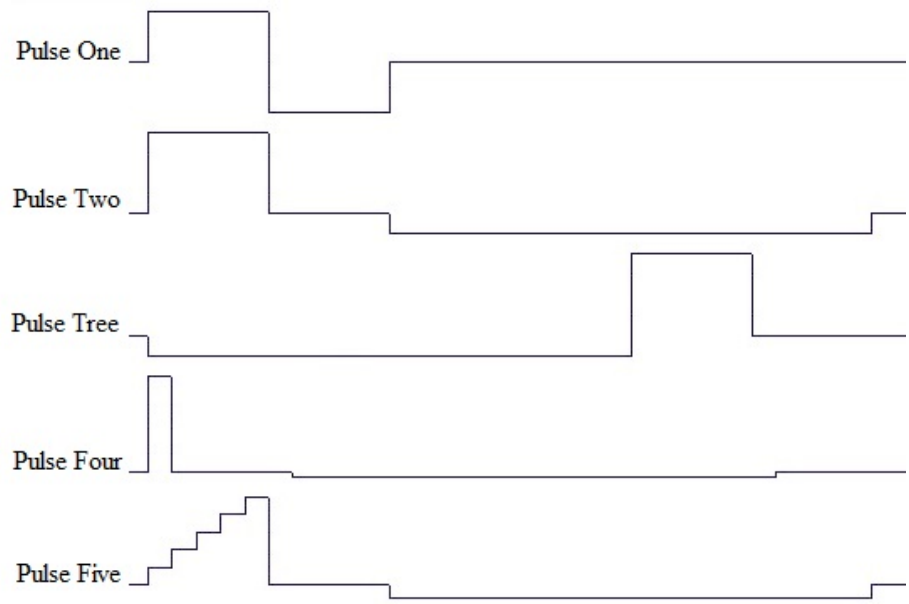

Figure 3.5: *Illustrates the five pulses used for the stimulation. Pulse one: Charge balanced biphasic. Pulse two: Charge balanced asymmetrical biphasic with intrapulse delay. Pulse three: Reversed charge balanced biphasic. Pulse four: Short charge balanced asymmetrical biphasic with intrapulse delay. Pulse five: Increasing step.*

3.3.2 Stimulations configuration

Different electrode configurations of the contact sites in the CICE were used for the investigation of how selectivity. As mentioned the CICE is build up with three rings (Figure 3.3). Three different electrode configurations were used for the stimulation and were as follows:

1. Maximal recruitment, ring one and three (cathodes), ring two (anode)
2. Longitudinal stimulation, Ring one and three (anodes), ring two (cathode)
3. Transversal stimulation, ring one and three (anodes), interfascicular electrode (cathode)

The maximal recruitment performed the recruitment when the whole nerve was stimulated at once. For the maximal recruitment all of the fascicles of the nerve should be maximally recruited. This was performed at the beginning and end of each experiment and after every fifth stimulation. The stimulation from the contact side in the middle (e.g A2) to the two contact sites furthest apart (e.g. A1 and A3) was used for the stimulation of the outermost fascicles of the nerve. For the stimulation from the interfascicular electrode (I) to the multi-contact cuff electrode (e.g. A1 and A3) the innermost fascicles were stimulated.

For the configuration of the stimulation the six sets of contact sites that run in parallel with the nerve were used. These were used along with the six sets of contacts sites from ring one and three to the interfascicular electrode 3.3.

Configuration	Anode	Cathode
1	A1 and A3	A2
2	B1 and B3	B2
3	C1 and C3	C2
4	D1 and D3	D2
5	E1 and E3	E2
6	F1 and F3	F2
7	A1 and A3	I
8	B1 and B3	I
9	C1 and C3	I
10	D1 and D3	I
11	E1 and E3	I
12	F1 and F3	I

Table 3.3: *Shows the different electrode configurations for the stimulation. It is seen that the contact sites of ring one and three were the anode for every electrode configuration where the contact site of ring two and of the interfascicular electrode were the cathode.*

The 12 different stimulation configurations were randomized during the experiment. Every time a stimulation configurations was randomly selected all five stimulation pulses was used for the stimulation each time with a randomized order. It is all randomized to avoid systematic bias.

3.4 Recording Design

To investigate the selectivity of the different stimulation pulses costume made tungsten rod electrodes were used for recording of data. The electrodes consisted of a tungsten rod (12 mm) covered by plexiglas attached to a copper wire (130 mm). The plexiglas was removed from the tip of the tungsten rod (4 mm) for the insertion of the tungsten electrode into the fascicle. To measure the selectivity it is necessary to measure the induced activity in each fascicle of the nerve. For this the tungsten rod electrodes were used because they easily can be inserted into each fascicle. The recording with tungsten rod electrodes provided a measure of the electrical potential. Furthermore the tungsten rod electrodes was placed outside of the nerve fascicles to record the surrounding noise. The collected data was analyzed offline using MATLAB® to give a measure of the selectivity of the stimulation.

3.5 Experimental Setup

For the first paper the experimental setup was consisting of:

- Median nerve from pig
- CICE
- Tungsten rod electrodes
- Custom made switch box
- STG4008 stimulator (AD Instruments Australia)
- Ni-DAQ (PCI-NI-6221, National Instruments, Austin, USA)
- Amplifiers and pre-amplifier (Multi-channel systems)
- Computer with MatLab® and MC-stimulus II (Multi-channel systems)
- Thermometer

- Nerve bath
- Na-Krebs solution
- Carbox tank
- Peristaltic pump (913 MITY FLEX)
- Lauda ecoline re 104 (heater/cooler)
- Medical power supply

The experimental setup are divided into two circuits: The electrical circuit (Figure 3.6) with the stimulation and recording and the nerve circuit (Figure 3.7) with the water flows of this.

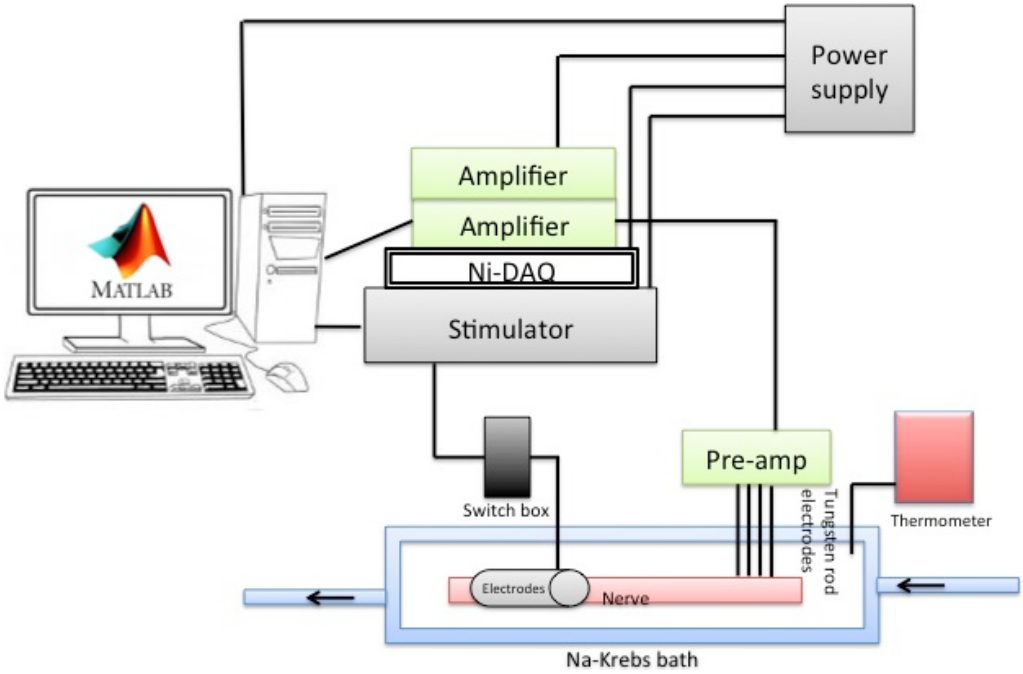

Figure 3.6: *Illustrates the electrical circuit of the experimental setup.*

For the electrical circuit the stimulation is generated with the STG4008 stimulator (AD Instruments Australia). The stimulation program was custom made in MatLab® and send to the stimulator through the MC-Stimulus II. To have a simultaneous stimulation and recording the Ni-DAQ controls this. The stimulation pulses were set to stimulate for the specific configuration of contact sites by using the switch box, that are connected to the CICE which are placed around and in the pig median nerve. This would then stimulate the nerve. For the recording the tungsten rod electrodes that are placed in each fascicle of the nerve are connected to the pre-amplifier (gain 10) and then the amplifiers (gain 500-5000). From the amplifiers the signals were recorded and saved in the computer. Besides this a thermometer through the whole experiment monitors the temperature of the Na-Krebs bath that the nerve lies in.

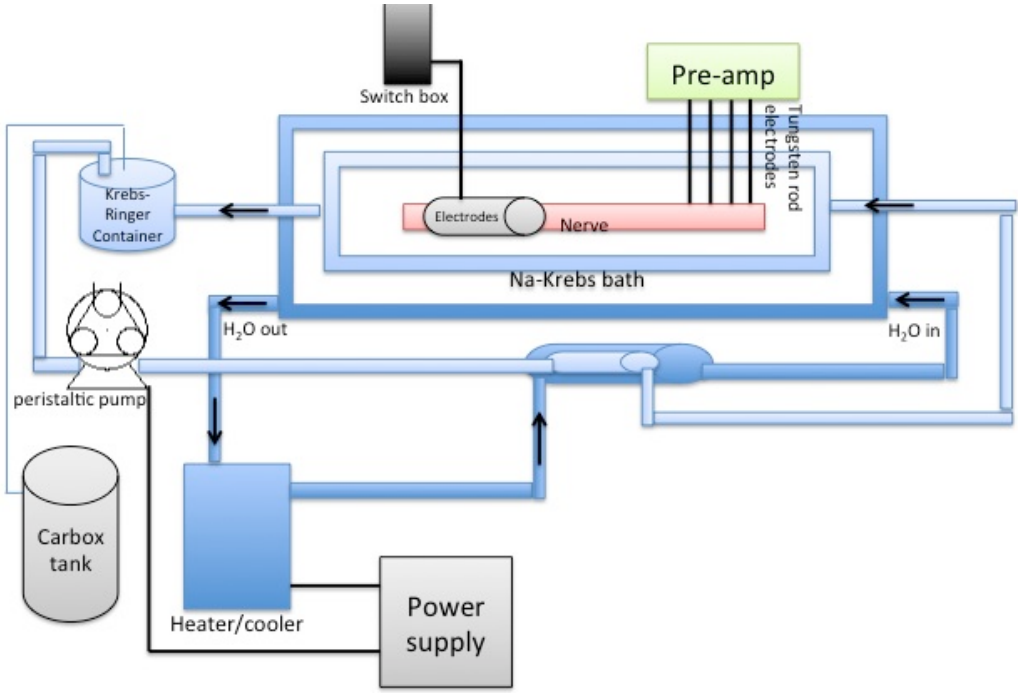

Figure 3.7: *Illustrated the nerve circuit with the flow of the Na-Krebs solution and the water.*

For the nerve circuit the nerve is placed in a Na-Krebs solution to have a more suitable environment with nutrients and oxygen. There are two circuits, one with the Na-Krebs solution and one with regular water to regulate the temperature of the nerve and the Na-Krebs solution. The nerve was placed in a custom made container where it was lying in the Na-Krebs solution and the water was flowing in a compartment surrounding the Na-Krebs bath. From the nerve bath the Na-Krebs solution was flowing to a container where it was oxygenated with carbox. From here a peristaltic pump pumps the water back to the nerve bath through a heat exchanger and back to the nerve bath. Whereas the water flows from the custom made container through a heater/cooler to the heat exchanger to regulate the temperature of the Na-Krebs solution.

3.6 Experimental Procedure

3.6.1 Solutions for the construction of the electrodes

The pre-work before the actual experiment consisted in constructions of electrodes. Before the actual electrode construction could take place some solutions had to be prepared for the stabilization and fixation for the electrodes.

Materials for silicone dipping solution

- Plastic container
- 30 g H-heptane
- 50 g silicone

Procedure for silicone dipping solution

30 g H-heptane was poured into a plastic container under a fume hood. Afterwards 50 g silicone was squeezed into the container. The container was then shamed very roughly for about 5 min.

After this the container was further shaken for 5 more min in a regular pace. The mixture was then left to stay overnight before use. To avoid evaporation leave the solution in the fume hood with a tight lid.

Materials for plexiglass solution

- Wire cutter
- Flat pliers
- Plexiglass
- Glass container
- Chloroform

Procedure for plexiglass solution

Plexiglass was broken down to small pieces. The pieces were filled in a glass container and afterwards chloroform was mixed together with the plexiglas. This was afterwards shaken together and the mixture was left to stay overnight before use.

3.6.2 Construction of electrodes

For the experiment two electrodes were constructed for the stimulation and recording. A multicontact cuff electrode and an interfascicular electrode was used for the stimulation part were a tungsten rod electrode was used for the recording of the signals.

Materials for cuff electrode

- Silicone dipping solution
- Fume hood
- Platinum foil
- Sandpaper
- Micro scissor
- Spacer clip
- Spot welder
- Teflon coated wires
- Microscope
- Silicone tubes
- Scalpel
- Multimeter
- Teflon coated mandrels (2.6, 3.4 and 4.2 mm)
- Marking for the wires
- Syringe with blunt needle
- Plug

Procedure for cuff electrode construction

A platinum foil was brushed over with sandpaper to increase the surface area. 18 platinum foil pieces were cut for the desired contact size. A spacer clip was then used to hold the platinum foil contact so that these pieces could be further cut to get small flaps on each side. These were made to give the contact the ability to be wrapped up on a silicone band for mechanical stabilization. Afterwards the platinum foil was spot welded with a Teflon coated wire under a microscope. The connection of the wire was then tested with a multimeter. Further silicone

tubes with the diameter of 2.6, 3.4 and 4.2 mm respectively to the teflon coated mandrels size, were cut to use for mechanical stabilization of the contacts. The 1 mm silicone tubes were then placed on the Teflon coated mandrel and the contacts were placed under the tubes at the desired place. The contacts and silicone tubes were then aligned for the final design. For reference the different wires were marked. The wires were then bundles to make it easier to finish the electrode. A syringe was now filled with a dipping solution (Section 3.6.1 on page 57) and a blunt needle was attached to it. This was then used to seal the contact sites to prevent silicone from flowing under the contact sites during dipping. This was let to dry for 1 hour. After this the mandrel was dipped in the silicone dipping and hanged to dry with the electrode pointing downwards. In case of a need for more dipping the drying time between the dipping was 1 hour. After final dipping the electrode was let to dry overnight. When the silicone was dry the excess was removed and a plug was soldered to the wires.

Materials for interfascicular electrodes

- 0.4 mm needle
- Wire cutter
- 0.3 mm Teflon coated wire
- Flat plier
- Scalpel
- Microscope
- Solder
- Multimeter
- Plug

Procedure for interfascicular electrode construction

A needle was separated from its plastic hut with a wire cutter. Afterwards a Teflon coated wire was placed in the needle. The wire was de-isolated in one end. The needle was then compressed around the wire using a flat plier. 2 mm was then de-isolated approximately 2 cm from the needle using a scalpel and a microscope. The connection of the electrode was then tested with a multimeter and then a contact was soldered to the wire.

Materials for tungsten rod electrodes

- Plexiglass-chloroform dipping solution
- Tungsten rod
- Copper wires
- Scissor
- Wirer cutter
- Microscope
- Solder
- Multimeter
- Sharpening tool

Procedure for tungsten rod electrode construction

Tungsten rods and copper wires are cut. Here the ends of the copper wires are stripped so that the tungsten rod could be placed in the copper threads. These were then soldered together. After the solder was cooled, the connection was first seen by pulling the tungsten rod lightly and afterwards tested with a multimeter. The electrodes were the dipped in a plexiglass solution

(Section 3.6.1 on page 58). The electrodes were let to dry overnight so the plexiglass can harden. A few mm of the end of the tungsten rod was then stripped and the needle was then sharpened with a sharpening tool.

3.6.3 Na-Krebs solution

When working with an in vitro experiment there was a need for substances to keep the tissue alive. For this purpose a Na-Krebs solution was made based on the paper [Lontis et al., 2009]. This will supply needed ions, nutrients and oxygen to keep the functions intact during the experimental work.

Materials for the Na-Krebs Solution

Chemicals	Molecular Formel	Amount
Sodium Chloride	NaCl	34.77g
Sodium Bicarbonate	$NaHCO_3$	6.3g
Potassium Chloride	KCl	1.71g
Magnesium Chloride	$MgCl_2 \cdot 6H_2O$	1.22g
Monosodium Phosphate	$NaH_2PO_4 \cdot H_2O$	0.83g
Calcium Chloride	$CaCl \cdot 2H_2O$	1.1g
Deionized water	H_2O	5L
Glucose	$C_6H_{12}O_6$	10g
Carbox	O_2 and CO_2	95% and 5%

Procedure for the Na-Krebs solution mixture

Each chemicals were separately weighed in a tray, except for glucose. 1L deionized water was prepared in a sterile container. The weighed chemicals were added to the deionized water. A magnetic stirrer was placed in the container to mix the chemicals until it dissolved. The solution was Saturated with Carbox. The pH of the solution was adjusted to 7.4-7.45 with hydrochloric acid (HCL) or potassium hydroxide (KCL). The solution was stored in a fridge. On the day of the experiment glucose was added and mixed with the magnetic stirrer.

3.6.4 Procedure of the nerve explant and nerve preparation

For the experiment a nerve was explanted from a pig and afterwards prepared for the actual experiment.

Material for the nerve explant

- Gloves
- Scalpel
- Gauze
- Scissors
- Forceps
- Tape
- Bandage
- Towel clamps
- Custom made glass pipette
- A container with Na-Krebs solution

Procedure for the nerve explant

The pig was euthanized beforehand and was placed on an operation table laying on the back. The preferred forelimb was then taped down or bound with a bandage to stretch it out. An incision from the axilla to the middle of the lower front limb was made. This procedure was continued until the median nerve was exposed. Towel clamps were used to separate the skin layer in the sides. It is important to orient where the median nerve is located. It is connected to the ulnar nerve at the proximal end and is the most anterior. A forceps was used to pull the connective tissue and then cut trough with a scissor for a more clean cut. This was continued along the nerve from the axillia to where it branches out to two, below the joint. A glass pipette was used to gently place it under the nerve to hold it a little upwards. The tissue underneath was then separated from the nerve. Orient where the ulnar and median nerve is branching and a cut is made close to the branch. The same was done in the distal end and a cut was made a few cm below the branching. As the final step the nerve was placed in a container filled with Na-Krebs solution (Section 3.6.3).

Materials for the nerve preparation

- Pins
- Tweezer
- CICE
- Micro scissor
- Microscope
- Tungsten rod electrodes

Procedure for the nerve preparation

During the preparation of the nerve it was important to have a setup that gave the nerve the needed environment to survive during the experiment. The nerve was placed in a bath with Na-Krebs solution. A pin was placed near the distal end for fixation of the nerve during the separation of the fascicles. Separation of the nerve fascicles was performed at the distal end with the different branched and for the proximal branch. The interfascicular electrode was placed in the middle of the nerve near the proximal end. Following this the multi-contact cuff electrode was then placed with the contact sites at the same place as the contact site for the interfascicular electrode. Afterwards the tungsten rod electrodes were placed in each of the separated fascicles. During the insertion of the tungsten electrodes each one was investigated during a stimulation to see if the placement gave a response, otherwise the tungsten electrode was moved more proximal until a response was observed.

3.7 Data Analysis

The data analysis for the recorded nerve signals were as illustrated in the following figure:

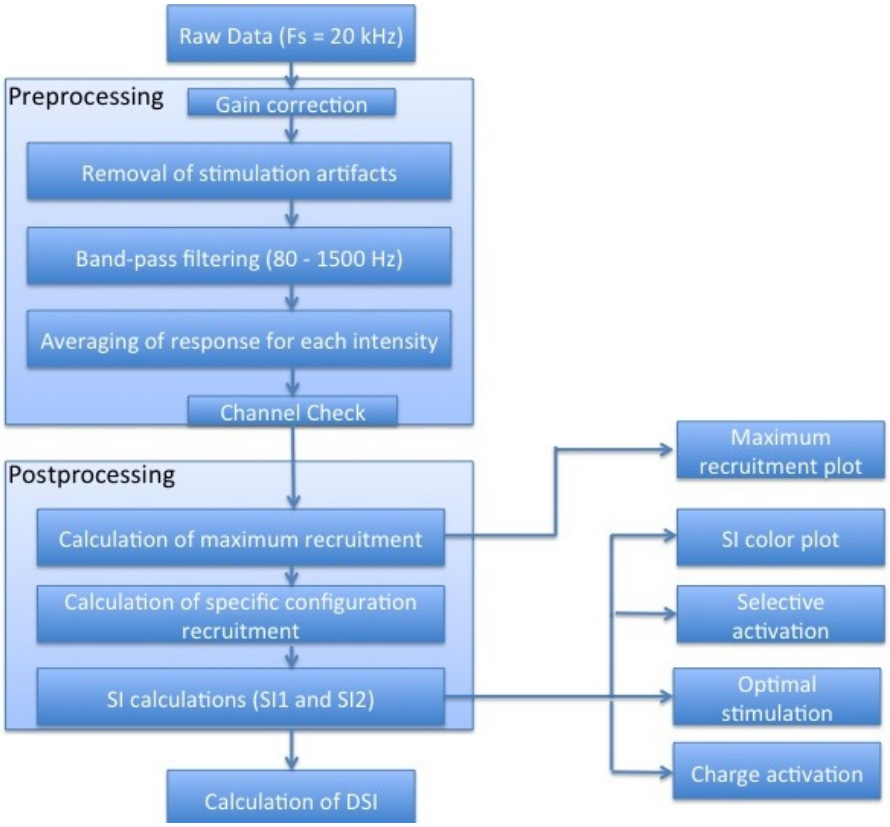

Figure 3.8: *Illustrates the flow of the data analysis for the recorded signals. As a part of the preproscessing of the data there was a correction of the gain followed by a removal of the stimulation artifacts. Then the signals were band-pass filtered and an average of the repetitions for the individual stimulation intensities were created. As the last part of the preprocessing all the channels for each nerve were checked to see if they were working properly, otherwise they were discarded. As a first step in the postprocessing the maximum recruitment for each nerve was calculated from were a plot was made for the supra maximal stimulation. This was followed by a calculation of the specific configuration recruitment, and at last a calculation of the three specific SI's. From the calculated SI color plot, optimal stimulation and charge balanced and an optimal stimulation were constructed. As the final step device SI (DSI) was calculated.*

3.7.1 Preprocessing

One synchronization and 20-28 channels each representing one fascicle, was recorded at 20 kHz. For the preprocessing the data was imported to MatLab® (Figure 3.9). The first step of the preprocessing was to correct the gain applied to the different channels, since these were not the same for all channels.

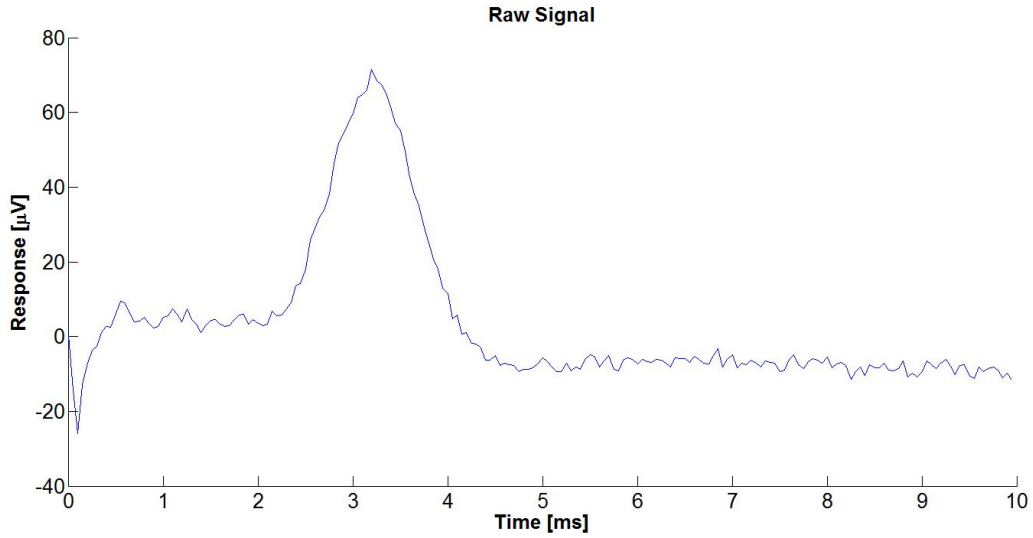

Figure 3.9: *Illustrates the recorded raw signal for nerve 1 with configuration 11 and pulse 5. The stimulation takes place at time 0, where the first peak (negative) is the stimulation artifact. (Fs = 20 kHz, high-pass filtered at 80 Hz)*

During the recording of the nerve signals the stimulation artifacts were recorded (Figure 3.10). These were removed since they would effect the later processing of the data. The stimulation artifacts were removed by being blanked out. This was done by forcing the samples during the stimulation to be equal to the average background level activity 0.5 ms (10 samples) just before the stimulation. The points that were blanked out were from 0.5 ms (10 samples) before the start of stimulation pulse until 1 ms (20 samples) after this.

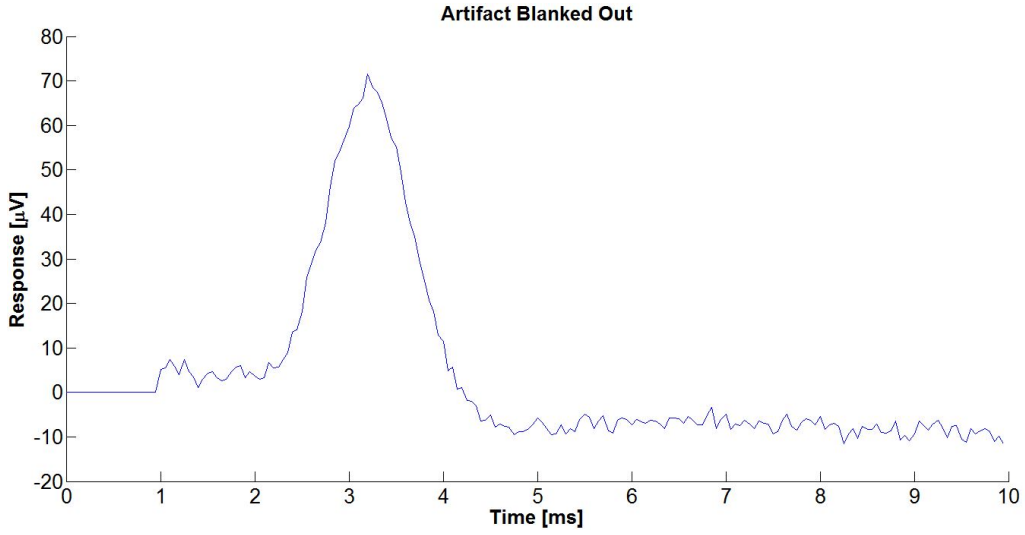

Figure 3.10: *Illustrates the effect of blanking out of the stimulation artifact for nerve 1 with configuration 11 and pulse 5. The stimulation takes place at time 0, where it is seen that the stimulation artifact is blanked out. (Fs = 20 kHz, high-pass filtered at 80 Hz)*

After removing the artifacts, the data was filtered (2nd order Butterworth band-pass filter with cut-off frequencies of 80-1500 Hz, MatLab® function `filtfilt`) (Figure 3.11). The cut-off

frequencies selected for the filtering were determined based on the filter frequency from the preamplifier, previous studies in the same area and setup, and the power spectral density after the artifact blank out.

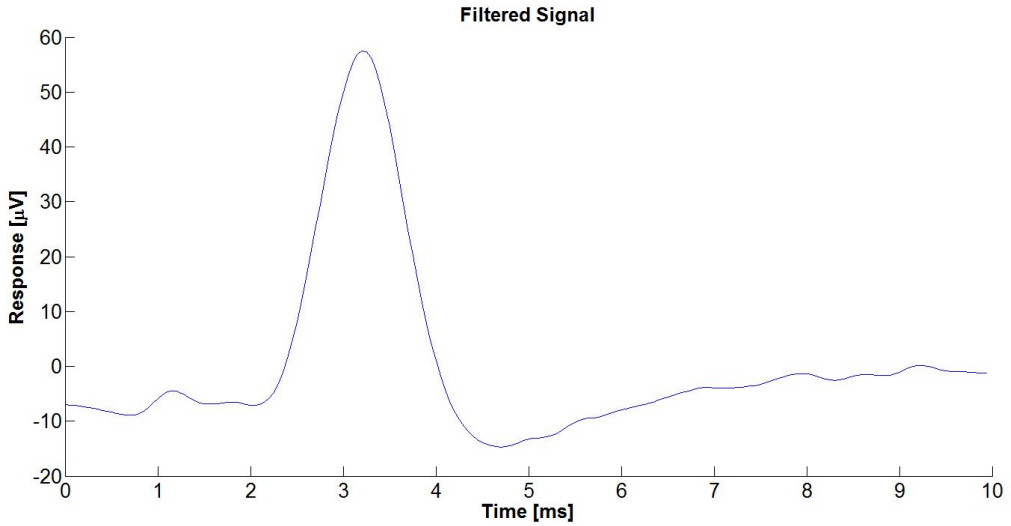

Figure 3.11: *Illustrates the signals after the filtering for nerve 1 with configuration 11 and pulse 5. (Fs = 20 kHz, band-pass filtered at 80-1500 Hz)*

Following the filtering an averaging of the different stimulation intensities were applied for the given repetitions for all channels (Figure 3.12). The averaging was for a time window of 10 ms from the onset of the stimulation. After the averaging an evaluation of the different channels for each stimulation configuration for each nerve was conducted. This was to investigate if the channel was working, if a response was reached, and if it was noisy.

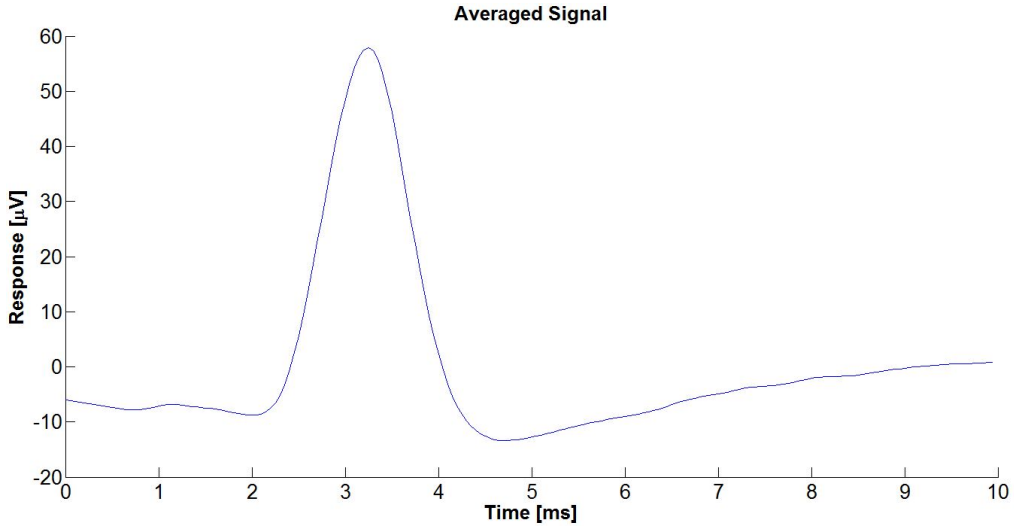

Figure 3.12: *Illustrates the signals after the averaging of the signals for each stimulation intensity for nerve 1 with configuration 11 and pulse 5 for 20 repetitions.*

The investigations of the channels were a combination of a visual inspection and a calculation of the ratio between the response and the noise. If the ratio between the response of the max-

imum intensity were more than 10 times the noise (the noise was set to an average of the last 0.5 ms) the channel was used for the further data analysis. If the responses of the channel was less than 10 times the noise the channel was discarded. Both the visual inspection and the calculated ratio had to be in accordance with each other to indicate which channels was working and which should be discarded. The filtered and averaged data was stored for the further data analysis.

Nerve	Recorded Channels	Working Channels
1	22	21
2	28	25
3	31	27
4	25	25
5	27	26
6	29	25
7	31	28
8	27	27
9	25	25
10	22	20

Table 3.4: *Shows how many fascicles that were recorded from during the experiments along with how many of the channels that were used for the data analysis. It is seen that relatively few channels have been discarded, which indicates that the experimental setup are reliable and stable.*

3.7.2 Postprocessing

For the postprocessing recruitment curves were calculated for the 12 configurations, five pulses, and 10 nerves (Figure 3.13). For the calculation of the recruitment curves the root mean square (rms) method was used for a period of 10 ms, where the data was normalized according to the prior supra maximal stimulation. These were used for the later calculation of the SI's.

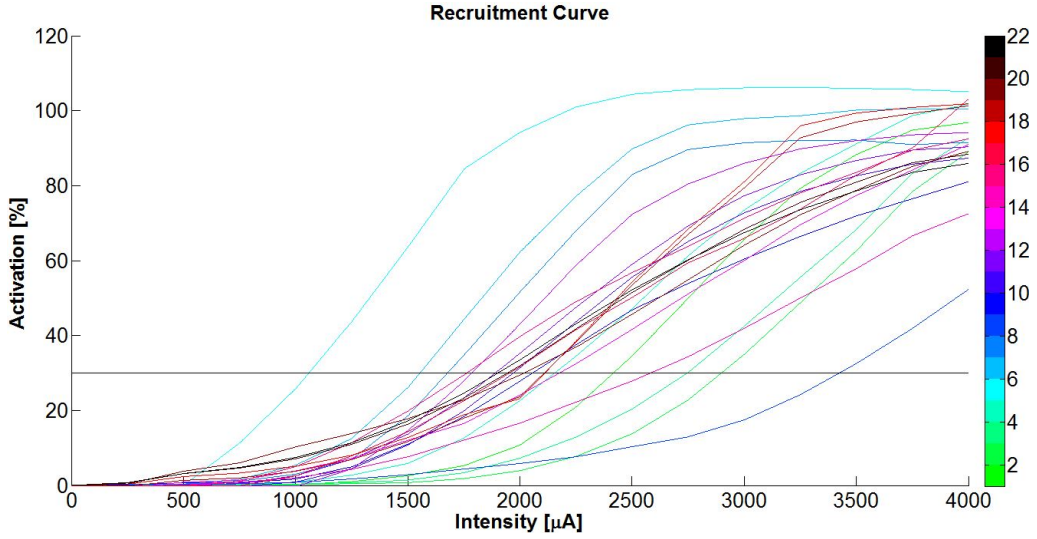

Figure 3.13: *Illustrates the recruitment curves for nerve 1 with configuration 11 and pulse 5. The different colored lines each represent one fascicle and the activation of this normalized to the prior supra maximal stimulation. The vertical black line at 30 % represents when the individual fascicles activation is above 30 % which are sufficient for a work task performance.*

During the calculation of the recruitment curves the data was smoothed (MatLab® function `smooth`, 5-point moving average) and interpolated (MatLab® function `interp1`). To improve the slope of the curves the signal was smoothed, which also removed some of the noise that was possibly not filtered out during the filtering of the signals. It was then investigated that the tendency of the recruitment curve was the same both before and after the smoothing (Figure 3.14). The following interpolation was used to calculate a data point for each 1 μA instead of having a resolution of 50 μA or 250 μA.

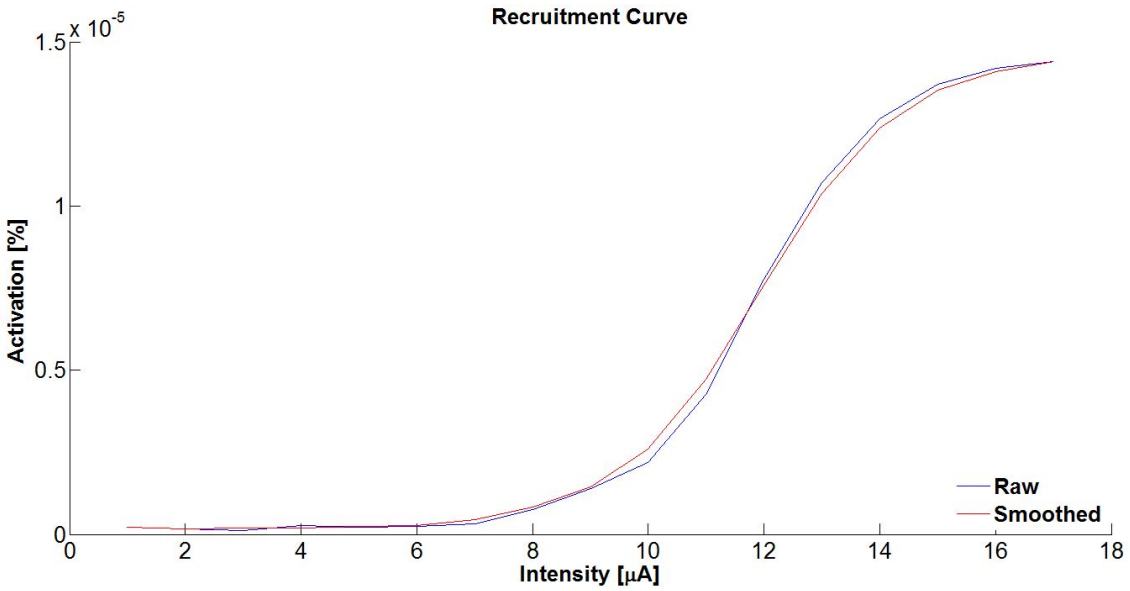

Figure 3.14: *Illustrates an example of the difference between the recruitment curve before and after smoothing. It is seen that the recruitment curve has the same tendency both before and after the smoothing.*

An activation threshold was set to 30 % for the activation of the individual fascicles during the stimulation. The threshold was set to 30 % since this has been reported to be sufficient for work task performance [Bao & Silverstein, 2005]. For the calculation of the activation threshold there was a calculation of the activation for each data point (1 μA) according to the prior maximal stimulation. This was calculated as a percentage according to the prior maximal stimulation. Following this calculation the highest obtained SI above the 30 % would be selected for the given fascicle, configuration and pulse.

3.7.3 Selectivity index

Two different SI's were used to estimate the stimulation selectivity of the CICE to investigate if they both gave the same indication on the selectivity. For this the two equations described in section 2.3 on page 47, were used which are the following:

$$SI1_F = \frac{t_j}{\sum_{i=1}^{N} t_i} \tag{3.1}$$

and,

$$SI2_F = t_j - \frac{\sum_{i=1, j\neq i}^{N} t_i}{N-1} \tag{3.2}$$

Following the calculation of the two SI's there was an investigation of how many fascicles each pulse could selectively activate. A selective activation was defined as the point when the SI for at least one of the configurations for a fascicle that was higher than $5 \times \frac{1}{N}$. For example for nerve 1 the fascicle had to have a SI above $5 \times \frac{1}{21} = 0.238$ for SI 1 (SI 1 has a range of 0-1) and 23.8 for SI 2 (SI 2 has range of 0-100). This was calculated to investigate how selectively the five different pulses could activate the different fascicles.

To give an overview of the optimal stimulation that should be used for each of the individual fascicles for each nerve an optimal stimulation table was constructed. This provides information on the specific configuration, pulse and intensity that should be used for each individual fascicle for each nerve, for both SI 1 and SI 2. Furthermore it was investigated which pulses that were used for the optimal stimulation for each fascicles. This was plotted, to give a better overview of the used optimal stimulation pulses.

Also the mean charge for each of the pulses to reach 30 % activation according to the prior maximal stimulation was calculated. This was calculated to investigate how the different pulses used different charge for the activation and to be able to compare if this was as expected from the theory.

Furthermore the DSI was calculated based on the equation (3.3). This was calculated to give an overview of the overall DSI, for both SI1 and SI2 were the best SI across the five pulses were used for the calculation. It was calculated for the CICE but also for the cuff configurations (1-6) alone, to investigate if the CICE enhances the selectivity according to the multi-contact cuff electrode.

$$DSI_D = \frac{\sum_{i=1}^{N} max(SI_i)}{N} \tag{3.3}$$

Supra Maximal Stimulation

The supra maximal stimulation was applied as the first and after every fifth stimulation (after one stimulation configuration with all five pulses). This was used to investigate if the nerve suffered any loss of function during the time of stimulation. For the calculation of the supra maximal stimulation during the full experiment the activation was calculated for each maximal stimulation. Afterwards these values were inspected to see if there was a decay in the response over time.

3.8 Results

3.8.1 Selectivity score

To get a visual overview of the results a color plot was constructed for each of the 10 nerves for both SI's. These color plots provided an overview of the maximum SI reached for each fascicle for all the different pulses used. On the x-axis the fascicles for the nerve is seen, and on the y-axis the different configuration is seen. Configuration 1-12 is stimulation used with the CICE, whereas configuration 1-6 is the stimulation only with the multi-contact cuff electrode part of the CICE. The colors on the right indicate the reached level of selectivity in the individual fascicle. The higher the selectivity the more red the bar plot will show and the lower the selectivity the more blue the bar plot will show. Two color plots for nerve 7 is shown below the other color plots can be seen in appendix A.

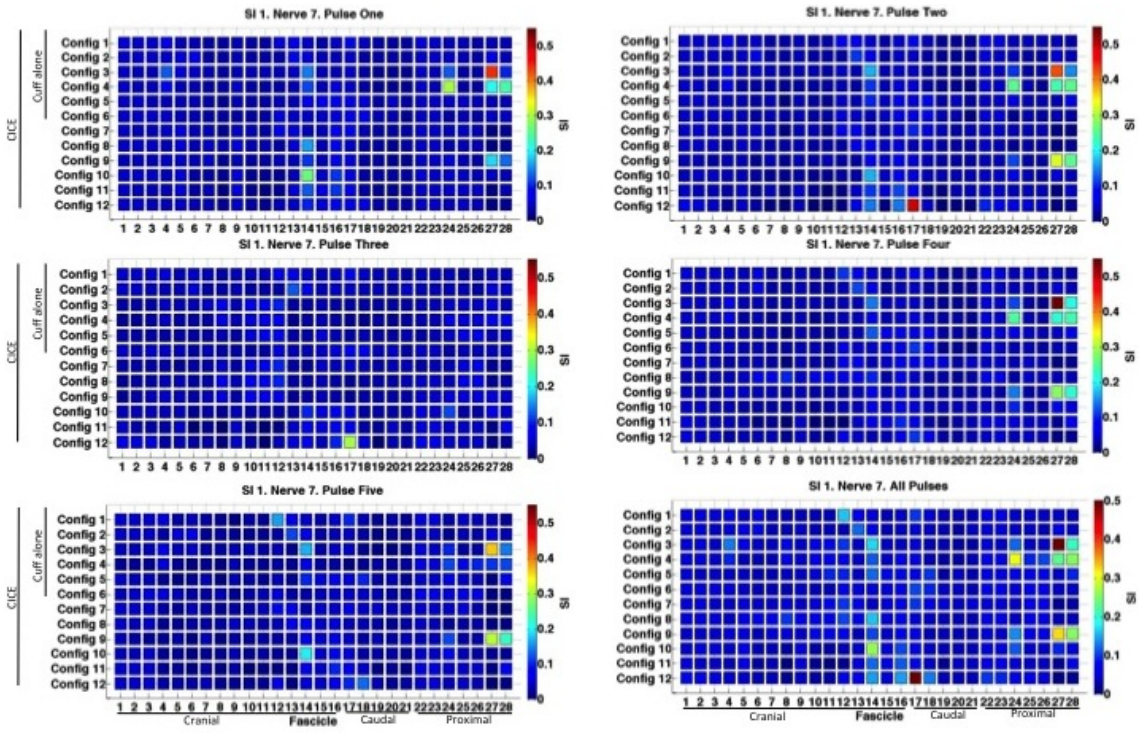

Figure 3.15: *Illustrates a color plot of SI 1 for nerve 7, with all five pulses used during stimulation, also a combination of all the five pulses is illustrated in the last bar plot. On the x-axis the fascicles is shown, with corresponding branches. On the y-axis the 12 configurations were listed, the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. Red color indicates high selectivity, while blue color indicates lower selectivity.*

It is observed that the tendency for the SI 1 for all 10 nerves were very similar (Appendix A on page 99 and figure 3.15 and 3.16). The highest selectivity was seen in the proximal branching, which correspond to the highest number of fascicles on the figure. Since the range varies for all the nerves, a specific range was not stated. It is also clearly seen that pulse three had the highest activation in the proximal end in most of the nerves, where the other pulses had a close similarity towards the pattern in this activation. In this particular nerve the mentioned tendencies were not exact. The most activation was seen with pulse one, where pulse three only had one activated fascicle. Another observation was the very low selectivity seen throughout the nerves. Overall for nerve 7 there was a low activation compared to SI 2 where more fascicles was activated during stimulation. The last plot with all of the five pulses combined gives a slightly better SI for the 10 nerves.

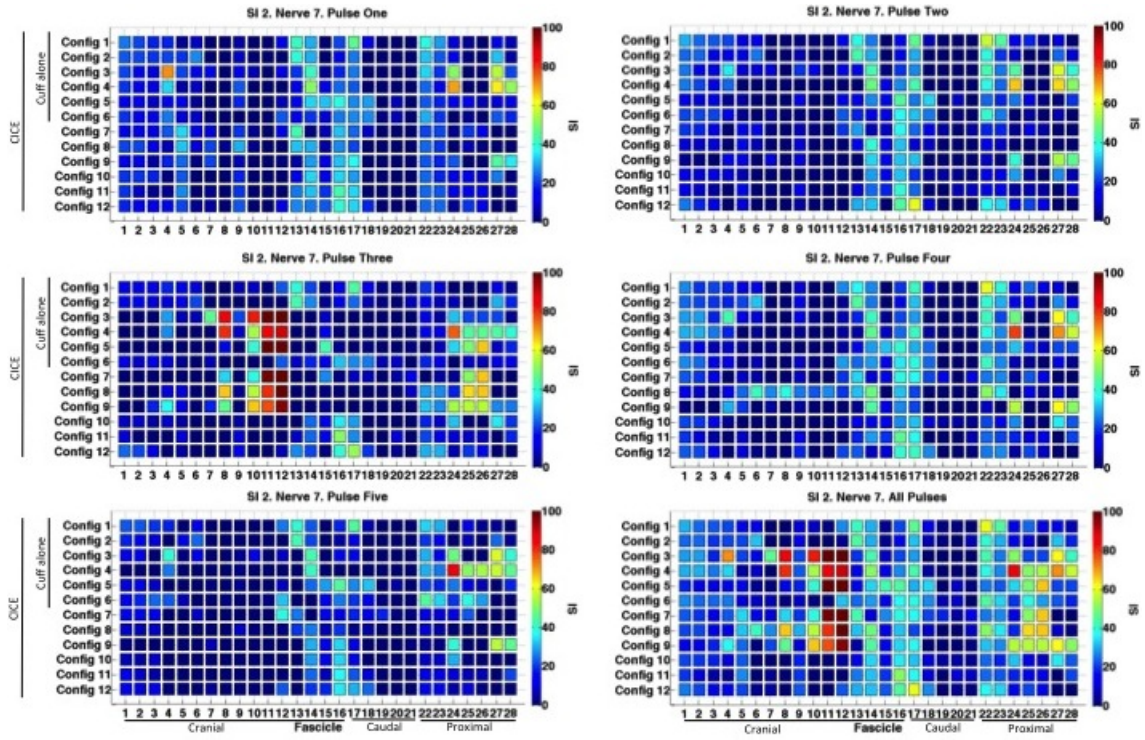

Figure 3.16: *Illustrates a color plot of SI 2 for nerve 7, with all five pulses used during stimulation, also a combination of all the five pulses is illustrated in the last bar plot. On the x-axis the fascicles is shown, with corresponding branches. On the y-axis the 12 configurations were listed, the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. Red color indicates high selectivity, while blue color indicates lower selectivity.*

The tendency of the SI level for all the 10 nerves (20-28 fascicles) was that pulse three seemed to have the highest SI in the part of the proximal branch of the nerve compared to the other pulses. This can also be seen in the color plot for nerve 7, where the difference from pulse three and the other pulses was clearly observed (Figure 3.16). There was a difference in the number of fascicles and arranging of these. There was seen a clear relation between the selectivity and anatomy. The area with the highest SI was seen in the proximal branch for all nerves except for nerve 10. In some nerves a high SI was also seen in some fascicles in the distal branches. For example a higher selectivity in nerve 7 was observed for four fascicles in the distal end. It was here seen that configuration 3-5 and 7-9 reached the fascicles during stimulation more than the other configurations. Furthermore it was observed that pulse one, two, four, and five had a high degree of resemblance in the activation patterns throughout the nerves. The last plot is a combination of all the five pulses. Here it was observed that combining all the different pulses the SI would increase, which was seen throughout all the 10 nerves. When observing the results when stimulating with (configuration 7-12) and without (configuration 1-6) the interfascicular part of the CICE it was seen that the fascicles activated during stimulation are different throughout the 10 nerves.

3.8.2 Selective activation of fascicles

For the investigation of how many fascicles there was selectively activated by the five pulses a plot was constructed. This plot shows how many of the fascicle was activated for each of the

five pulses for each of the SI's.

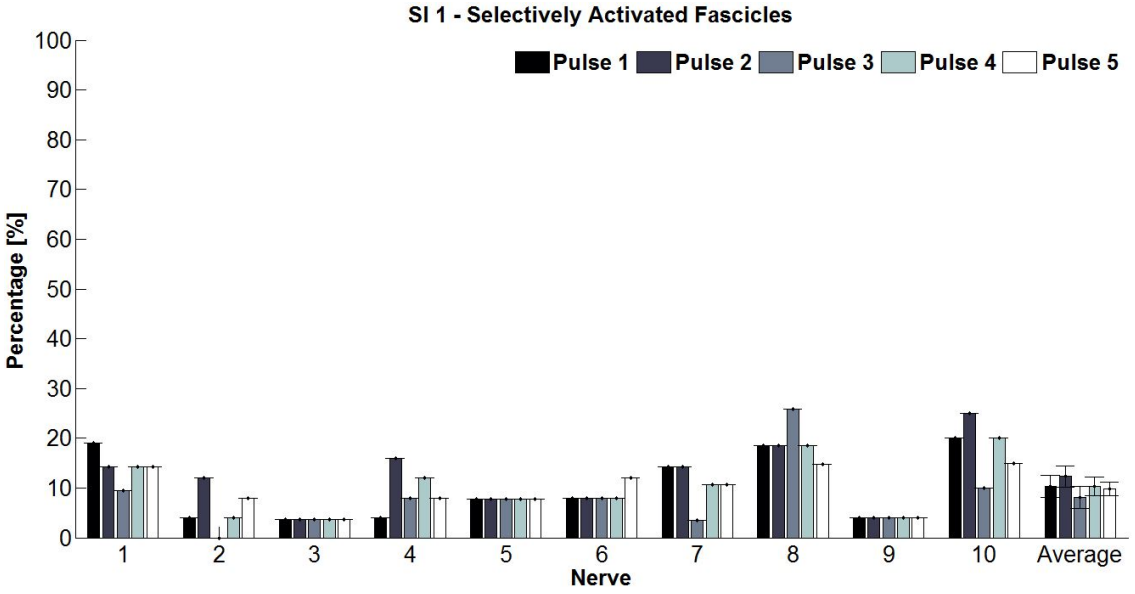

Figure 3.17: *Illustrates the selectively activated fascicles for all 10 nerves for SI 1. It is seen that the selectively activation as a percentage-wise activation was up to 30 %. Also the average for all 10 nerves were shown (mean ± SEM).*

For SI 1 the percentage of fascicles that was selectively activated was up to 30 % for all five pulses for the 10 nerves (Figure 3.17). For the average for all 10 nerves, it was seen that the percentage activation was between 5 % and 15 %. This shows that there was a relatively low selective activation of fascicles with all five pulses for SI 1.

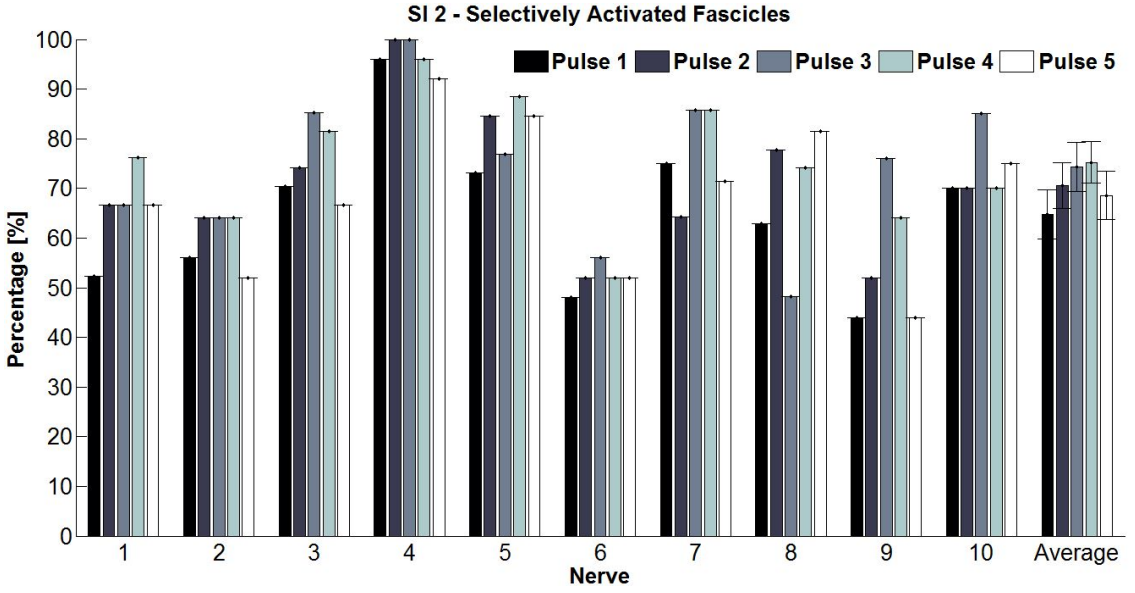

Figure 3.18: *Illustrates the selectively activated fascicles for all 10 nerves for SI 2. It is seen that there is a relatively high selectively activation as the percentage-wise activation is between 55 % and 100 %. Also the average for all 10 nerves were shown (mean ± SEM).*

For SI 2 the percentage of fascicles that was selectively activated was between 55 % and 100 % for all five pulses for the 10 nerves (Figure 3.17). The average for all 10 nerves shows that the percentage activation was between 60 % and 80 %, this shows that there was a high selective activations of fascicles with all five pulses for SI 2.

3.8.3 Optimal stimulation

For the optimal stimulation for each fascicle an optimal stimulation table was constructed with the maximal SI obtained, along with the stimulation parameters for this: configuration, pulse, and intensity. Furthermore a count of how many times each pulse was used to reach the highest SI for both SI 1 and SI 2 was plotted, to see which pulse contributed mostly to the optimal stimulation.

Fascicle	SI 1	Configuration	Pulse	Intensity [μA]
1	0.0784	12	2	1351
2	0.0597	12	3	1301
3	0.0523	12	3	1301
4	0.127	3	1	501
5	0.0595	11	1	1301
6	0.0794	2	2	679
7	0.0709	8	4	110
8	0.0976	8	4	101
9	0.0550	8	1	1151
10	0.0807	9	3	268
11	0.0875	5	3	651
12	0.159	1	5	384
13	0.122	2	3	590
14	0.266	10	1	495
15	0.0793	5	1	451
16	0.145	12	2	1251
17	0.493	12	2	781
18	0.132	12	5	1089
19	0.0519	7	4	651
20	0.0459	11	3	1651
21	0.0496	7	4	506
22	0.0970	12	2	1318
23	0.0872	12	2	1328
24	0.299	4	1	131
25	0.0908	4	5	426
26	0.102	4	5	409
27	0.545	3	4	206
28	0.253	4	2	406

Fascicle	SI 2	Configuration	Pulse	Intensity [μA]
1	30.5	4	4	10000
2	30.4	4	4	10000
3	25.3	4	4	10000
4	70.7	3	1	601
5	33.0	7	1	1101
6	42.0	8	4	9255
7	45.6	3	3	1751
8	85.8	3	3	651
9	30.5	8	1	1151
10	81.4	3	3	2000
11	105	5	3	1301
12	156	3	3	951
13	44.7	2	4	4005
14	51.0	4	1	601
15	42.4	5	3	1351
16	50.5	11	3	2000
17	59.9	12	2	2000
18	33.8	5	2	951
19	18.3	7	5	2000
20	10.7	11	3	2000
21	14.4	7	3	1001
22	59.6	1	4	10000
23	44.3	1	2	2000
24	86.3	4	5	1001
25	64.6	8	3	1101
26	67.2	7	3	1001
27	70.4	4	4	2255
28	57.8	4	4	2005

Table 3.5: Shows the maximum SI for each fascicle for nerve 7. Besides this it also shows the optimal configuration, pulse, and intensity to activate the individual fascicle as selective as possible.

For the optimal stimulation tables it is seen that SI 2 generally has a higher SI than SI1 (Appendix B on page 117 and figure 3.5). Besides this it was seen for SI 1 that the most used pulse was pulse five followed by pulse three and one respectively. Whereas for SI 2 the most used pulse was pulse three followed by pulse one and four respectively. This optimal stimulation table could be used as a reference for which parameters to be used for an optimal stimulation of a fascicle (e.g. muscle in the clinics).

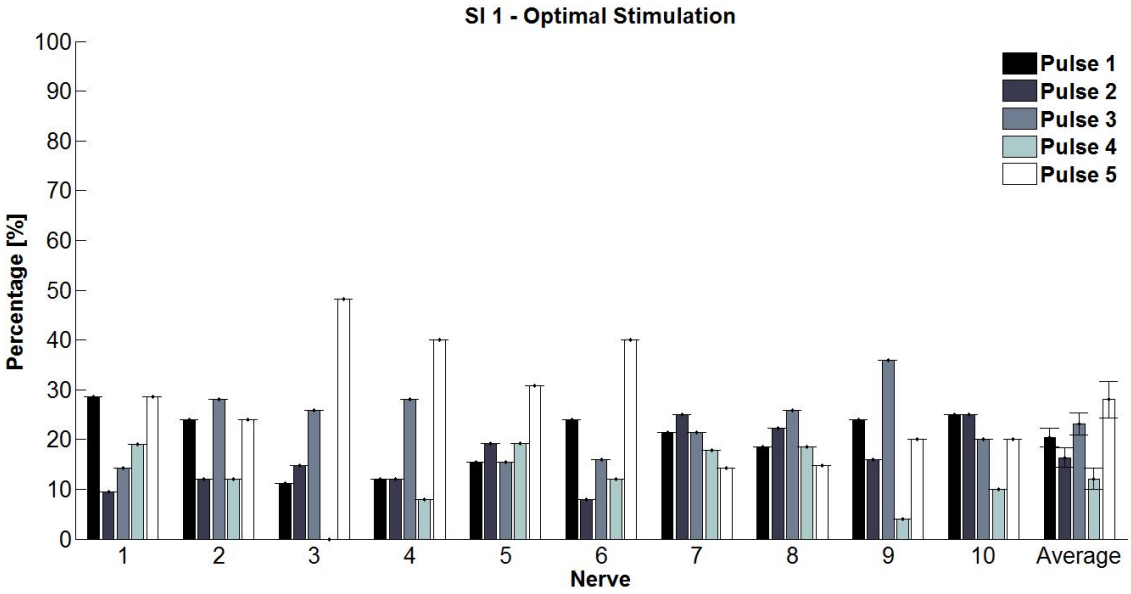

Figure 3.19: *Illustrates how many times percentage-wise each pulse was used to obtain the optimal stimulation of each fascicle for each nerve for SI 1. It is seen that pulse 5 is the pulse mostly used (mean ± SEM).*

The pulses that were mostly used for obtaining the optimal stimulation for all fascicles was for SI 1 pulse 5 (35 %) followed by pulse 3 (25 %) and 1 (20 %) respectively. Pulse 2 (15 %) and 4 (10 %) was not used as much as the three other pulses, with pulse 4 the least times.

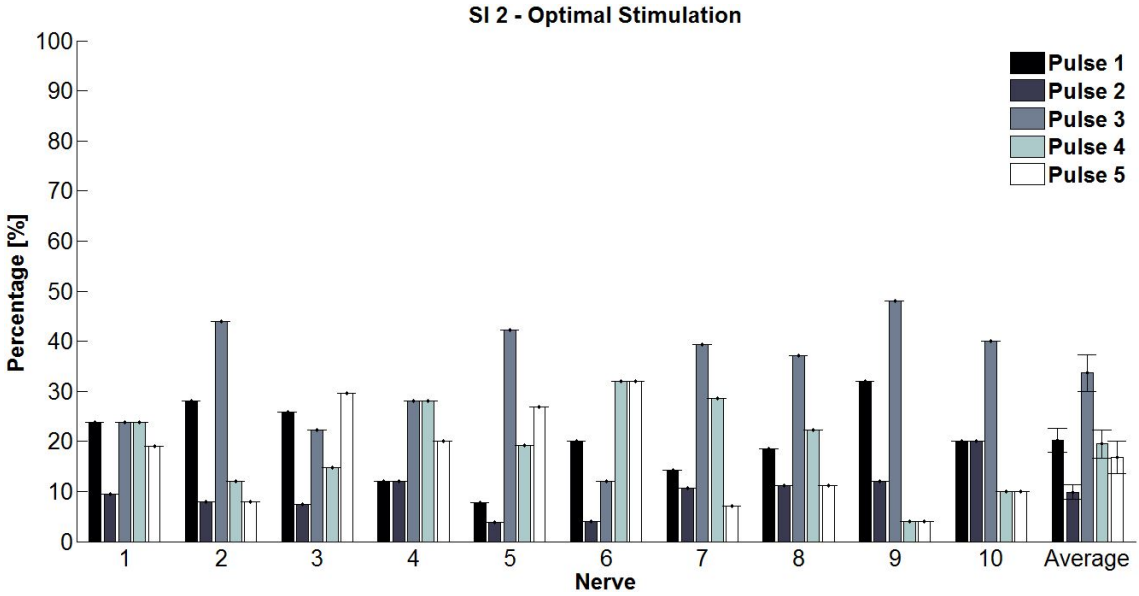

Figure 3.20: *Illustrates how many times percentage-wise each pulse was used to obtain the optimal stimulation of each fascicle for each nerve for SI 2. It is seen that pulse 3 is the pulse mostly used(mean ± SEM).*

Pulse 3 (45 %) was the pulse mostly used for obtaining the optimal stimulation for all fascicles with SI 2. Pulse 1 (20 %) and 3 (20 %) was used approximately the same times, followed by pulse 5 (18 %). Pulse 2 (10 %) was used the least amount of times for the optimal stimulation.

3.8.4 Charge activation

To visualize the charge induced for activation over 30 % during stimulation in the 10 nerves with the five pulses a bar plot was constructed. for each of the five pulses.

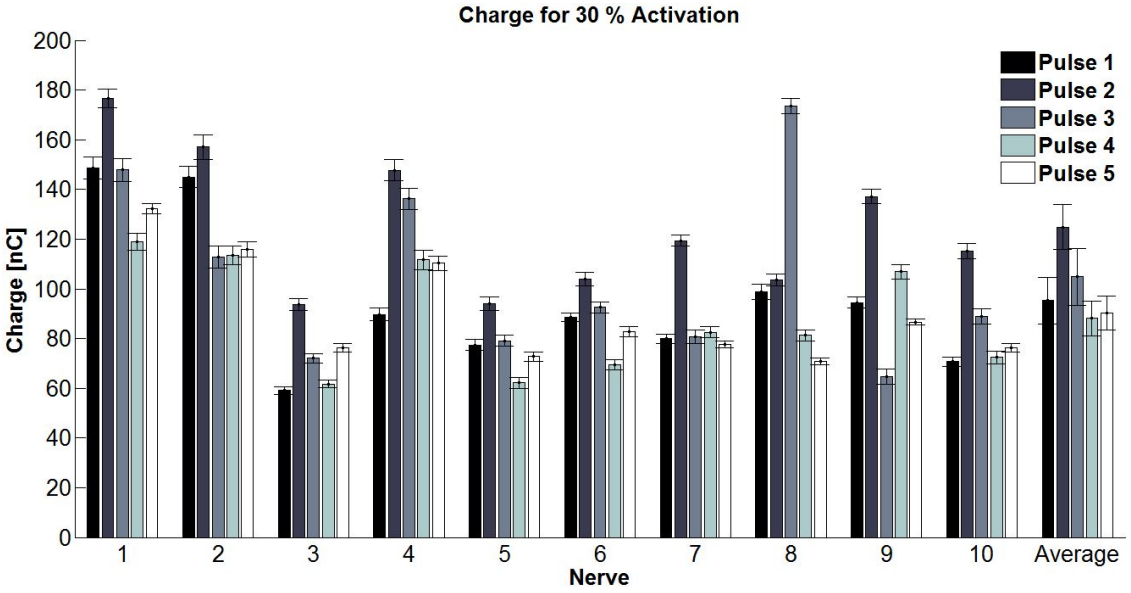

Figure 3.21: *Illustrates the charge induced for activation over 30 % during stimulation in the 10 nerves, with each of the five pulses and for an average of all the five pulses (mean ± SEM).*

The bar plot 3.21 for the individual 10 nerves has a varying amount of used charge in the five pulses. Nerve 1 and 8 both show a high level of charge in pulse two and three respectively. When looking at the average overview of the five pulses, it was seen that pulse two has the highest charge compared to the others, where pulse four showed the lowest level of charge in average.

3.8.5 Device selectivity index

To assess if the added interfascicular electrode increased the device selectivity, the DSI was calculated for both the CICE (configuration 1-12) and for the multi-contact cuff electrode part (configuration 1-6) of the electrode alone. For the calculation of the DSI the best SI across the five pulses were used.

	DSI 1	
Nerve	**CICE**	**Cuff**
1	0.171	0.164
2	0.118	0.081
3	0.0965	0.095
4	0.152	0.144
5	0.107	0.104
6	0.101	0.0999
7	0.138	0.112
8	0.229	0.204
9	0.104	0.0966
10	0.218	0.206

	DSI 2	
Nerve	**CICE**	**Cuff**
1	53.0	50.3
2	40.2	36.8
3	39.9	39.0
4	62.9	57.6
5	42.2	39.2
6	32.8	31.1
7	54.0	51.0
8	99.8	92.2
9	43.3	39.9
10	100	98.0

Table 3.6: Shows the DSI of the CICE (configuration 1-12) and multi-contact cuff electrode (configuration 1-6) during stimulation in all the 10 nerves across all five pulses.

The DSI was higher for the CICE than for the cuff electrode for both the SI 1 and SI 2. This means that the added interfascicular electrodes contribute with a higher selectivity to the CICE than what was seen for the multi-contact cuff electrode. It was seen for SI 1 that the average for the CICE was 0.143 and for the cuff 0.118 (difference of 0.0257). Where the SI 2 has an average for the CICE of 56.8 and for the cuff of 53.5 (difference of 3.4). This shows that there was an individual and overall increase in the DSI for both SI's.

3.8.6 Supra maximal stimulation

For the investigation of the endurance and loss of function of the nerve, the supra maximal stimulation was used. The supra maximal stimulation was used for plotting the maximum activation for each fascicle for each of the 13 supra maximal stimulations for each of the 10 nerves. These were plotted and show how the maximum activation over time changes for each fascicle. On the x-axis is the number of stimulation from 1-13 and on the y-axis is the percentage-wise activation all normalized to the first supra maximal stimulation.

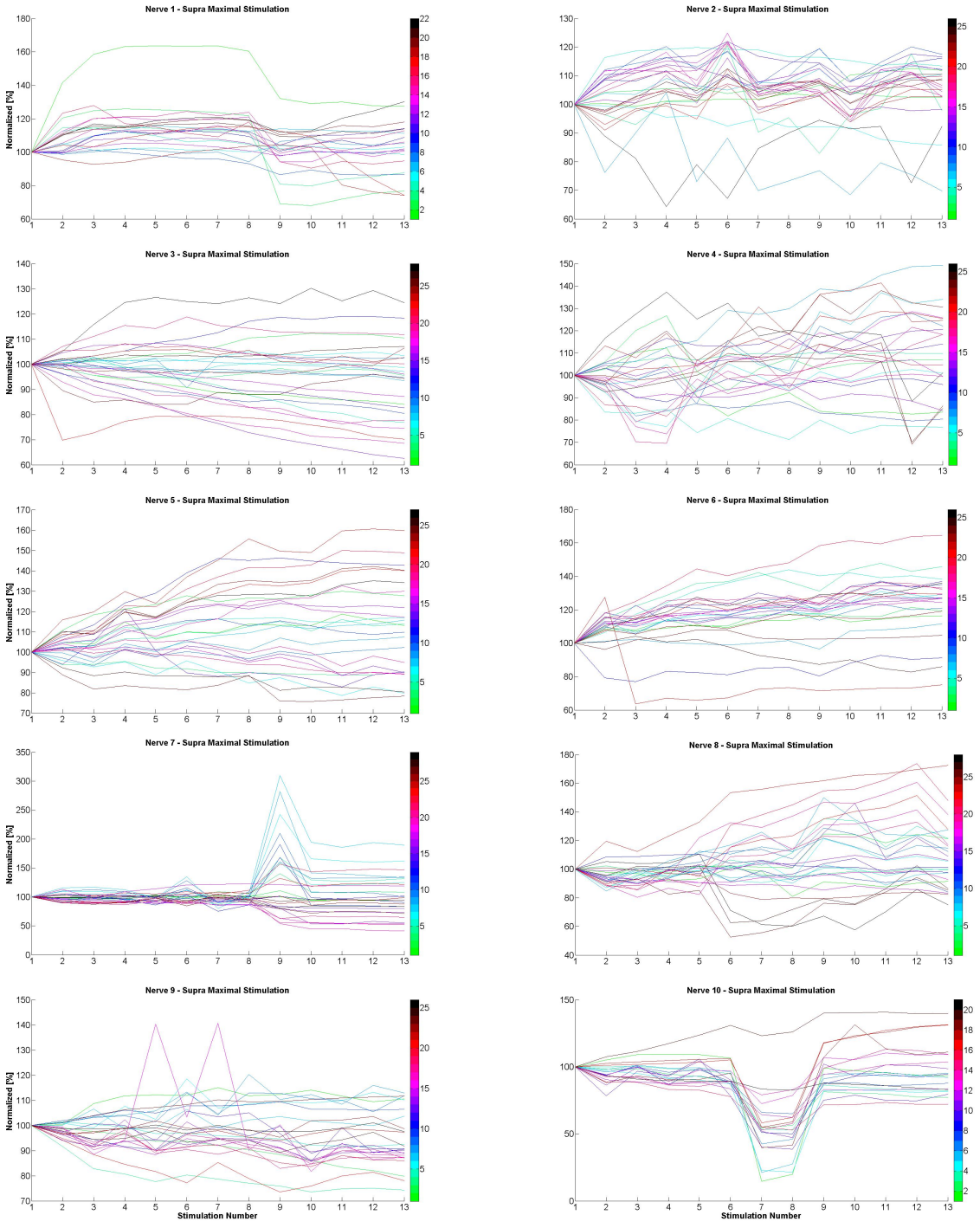

Figure 3.22: *Illustrates the supra maximal stimulation for each of the 10 nerves, with all of the fascicles. The 13 stimulations were normalized to the first supra maximal stimulation. The level of maximum activation throughout the experiment is seen and gives a description of the loss of function of the nerve during the experiment.*

The supra maximal recruitment where plotted (Figure 3.22) with the activation of the individual fascicles for each nerve. The tendency of nerve 2, 3, 4, 5, 6, 8, and 9 are more or less the same throughout the experiment. In these seven nerves some of the fascicles evoked responses were

increased while some are decreased or remained at the same level of activation. Nerve 1 remained the same until at stimulation 8-9 where all fascicles maximum activation decreased. Nerve 7 was stable throughout the experiment except for stimulation 8-10 where it increased rapidly and then decreased to the initial level of activation again. Whereas nerve 10 was also stable throughout the experiment except for stimulation 6-9 where it decreased rapidly and then increased to the initial level of activation again.
This gives an indication of the loss of function during the experiment and it can be seen (Figure 3.22) that the activation level is not stable throughout the experiment for all nerves.

The mean and standard deviation (SD) (Table 3.7) gives a overview of the activation for each fascicles in each nerve. For nerve 1, 2, 3, 5, 6, and 9 there are between 1 and 7 fascicles there has a SD above 10 %. This shows that these nerves were relatively stable throughout the experiment. The nerves 4 and 8 had between 12 and 14 fascicles with a SD above 10 % which is approximately half of the nerves, which indicates that these two nerves were not completely stable during the experiment. Whereas nerve 7 and 10 both had 17 fascicles with a SD above 10 %, this indicates that these nerves were not stable in their activation during the experiment.

Nerve 1

Fascicle	Mean ±SD
1	143±20
2	102±25
3	101±15
4	105±6
5	102±1
6	112±4
7	102±4
8	93.8±6
9	107±5
10	110±5
11	103±4
12	102±2
13	109±11
14	108±6
15	114±9
16	107±13
17	104±13
18	94.0±10
19	114±5
20	113±5
21	117±7

Nerve 2

Fascicle	Mean ±SD
1	105±5
2	104±2
3	99.3±10
4	116±5
5	93.3±4
6	107±3
7	80.4±12
8	112±5
9	114±6
10	109±5
11	114±6
12	105±6
13	104±6
14	111±5
15	108±8
16	109±5
17	107±7
18	107±6
19	105±4
20	101±5
21	99.6±4
22	104±4
23	104±4
24	106±4
25	84.5±11

Nerve 3

Fascicle	Mean ±SD
1	90.6±5
2	107±5
3	96.5±2
4	90.1±9
5	99.7±2
6	103±2
7	100±1
8	97.0±2
9	101±3
10	97.6±2
11	88.2±6
12	112±7
13	92.2±6
14	78.4±13
15	103±4
16	94.0±4
17	86.7±9
18	79.9±10
19	86.1±8
20	112±5
21	104±3
22	77.4±8
23	103±2
24	98.4±3
25	90.0±5
26	104±2
27	122±9

Nerve 4

Fascicle	Mean ±SD
1	91.9±11
2	107±9
3	108±3
4	79.9±7
5	95.7±9
6	113±16
7	127±17
8	107±16
9	89.0±9
10	95.7±3
11	112±5
12	110±6
13	93.9±6
14	97.6±5
15	111±11
16	94.3±9
17	101±12
18	99.3±14
19	99.9±10
20	114±12
21	121±13
22	102±13
23	120±13
24	98.6±11
25	117±14

Nerve 5

Fascicle	Mean ±SD
1	111±5
2	123±9
3	91.8±3
4	109±5
5	112±6
6	88.8±7
7	107±6
8	103±4
9	99.1±2
10	110±5
11	134±16
12	94.3±10
13	97.3±5
14	117±8
15	117±7
16	97.2±5
17	117±11
18	101±4
19	95.6±5
20	131±17
21	125±13
22	139±20
23	127±14
24	82.6±7
25	87.1±5
26	123±11

Nerve 6

Fascicle	Mean ±SD
1	111±5
2	114±6
3	133±14
4	131±14
5	118±8
6	119±9
7	103±5
8	114±7
9	123±10
10	85.9±6
11	125±10
12	118±8
13	118±7
14	118±7
15	120±9
16	119±8
17	125±10
18	124±10
19	143±20
20	121±9
21	77.1±17
22	112±5
23	103±3
24	93.9±7
25	

Nerve 7

Fascicle	Mean ±SD
1	98.2±3
2	98.4±5
3	107±11
4	112±23
5	110±20
6	132±44
7	149±63
8	124±52
9	116±20
10	115±30
11	103±28
12	100±22
13	97.4±3
14	87.6±7
15	84.3±21
16	86.0±10
17	115±8
18	77.5±18
19	74.9±25
20	76.8±19
21	83.4±15
22	122±22
23	111±11
24	94.2±4
25	93.6±6
26	85.5±10
27	98.3±3
28	92.1±8

Nerve 8

Fascicle	Mean ±SD
1	93.1±6
2	109±12
3	99.1±2
4	110±16
5	96.9±3
6	103±9
7	96.0±4
8	116±17
9	109±8
10	110±11
11	93.8±5
12	104±4
13	98.4±5
14	108±15
15	104±4
16	89.7±4
17	94.5±3
18	111±21
19	126±25
20	109±16
21	129±31
22	118±21
23	146±25
24	88.1±9
25	79.6±15
26	86.9±15
27	82.5±20

Nerve 9

Fascicle	Mean ±SD
1	110±5
2	91.0±7
3	80.2±8
4	96.1±3
5	107±6
6	102±3
7	109±6
8	107±5
9	106±3
10	94.5±4
11	97.5±6
12	92.4±3
13	94.4±4
14	92.1±4
15	100±19
16	92.0±4
17	92.5±4
18	90.5±5
19	90.4±5
20	83.0±8
21	97.5±2
22	99.4±2
23	108±4
24	94.8±3
25	98.8±1

Nerve 10

Fascicle	Mean ±SD
1	88.6±32
2	83.1±15
3	86.0±16
4	78.0±26
5	85.4±27
6	86.8±19
7	88.0±11
8	86.9±17
9	78.1±20
10	89.9±19
11	83.1±10
12	97.6±12
13	93.2±8
14	92.0±19
15	76.1±13
16	105±23
17	105±24
18	93.2±26
19	126±14
20	88.2±5

Table 3.7: Shows the mean and standard deviation (SD) of the supra maximal stimulations for all 10 nerves. This shows if the activation of the nerves remains stable throughout the experiment.

CHAPTER 4

NEUROPHYSIOLOGY OF GAIT CONTROL

Gait is the repetitive movement of the limbs during locomotion. Locomotion is important when moving from one place to another for all living creatures and includes e.g. starting, stopping, direction alterations, and speed alterations of gait. Gait consist of rhythmic movements of the legs, which is coordinated by muscular contractions and is controlled by the brain, spinal cord and the peripheral nerves. [Kandel et al., 2000], [Michael-Titus et al., 2007], [Rose & Gamble, 2006], [Latash, 2008]

Four interactive parts of the somatic nervous systems are controlling movement to produce gait (Figure 4.1). These are hierarchically ordered by the lower motor neurons (LMNs) (most distal), upper motor neurons (UMNs), cerebellum, and the basal ganglia (most proximal). The LMNs is the first system and consist of the primary neurons of the brain stem and the spinal cord. The LMNs are the ones that directly control, activate, and coordinate the skeletal muscles for movement. For this activation the LMNs interact with a single motor unit in the muscle. The UMNs is the second system and consist of the premotor neurons that are originating in the cortex. The UMNs modulates the activity of the LMNs by synapses from the descending pathways and do not have any directly affect on the skeletal muscle. [Kandel et al., 2000], [Michael-Titus et al., 2007], [Martini & Nath, 2009]
The cerebellum and the basal ganglia are working in parallel as the third and fourth systems. They are modulating the movement on a minute to minute basis. The cerebellum is mostly excitatory whereas the basal ganglia are mostly inhibitory. The cerebellum and the basal ganglia are directly influencing the UMNs and the LMNs. They both receive input from the motor cortex and sends back input to the motor cortex through the thalamus. This provides the smooth and coordinated movement due to the two systems excitatory and inhibitory effects. [Kandel et al., 2000], [Michael-Titus et al., 2007], [Martini & Nath, 2009]

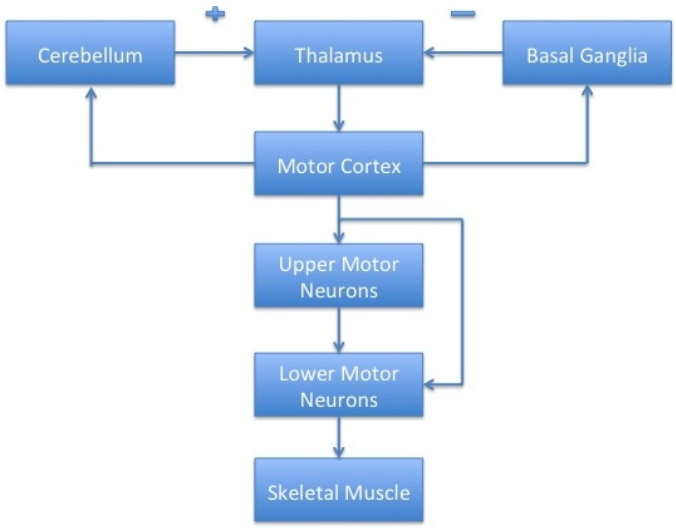

Figure 4.1: *Illustrates the control of the motor functions from the four interactive systems: lower motor neurons (LMNs), upper motor neurons (UMNs), cerebellum, and the basal ganglia. The LMNs directly activates and coordinates the skeletal muscle contractions. The UMNs modulates the activity of the LMNs. The cerebellum and the basal ganglia are working in parallel where they are excitatory and inhibitory respectively. This balance between the excitation and inhibition makes sure that the movement is smooth and coordinated.*

4.1 Gait Cycle

A gait cycle is defined as the time from the first heel contact to the next heel contact of the same foot (Figure 4.2). During the gait cycle there is a stance (extension) and a swing (flexion) phase. During the stance phase were the weight is on the leg the extensor muscles of the hip, knee and ankle are activated. In the early stance phase (first 1/3 of the stance phase) also the flexor muscles of the knee and ankle are activated, whereas in the late stance phase (last 2/3 of the stance phase) all the joints are extending to provide force to move forward. The swing phase commences with flexion of the hip, knee and ankle, whereas midways through the swing phase the knee and ankle starts to extend. This is moving the foot in front of the body and prepares the leg to stand again and bear the weight of the body. [Kandel et al., 2000], [Squire et al., 2008]

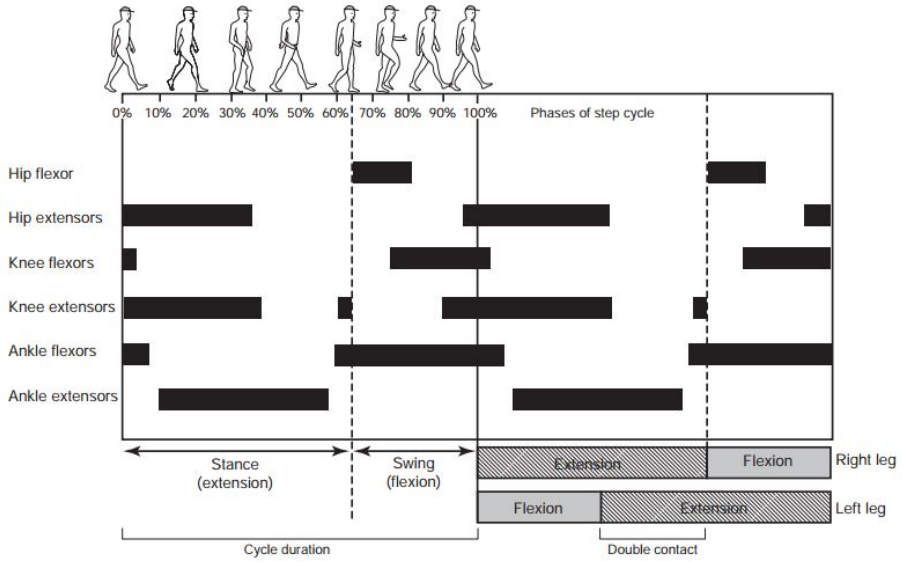

Figure 4.2: *Illustrates a gait cycle. First there is a stance phase (extension), where the extensor muscles of the hip, knee, and ankle are activated. The flexor muscles of the knee and ankle are also activated during the early stance phase. The second phase is the swing phase (flexion), where the flexor muscles of the hip, knee, and ankle are active. During the late swing phase the extensor muscles of the hip and knee are activated to prepare for the stance phase.* [Squire et al., 2008]

4.2 Methods for Analysis of Gait

EMG and recording of kinematics are some of the most common methodological approaches to measure and analyze gait. EMG detects the electrical activity associated with muscle force produced during movement by recording of muscle activity with electrodes placed on the muscle (surface EMG (sEMG)) or in the muscles (iEMG) that are of interest for the specific movement. The kinematic analyzes is the geometry of the movement without taking the forces into consideration, and is obtained through capturing video of the movement. This video is later digitized to the placed markers on the captured video. [Back & Clayton, 2001], [Rose & Gamble, 2006]

4.2.1 Electromyography

EMG recordings that are representing muscle action potentials record the activity of the investigated muscle. This is used in the investigation of gait since it can assist to describe the activity patterns of muscles during gait. For this the amplitude is representing the muscle force produced during a muscle contraction. [Webster, 2010], [Bronzino, 1999], [Latash, 2008], [Back & Clayton, 2001]
Both iEMG and sEMG electrodes are available for recording of EMG signals, but only the sEMG are routinely used in the clinics due to the invasiveness of the iEMG electrodes. For the selection of electrode type the goal for the investigation have to be taken into consideration. iEMG electrodes are preferable in the investigation of fine motor activity in both the extensor and flexor muscles. The iEMG electrodes are more appropriate for the investigation of separate muscles or fibers and for muscles that lies in deeper structures of e.g. the limb. Whereas the sEMG electrodes are more useful for superficial muscles where only the gross activity is desirable to investigated. [Webster, 2010], [Bronzino, 1999]

The difference in activation of both flexor and extensor muscles during gait are clearly recognizable in the EMG signals due to different bursts activity. Raw EMG signals might not be as easily distinguishable due to artifacts (e.g. stimulation or movement artifacts) and surrounding noise (e.g. background noise at 50 or 60 Hz). Therefore preprocessing of the EMG signals is necessary before analyzing the signals. The preprocessing could consist of artifact removal and filtering. After this the desirable information of the muscles activation can be extracted. This information can e.g be the recruitment level. [Webster, 2010], [Bronzino, 1999]

4.2.2 Kinematics

Kinematic analysis is used in the analysis of gait since it studies the relative movement of rigid bodies (each limb segment is considered as a rigid body). The kinematics describes the movement in terms of acceleration, velocity, and displacement without taking the forces that are causing the movement into consideration. One method for recording of kinematics is with the use of videographic analysis. This involves the use of markers placed on the subject, setup and calibration of cameras, capturing of video and digitization. All these factors are important for the following processing of the collected data, since all of them have an influence on the quality of the data. The kinematic analysis can both be two-dimensional (2D) and three-dimensional (3D). The 2D analysis are relatively easy to perform, but is easily affected by image movement caused by out-of-plane movement of the body segments. Whereas the 3D analysis overcomes theses problems but are more complex to perform. This is especially complex for the calibration of the movement space. [Back & Clayton, 2001], [Rose & Gamble, 2006], [Latash, 2008]

External markers placed on the body at the points of interest are needed to detect the line segments of the body segments. The placement of the markers has to be determined by the purpose of the analysis and therefore be placed on body segments that are involved in the investigated movement. For e.g. the investigation of hindlimb movements of gait the points of interest are the toe, ankle, knee, and hip. With the use of these points the joint angles linear movement can be determined of the hindlimb. [Back & Clayton, 2001], [Rose & Gamble, 2006]
To be able to make an automatic digitization of these markers, the markers have to be well defined and stand out from the background. Therefore markers that have a great contrast to the surroundings have to be used for the recording (Figure 5.6 on page 93) and also the lightning has to be controlled to provide a sufficient contrast between the markers and the surroundings. Furthermore the repeatability of positioning the markers at the same place for each experimental subject, and to replace the markers if they fall of during the experiment, has to be considered. [Back & Clayton, 2001], [Rose & Gamble, 2006]

For the video recording of the movements for 2D analysis the camera have to be oriented precisely perpendicular to the plane of interest whereas for the 3D analysis the markers just have to be visible to at least 2 cameras at a time without a specific angle in between them. Although there is no need for a specific angle in between the cameras the accuracy is decreasing if the ankle becomes too small. [Back & Clayton, 2001], [Rose & Gamble, 2006]
The cameras are tracking and recording the markers placed on the body while the experimental subject perform the desired movement. The number of frames per second should be considered and chosen dependent on the movement that is being investigated. For e.g. locomotion recordings of 60 frames per second have shown to be adequate. To make sure no information is lost higher frequencies have to be used for fast movements of short duration. Whereas lower frequencies can be used for slower movements that last for an extended period of time. [Back & Clayton, 2001], [Rose & Gamble, 2006]

The digitization of the reflective markers that indicates the joints that are activated during the movement is through an offline digitization program. When the points have been determined the body segments can be identified. The digitization is often performed automatically by the digitization program (after a manual marking on the first frame of the different markers), but can also be performed manually if the program has difficulties in differentiation of the markers or when markers are obscured. The user must also always check the accuracy of the digitization performed by the program before further analysis. After the digitization it is possible to determine the movement of the body segments along with the angle between these. The joint angles can then be plotted where a recognizable pattern of the gait cycle should be visible. [Back & Clayton, 2001], [Rose & Gamble, 2006]

There are different things to have into consideration for each of the steps for the use of kinematic recordings. These are the marker placement on the subject, capturing of video and digitization (Table. 4.1)

Parameter	What to have in mind
Marker placement	- Placed on body segments involved in the movement - Great contrast between the markers and the surroundings - Repeatability of positioning the markers
Capturing of video	- Markers have to be visible to at least two cameras at all times - Number of frames per second recorded have to chosen in regard to the specific movement - Lighting of the captured picture for contrast to markers
Digitization	- Automatically digitization might have difficulties in differentiation of the markers or when markers are obscured - Always inspect the automatically digitization for errors

Table 4.1: *Shows the different parameters that have to be considered for the use of kinematic analysis.*

CHAPTER 5

EXPERIMENTAL METHODS FOR INVESTIGATION OF INDUCED MUSCLE FATIGUE

5.1 Rat Model

For the current investigation a rat model was used because the dysfunction and damage process of a SCI in rats are similar to what occurs in humans. Besides this it is also an advantage that the rats are big enough to easily conduct the surgery, and is easy to manage and is not that costly, compared to other animals. [LeDoux, 2005], [Chen, 2009]

The model used for the current study consisted of an anesthetized rat. When working with an anesthetized rat model the only output seen, would be from the FES. This means that it would only be the induced movement without any voluntary movement. This was preferred when the investigation was about inducing the rhythmic movement and when fatigue occurs.
For the anesthesia of the rat Isoflurane gas and Sodium Pentobarbital were used. The Isoflurane gas is one of the standard inhalation anesthetics for laboratory animals. This is normally used because it has a rapid induction time and recovery time. It is also easy to administer trough inhalation and to adjust the level of anesthesia. Sodium Pentobarbital is one of the most used barbiturates. It is most often longer lasting than other injectable agents. The Sodium Pentobarbital makes sure it only was the movement that the stimulation produced, which was observed. [Services, 2013]

5.2 Electrode Design

To be able to stimulate, induce movement and induce fatigue a neural interface for selectively activating the muscles for the movement is needed. Besides this it was preferred to mimic the normal physiology as much as possible for the stimulation. Under normal conditions the small, fatigue resistant muscle fibers are recruited before the recruitment of the larger fibers. When electrical stimulation is applied to the body the larger fast fatigable fibers are the first to be activated. Therefore it was desirable to use a PNE, which can use lower currents to obtain the same effect as other electrodes that use higher currents.

The electrodes used for the stimulation were LIFEs. These were used because intrafascicular electrodes, gives a higher selectivity relative to e.g. surface or cuff electrodes that also are available (Section 2.2 on page 45). The higher selectivity should make it easier to only stimulate the fascicles that innervate the desired muscles. Another benefit with the LIFEs was that they are stimulating at the nerve and thereby the needed current for the activation of the muscle is lower than seen with a surface electrode. This is an important quality of the electrodes because

with low current it should take longer to induce fatigue.

The paper from Bourbeau et al. describes that the diameter of the axon has no influence on the low current recruitment. This is because a neuron will fire as long as the electrode is near to the node of ranvier. It is known that there is a shorter distance between the nodes of ranvier in smaller diameter axons than larger diameter axons. It is also known that there are a higher number of smaller diameter axons than the larger diameter ones. Therefore by using LIFEs with low currents it is more likely to stimulate the nodes of ranvier of smaller diameter axons. This also means that the larger fast fatigable fibers might not be the first to be stimulated and this might postpone the fatigue until higher currents are needed. [Bourbeau et al., 2011]

To be able to induce movement of the ankle of the rat an extensor and a flexor muscle have to be activated. To activate the muscles the LIFEs have to be placed in the nerve fascicles that innervate the two muscles. The criterion for the selection of muscles required main muscles for the movement and that there was a relatively easy access to the innervating nerves. The Gastrocnemius Medialis (GM) was chosen as the extensor muscle and the Tibialis Anterior (TA) as the flexor muscle. These two muscles are two of the main muscles involved in the movement of the hindlimb of the rat, which makes them preferable. The GM and TA fascicles can both be accessed in the sciatic nerve so only one nerve had to be exposed to implement the electrodes.

5.3 Stimulation Design

For the stimulation used for the current study an open-loop paradigm was applied to the FES system. It was desirable to investigate the development of fatigue during normal conditions as in the clinics where the open-loop design is used today. With the investigation of the stimulation used in the clinics today, it could be investigated if this could be improved and if the methods used are providing reliable information for a later implemented closed-loop system.

For the use of the open-loop controller, the FNS-16 Multi-Channel Stimulator (Cwe inc. commercial available stimulator) provided electrically isolated rectangular current pulses. The stimulation levels were in the range of 0-300 μA with a pulse width of 30-300μs. This range was based on data from previous clinical and non-clinical studies [Fairchild et al., 2010], [Kim et al., 2009]. The current open-loop stimulation system, manually set the parameters to stimulate the muscles rhythmically over 100 cycles (Figure 5.1). A cycle was defined as being one stimulation of the TA and of the GM with a pause in between them. First the TA was stimulated for 110 ms, and then there was a 20 ms silence period, followed by GM stimulation for 310 ms. This would then be followed by a 60 ms silence period before the next cycle started again with stimulation of the TA. In each cycle the muscles were stimulated with biphasic charge balanced pulses.
The biphasic pulse gives an overall zero net charge. This was to damage the surrounding tissue as little as possible and not to damage the electrodes as well. The first pulse was set to be negative because it was desired to activate the neurons, which is done trough depolarization. [Merrill et al., 2005], [Cogan, 2008]

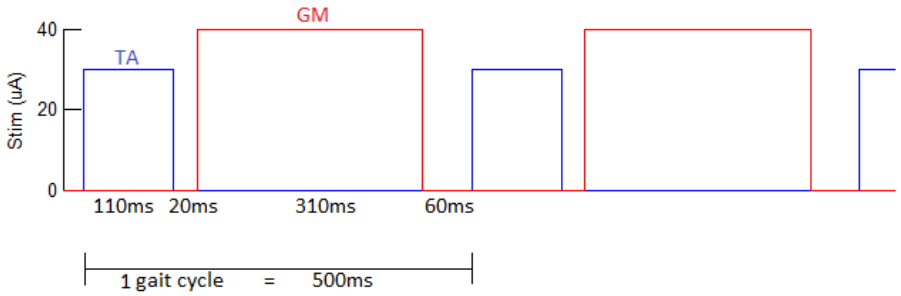

Figure 5.1: *Illustrates the stimulation cycles for the stimulation. The blue line is representing the stimulation of the TA and the red line the GM. It is seen here that there are a difference in the amplitudes of the two stimulations. This is due to the amplitudes selected are specific to the individual muscle. The cycle starts with the TA being stimulated for 110 ms, followed by a 20 ms pause before 310 ms stimulation of the GM. This is followed by a 60 ms pause before the next cycle starts again with a stimulation of the TA.*

The electrode placement was tested before closing the rat since the stimulation parameters depends on successful implementation. To test if the electrodes were placed correctly the charge balanced symmetric biphasic pulse was used to see if a twitch was seen in the hindlimb (Figure 5.2). To find the optimal location of the electrodes the amplitudes of the stimulation was tested between 10, 20, 30 and 40 μA. The range of amplitude gives an indication on how well the electrodes were placed.

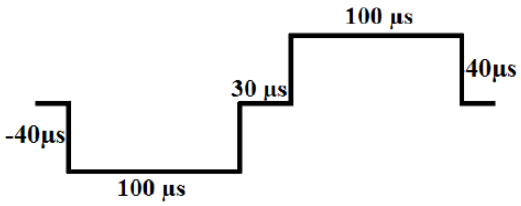

Figure 5.2: *Illustrates the biphasic pulse for the test of the electrodes correct placement. The amplitude of the pulses varies between 10, 20, 30 and 40 µA according to how well the electrodes were placed.*

5.4 Recording Design

To investigate if iEMG recordings can provide a reliable information on functional movement during development of fatigue, iEMG and kinematic recordings were used.

iEMG recordings were used to give a measure of the produced movement of the GM and TA muscles during the experiment. This was done to investigate the muscle force that was produced in the TA and GM muscles. This would provide information about when fatigue was occurring during the experiment. A single differential electrode was designed to avoid noise (e.g. 50 or 60 Hz noise or crosstalk) in the signals. The collected data would be further analyzed offline using MATLAB® to give a measure of the produced muscle force.

Another method for measuring the movement and when fatigue was produced was kinematic recordings. To measure the angle of the ankle a kinematic system (Peak system, Peak Perfor-

mance Technologies, Inc. Centennial, CO) was used. This gave a description of the joint angle data for the stimulated limb. To obtain the kinematic data cone shaped three dimensional reflective markers made from 3M infrared reflective tape was placed on the rat. These were placed on the bony projections of the hip, knee, ankle and toe of the rat hindlimb (Figure 5.6 on page 93). The two infrared cameras of the Peak Motus system was focused to capture the reflective markers during hindlimb movement. The captured video was further analyzed offline using MATLAB® to determine the joint angles.

For the recording there were two parts, both the iEMG recordings and the kinematic recordings. Custom made iEMG electrodes were inserted into the GM and TA of the hindlimb for the recording of the iEMG signals. The iEMG were connected to the A-M systems differential AC amplifier (Model 1700) with a gain of 100. This was then connected to the DAC (NI USB-6259 16-bit), then to the computer and seen in LabView on a computer. The iEMG signals were sampled at a sample frequency of 10 kHz. For the kinematic, two infrared cameras was used for capturing the video of the cone shaped three dimensional reflective markers. This was recorded by the Peak Motus system, with a sampling frequency of 60 Hz.

5.5 Experimental Setup

The experimental setup was consisting of:

- Gas anesthesia (isoflurane gas (0.5-5.0 %) and sodium pentobarbital (40 mg/kg ip))
- Four LIFEs
- Two iEMG electrodes
- FNS-16 Multi-Channel Stimulator (commercial available stimulator)
- Two infrared cameras
- Computer with LabView and Peak Motus system
- Body and tail restraints

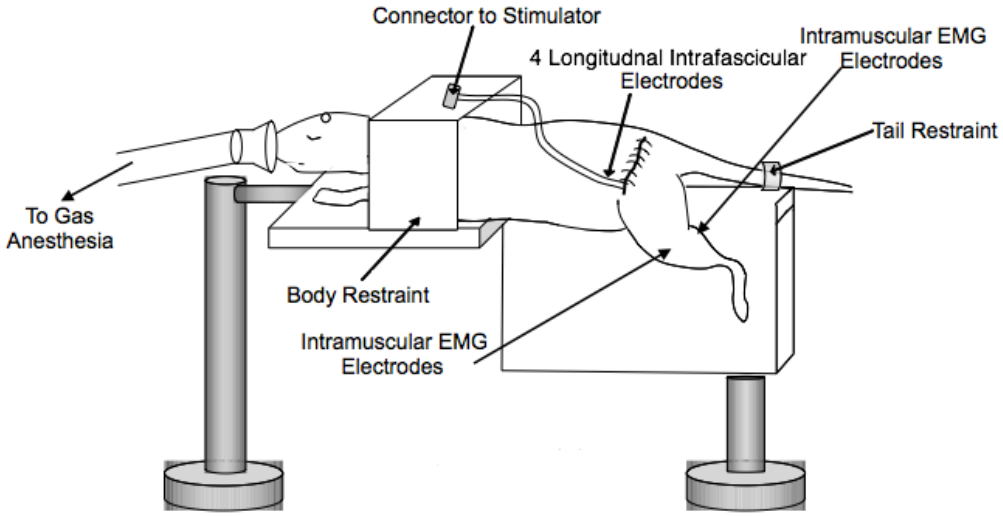

Figure 5.3: *Illustrates the experimental setup. It is seen that the rat was placed in a prone position with the legs hanging freely. The rat was anesthetized using gas anesthesia, iEMG electrodes were inserted in the GM and TA muscles of the left hindlimb, and LIFEs were inserted in fascicles of the sciatic nerve innervating the GM and TA muscles.*

After induction of anesthesia with isoflurane gas (5 %), a single injection of Sodium Pentobarbital (40 mg/kg ip) was given. Anesthesia was maintained with isoflurane (0.5-3.0 %), throughout the experiment. The level of anesthesia was assessed with toe pinch, and observation of eye blink and the respiration rate. To prevent dehydration regular subcutaneous injections of isotonic saline in the dorsal cavity were administered.

To generate the stimulation the FNS-16 Multi-Channel Stimulator was used. To control the stimulation a custom made LabView program was used.

5.6 Experimental Procedure

5.6.1 Construction of electrodes

Before the experiments took place the LIFEs and iEMG electrodes were constructed. For the fabrication of the LIFEs the method from the paper by Malagodi were used [Malagodi et al., 1989].

Materials for longitudinal intrafascicular electrodes

- 50 μm diameter Isonel insulated platinum wire (Pt-Ir wire)
- 50 μm diameter Teflon insulated wire
- 7 μm diameter carbon fiber
- 75 μm diameter tungsten needle
- Insulating varnish
- Cyanoacrylate adhesive
- Dilute solution of NaOH
- 2x3 mm Aluminum plate

Procedure for longitudinal intrafascicular electrode construction

For the construction of the LIFEs the tungsten needle was welded to the Pt-Ir wire using a micro-spot welder. Then the end of the electrode was attached to the tungsten needle with cyanoacrylate adhesive. The Teflon wire was stripped from a zone approximately 20 mm back from the end of the wire. The tungsten needle was then heated with a voltage source and following this it was sharpened in a electrochemical etching dilute solution of NaOH. The aluminum plate is welded to the end of the electrode for the connection to the amplifier.

Materials for intramuscular electromyographic electrode

- 0.7 mm needle
- Tape
- 0.1 mm stainless steel wire
- Micro scissors
- Tweezer

Procedure for intramuscular electromyographic electrode construction

For the construction of the iEMG electrodes two needles were taped together at the plastic hut. A stainless steel wire was lead through each needle and de-isolated at both ends. The wire was bended to form a hook at the needle end.

5.6.2 Kinematic camera setup

For the experiment the first step was to calibrate the two infrared cameras for the kinematic recordings. This was done with a calibration setup made at the ANS Lab at FIU. (Figure 5.4). Following this the data acquisition could take place right after the preparation of the rat.

Materials for kinematic camera setup

- Computer with Peak Motus system
- Calibration device
- Two infrared cameras
- Colored tape

Procedure for kinematic camera setup

The Peak Motus system was turned on. The control object was placed where the rat was going to be placed during the experiment. The cameras were placed so that they captured the control object with an approximate angle of 90 °. The focus, lighting and zoom of the cameras were adjusted for the most optimal picture. The calibration was captured, followed by a marking of all the rods (2-9) for each point (1-5) in the Peak Motus system.

Figure 5.4: *Illustrates the calibration device that was used for the calibration of the two infrared cameras, which was used to collect the kinematic data. Each of the reflective points on the 8 pins was marked in the Peak Motus system.*

5.6.3 Preparation of rat and nerve separation

Following this the rat was anesthetized and prepared for the experiment. After the preparation of the rat, the rat was opened and the sciatic nerve was exposed in the hindlimb for the insertion of the four custom made LIFEs (Figure 5.5).

Materials for preparation of the rat

- Weight
- Isoflurane
- Anesthesia machine
- Oxygen
- Sodium Pentobarbital

- Shaver
- Alcohol swabs
- Refractory system
- Heating blanket
- Thermometer
- Warming pads
- Sodium chloride
- Needle
- Syringe

Procedure for preparation of the rat

For the preparation of the rat, the rat was first weighted for the calculation of the appropriate amount of anesthesia. The first anesthesia administered was the Isoflurane gas anesthesia (5.0 %). The rat was then observed to verify when it was sleeping, this was investigated through toe pinching, and observation of respiration rate and eye blinking. When the rat was sleeping 40 mg/kg ip injection of Sodium Pentobarbital were given. Afterwards the rat was shaved, wrapped in a heating blanked and placed on the refractory system. Then gas anesthesia was given through a tube with a constant flow of anesthesia (0.5-3.0 %) throughout the rest of the experiment. As the last part a thermometer probe was placed in the rectum of the rat to monitor the temperature throughout the experiment. If the temperature was to low a heating pad was placed under the thorax of the rat, and if it got to warm the heating blanked was removed. Sodium chloride was injected to avoid dehydration throughout the experiment.

Materials for nerve separation

- Scalpel
- Forceps
- Tweezers
- Scissor
- Saline
- Syringe
- Custom made glass pipette
- Rubber band
- Four LIFEs
- Computer with LabView program for control of the FNS-16 Multi-Channel Stimulator
- Suture

Procedure for nerve separation

An incision was made in the hindlimb, where the muscles were separated to reach the sciatic nerve. When the nerve was visible saline was poured over the nerve to avoid dehydration. This was done during the whole experiment. Then the sciatic nerve was separated from its connections, where attentions had to be payed not to touch the nerve with metal. The perineum of the nerve was separated to be able to get a better view of the fascicles. A rubber band was placed underneath the nerve to stretch the nerve a little bit to be able to see the individual fascicles. The four LIFEs where then inserted, two in the GM fascicles and two in the TA fascicles. After the insertion the placement of the electrodes were investigated by using the LabView program to control the FNS-16 Multi-Channel Stimulator. The current was changed up and down (10-40 μA) until a twitch was observed in the rat's foot, this was done for all four electrodes. The LIFEs

for both muscles with the lowest needed current to obtain a twitch was chosen and used for the rest of the experiment. Following this the electrodes were sutured to keep them in place during the experiment and the rat was closed.

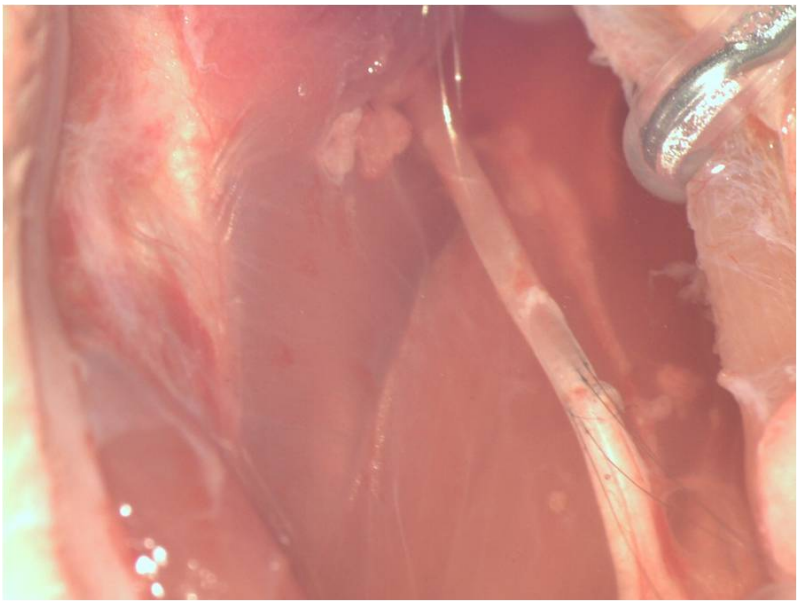

Figure 5.5: *Illustrates how the four LIFEs were placed in the sciatic nerve. Two of the electrodes in the TA and two in the GM.*

It was investigated if the LIFEs were placed correctly in the fascicles for the TA and GM. During this saline was poured over the nerve regularly to avoid drying. The iEMG electrodes were inserted into both the GM and the TA (Figure 5.6). The cone shaped three dimensional reflective markers were placed on the hip, knee, ankle and toe (Figure 5.6). This was done so that the joint angle of the hindlimb could be calculated with kinematic data analysis.

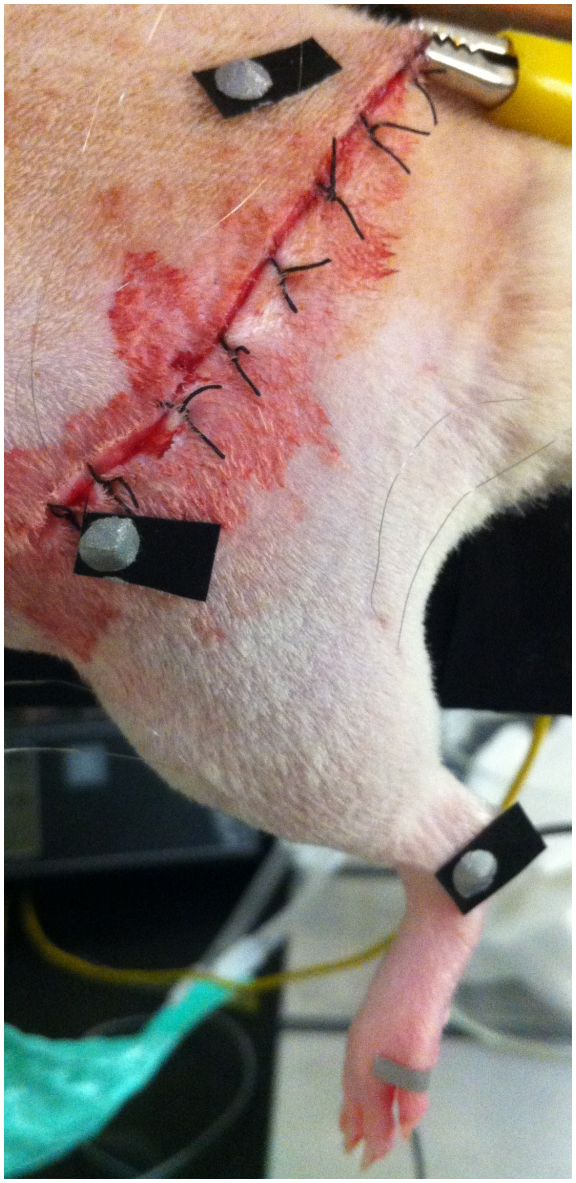

Figure 5.6: *Illustrates how the iEMG electrodes were placed in the TA and GM muscles. It is also seen how the cone shaped three dimensional reflective markers were placed on the hip, knee, ankle, and toe. These markers were used for the kinematic data analysis.*

5.6.4 Strength duration curve

When all of the preparations were finished a Strength Duration Curve (SDC) was made (Figure 5.7) to investigate which pulse width and amplitude was appropriate for the specific rat.

Materials for strength duration curve

- Computer with LabView program developed at the ANS Lab at FIU
- FNS-16 Multi-Channel Stimulator

Procedure for strength duration curve

First the LabView program developed at ANS Lab at FIU for the SDC stimulation was turned on. The four LIFEs were connected to the FNS-16 stimulator one at a time to create a SDC curve for each of them. The pulse widths used was between 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 and 300 μs. For each of the pulse widths the amplitude was raised by 10 μA until a twitch was seen in the hindlimb. Following this the SDC was plotted and from the SDC appropriate combination of pulse width and amplitude was selected as stimulation parameters for the experiment. The pulse width used for the rest of the experiment was the pulse width, which reached a plateau. The amplitude used was the amplitude from the plateau multiplied by 1.5. The electrodes used for the rest of the experiment were the ones with the lowest charge for both the TA and GM. The frequency of the stimulation was determined by visual inspection of when a twitch was seen.

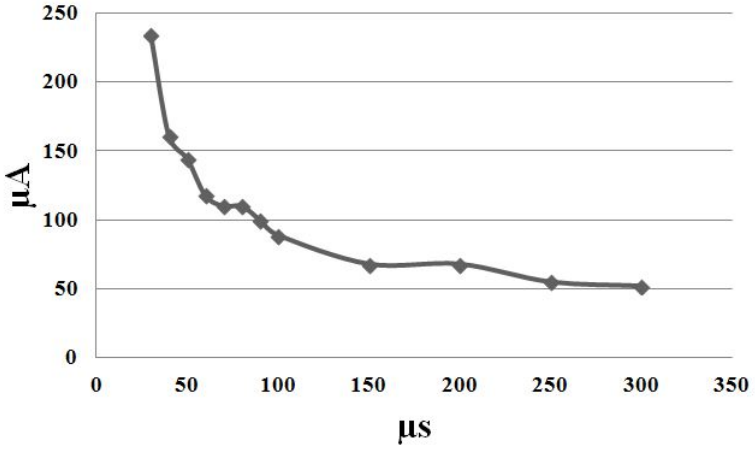

Figure 5.7: *Illustrates the strength duration curve that was made from the stimulation to investigate when a twitch occurs with different pulse width (30-300 µs) with increasing amplitude (0-300 µA). From this the pulse width and amplitude was chosen according to when a plateau was reached.*

5.6.5 Collection of data

Following there was a data collection with the set parameters of the pulse width and amplitude but with increasing frequencies. This was applied to both the best GM and TA channels. This stimulation was given to investigate, which frequency gave a fused contraction. This data was only investigated to see when a fused contraction was seen.

For the data acquisition of the open-loop controller the determined pulse width and amplitude was used with alternating frequencies of 30-70 Hz. Each frequency was recorded for 100 cycles followed by a 15 min resting period without any stimulation. The open-loop stimulation was with both the TA and GM muscle activation. This was to investigate the coordinated movement of the hindlimb.

5.7 Data Analysis

The data analysis of the recorded signals were as illustrated in figure 5.8. The preprocessing was divided in two: Kinematic data and iEMG signals.

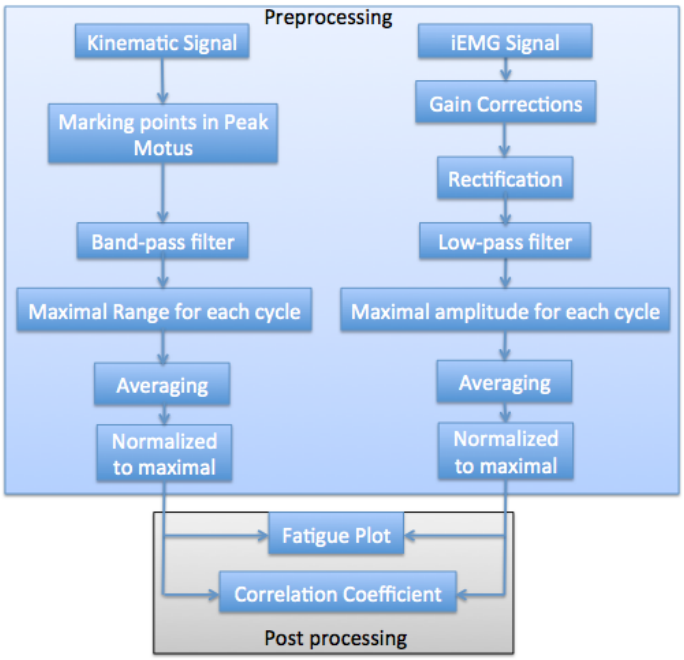

Figure 5.8: *Illustrates the flow of the data analysis for both the kinematic and iEMG data. For the kinematic data during the preprocessing that the points were marked in the Peak Motus System, and was afterwards band-pass filtered. Following this the maximal range of the GM and TA (from rest) for each cyclic movement was calculated and then averaged for all three rats, afterwards it was normalized to the maximal range. For the iEMG data there was first a correction of the gain followed by a full wave rectification and then a low-pass filtering of the signal. Hereafter the maximal amplitude for both the GM and TA for each cyclic movement was calculated, this is followed by an averaging of the three rats. For the postprocessing a fatigue plot was made for the maximal ankle angle and iEMG amplitude for both the GM and TA along with the correlation coefficients between these.*

5.7.1 Preprocessing of kinematic data

The kinematic data was analyzed in the Peak Motus System as a part of the preprocessing. Here a marking of the points representing the markers placed on the hip, knee, ankle and toe for both camera views were made (Figure 5.9). First they were marked manually in the first frame afterwards the Peak Motus System would go through the rest of the frames and automatically mark all four points in the frames. An investigation of the frames was performed manually to verify that the markers were correctly detected. The data was digitized and the Peak Motus System filtered the data (4th order band-pass Butterworth filter). The Peak Motus System determined the cut-off frequencies automatically. If there were any digitizing filtering errors these would then be filtered out. Following this the kinematic data would be analyzed in MatLab®.

Figure 5.9: *Illustrates how the four markers of the toe (blue), ankle (red), knee (black) and hip (orange) were marked in the Peak Motus System.*

The ankle angle for both the GM and TA was calculated for each cyclic movement to determine when fatigue occurs in the kinematic data. This was calculated from the resting point, which was set at the angle the hindlimb had before any stimulation was induced, to either the maximal extension or flexion. The ankle angle was calculated for all three rats, which was afterwards averaged. When this was calculated the maximal range of the muscles was set to 100 % and the data was normalized to this.

5.7.2 Preprocessing of intramuscular electromyography signals

The iEMG signals were loaded into MatLab®. The first part of the preprocessing was a correction of the gain that was applied during the recordings of the signal. After this the signal was full wave rectified and filtered (3rd order low-pass Butterworth filter with 0.5 Hz cut off frequency). The amplitude for both the GM and TA was calculated for each cyclic movement to determine when fatigue occurs in the iEMG signals. This amplitude was calculated for all three rats, which was afterwards averaged. Following this the maximal range of the muscles was set to 100 % and the data was normalized to this.

5.7.3 Evaluation of fatigue based on iEMG signals

The representation of the kinematic data and iEMG signals were a computation of a fatigue plot. The ankle angle and the iEMG amplitude envelope of both the GM and TA were plotted. Fatigue was defined as a decrease in the ankle angle and the iEMG amplitude envelope.
Furthermore a correlation coefficient was calculated between the ankle angle and iEMG for both the GM and TA muscles for each frequency.

Appendix

A. Selectivity Score Appendix

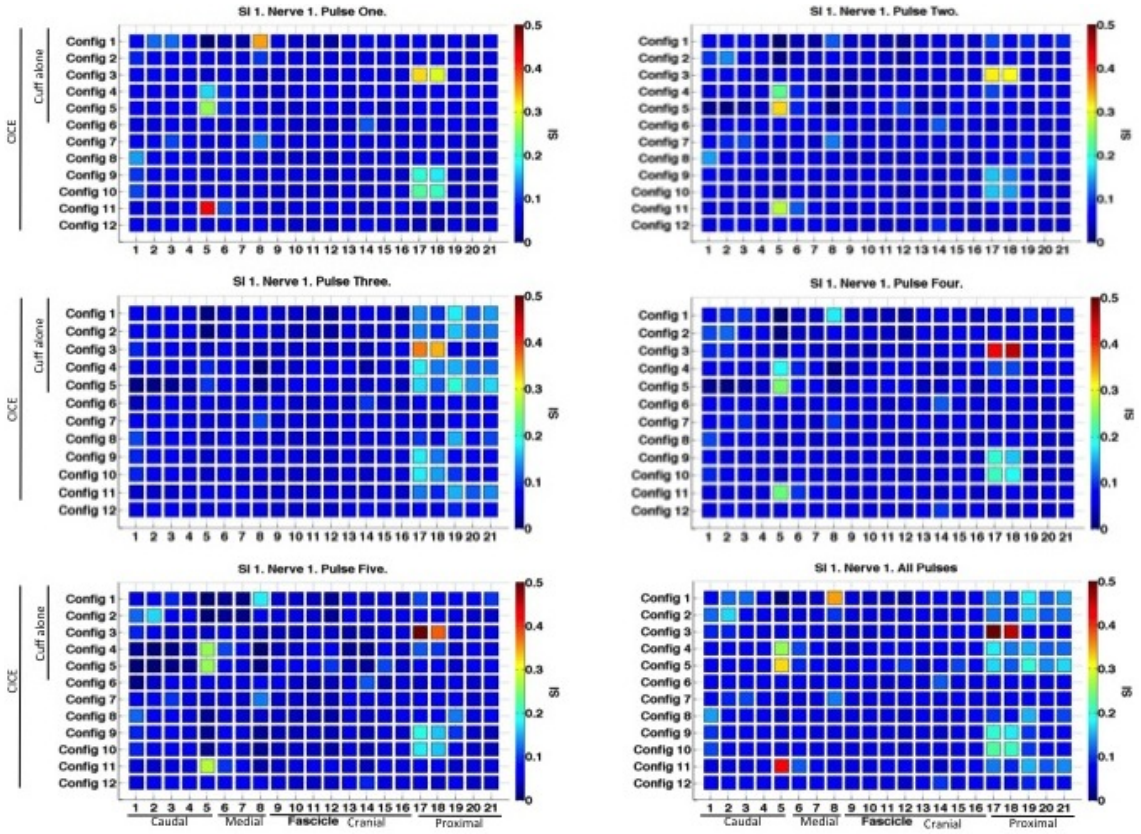

Figure 1: *Illustrates a color plot of SI 1 for nerve 1, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. For all the pulses except pulse three the same tendency is observed. The difference is the level of SI there is reached for the individual fascicles in the nerve. Pulse three has some noticeable activation in mostly the proximal branch of the nerve.*

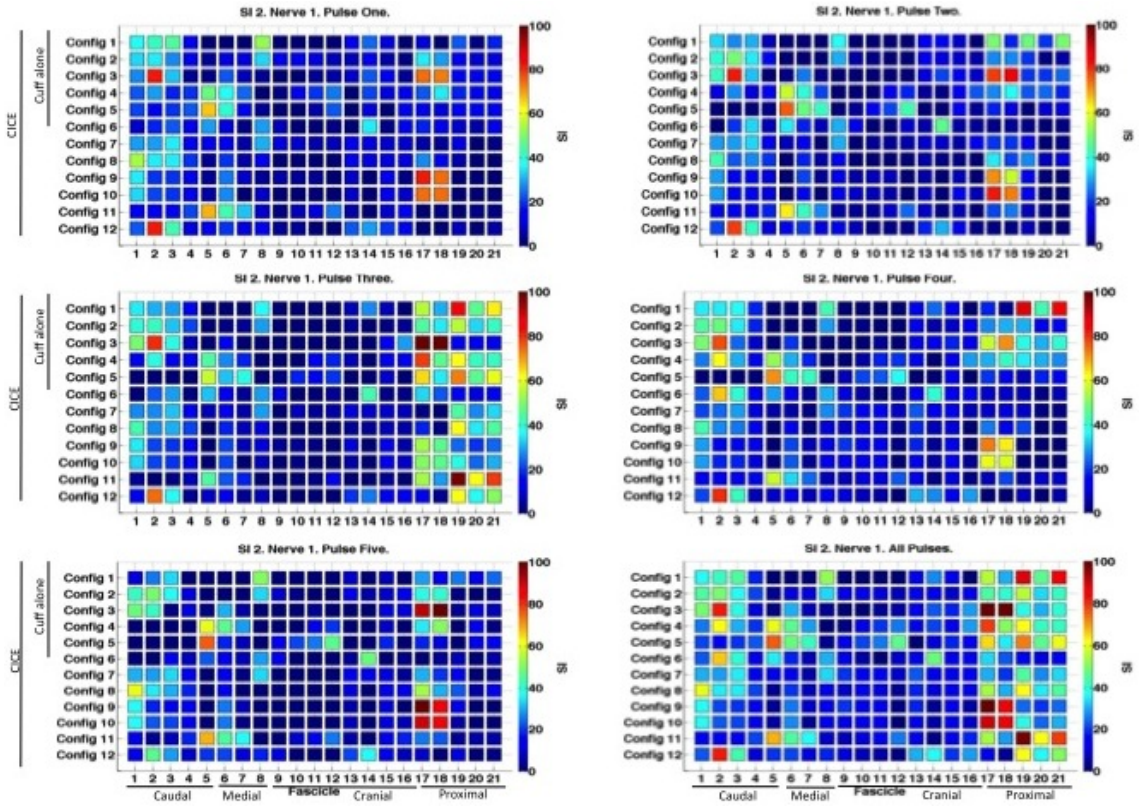

Figure 2: *Illustrates a color plot of SI 2 for nerve 1, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. For pulse three a higher SI is seen in the proximal branch.*

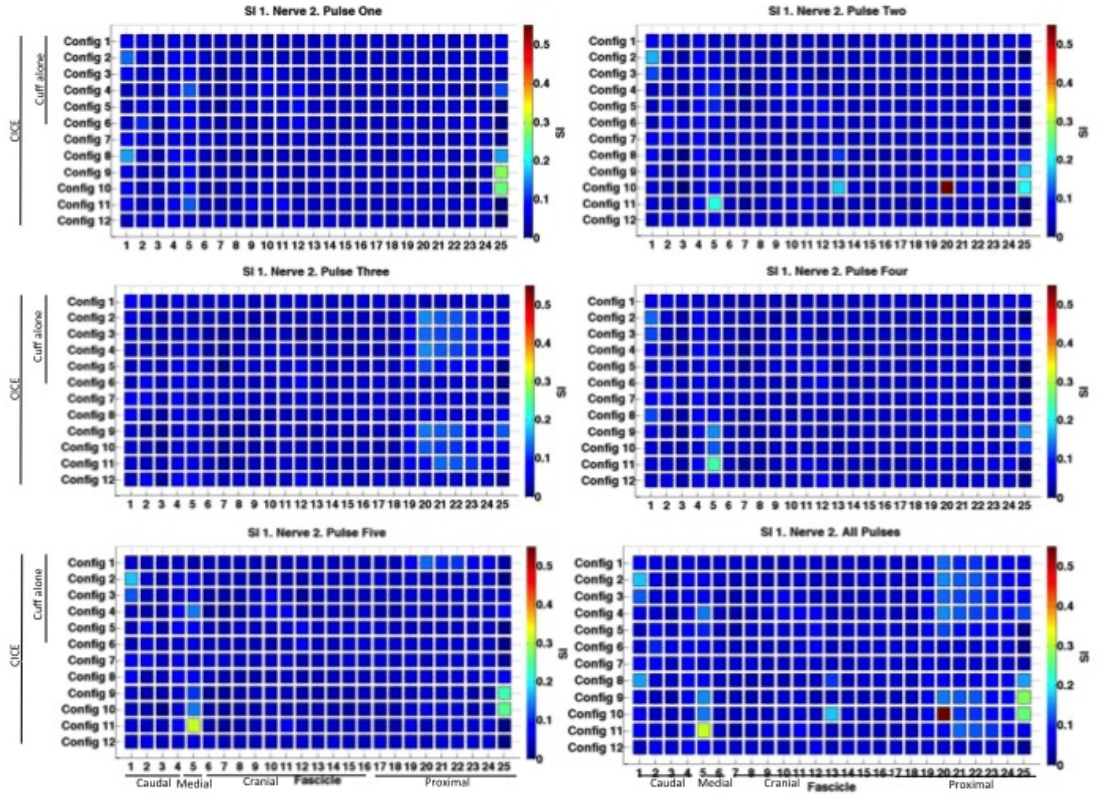

Figure 3: *Illustrates a color plot of SI 1 for nerve 2, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. A little activation and low SI is seen for all five pulses.*

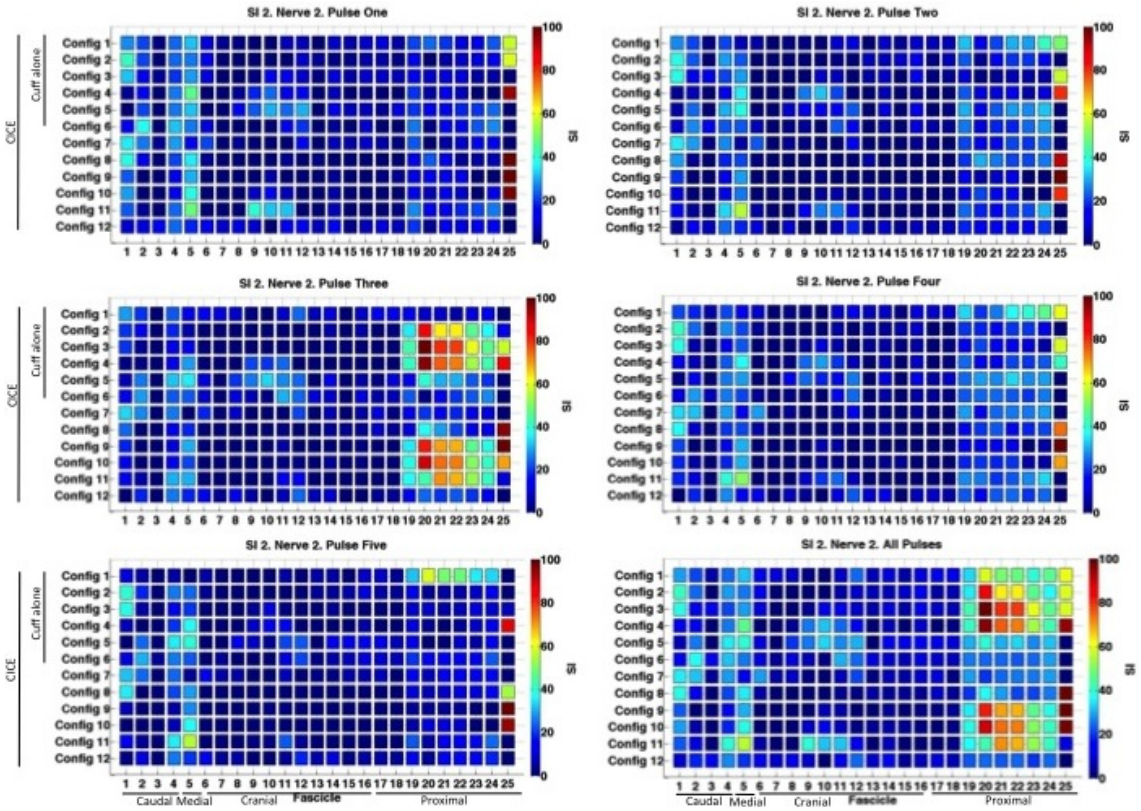

Figure 4: *Illustrates a color plot of SI2 for nerve 2, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. Pulse one two, four and five has a higher SI in fascicle 25, where as pulse three has a high SI for most of the proximal fascicles.*

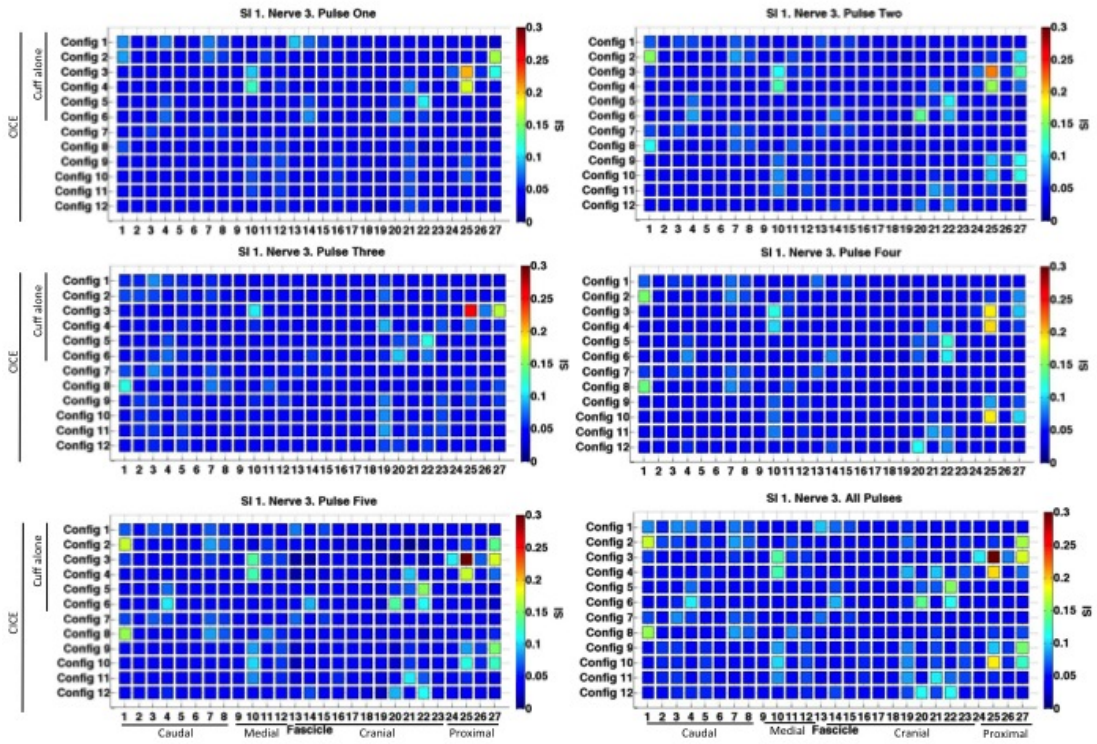

Figure 5: *Illustrates a color plot of SI 1 for nerve 3, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. Here an overall low SI is seen except for in few fascicles*

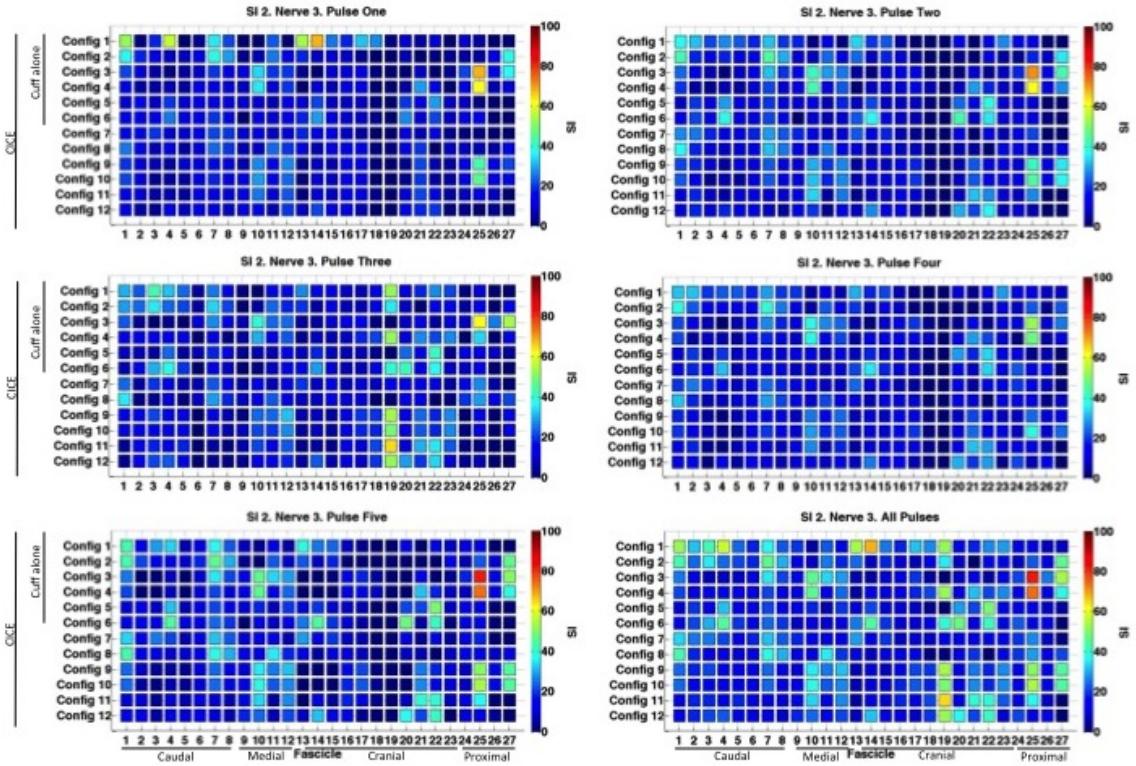

Figure 6: *Illustrates a color plot of SI 2 for nerve 3, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. The tendency for all five pulses are similar, but with a difference in the level of SI.*

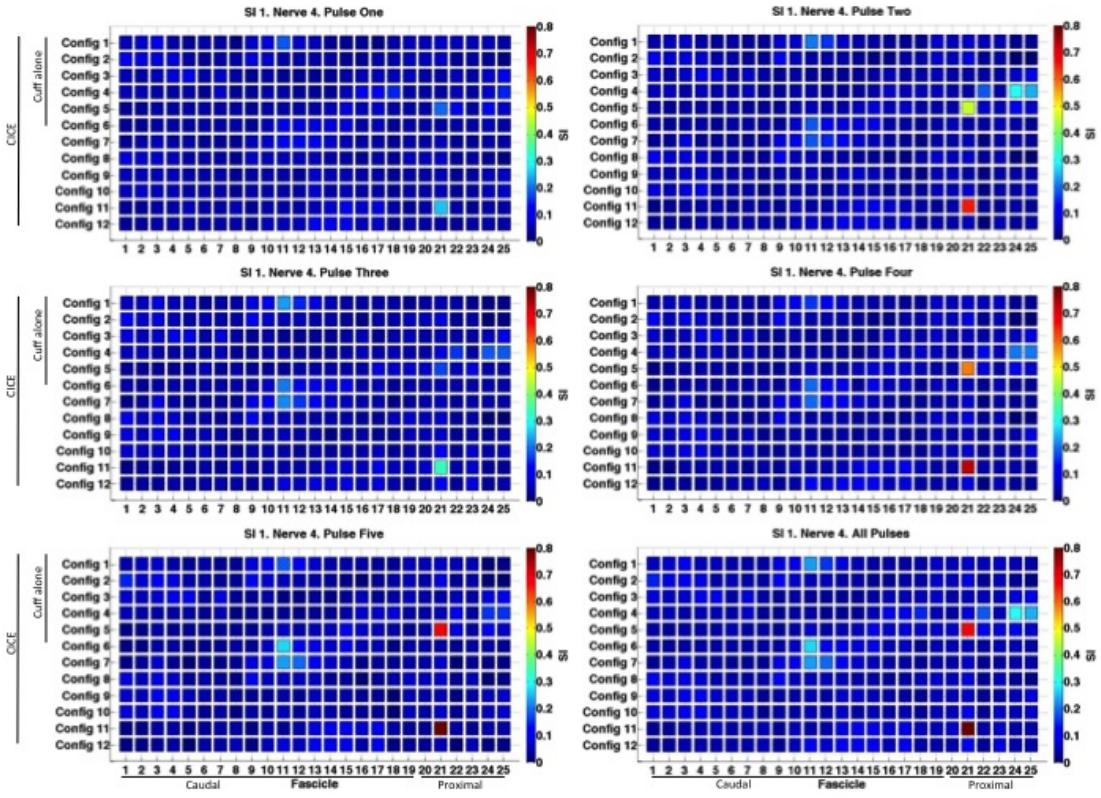

Figure 7: *Illustrates a color plot of SI 1 for nerve 4, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. Si was low in five pulses except for very few fascicles.*

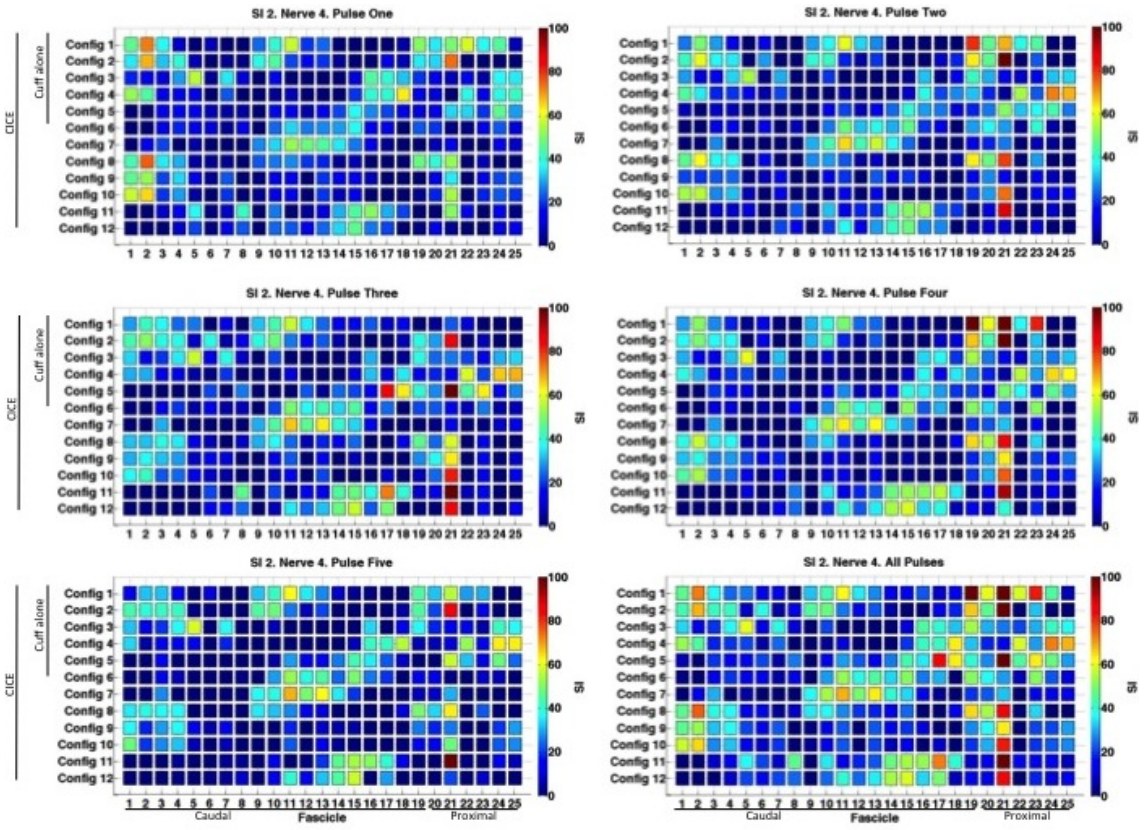

Figure 8: *Illustrates a color plot of SI 2 for nerve 4, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. Higher SI was seen for most fascicles and the tendencies for all five pulses was the similar.*

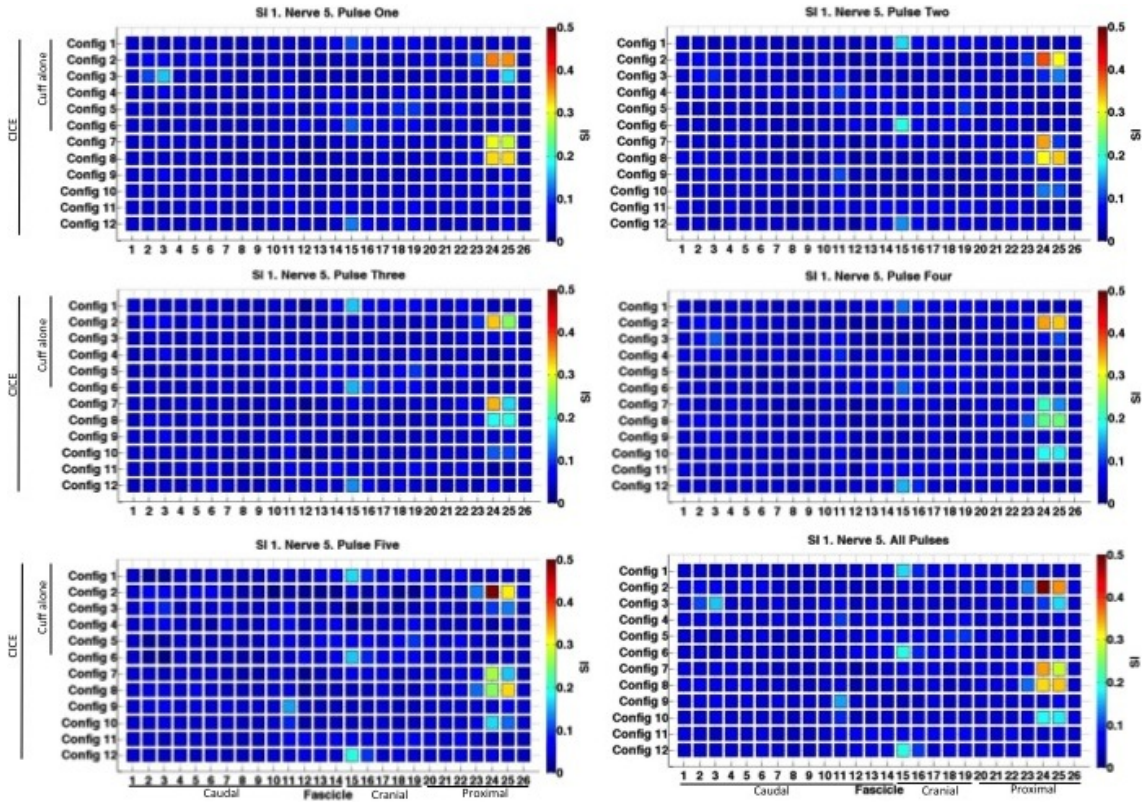

Figure 9: *Illustrates a color plot of SI1 for nerve 5, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. The SI was low for all five pulses except in fascicle 24 and 25.*

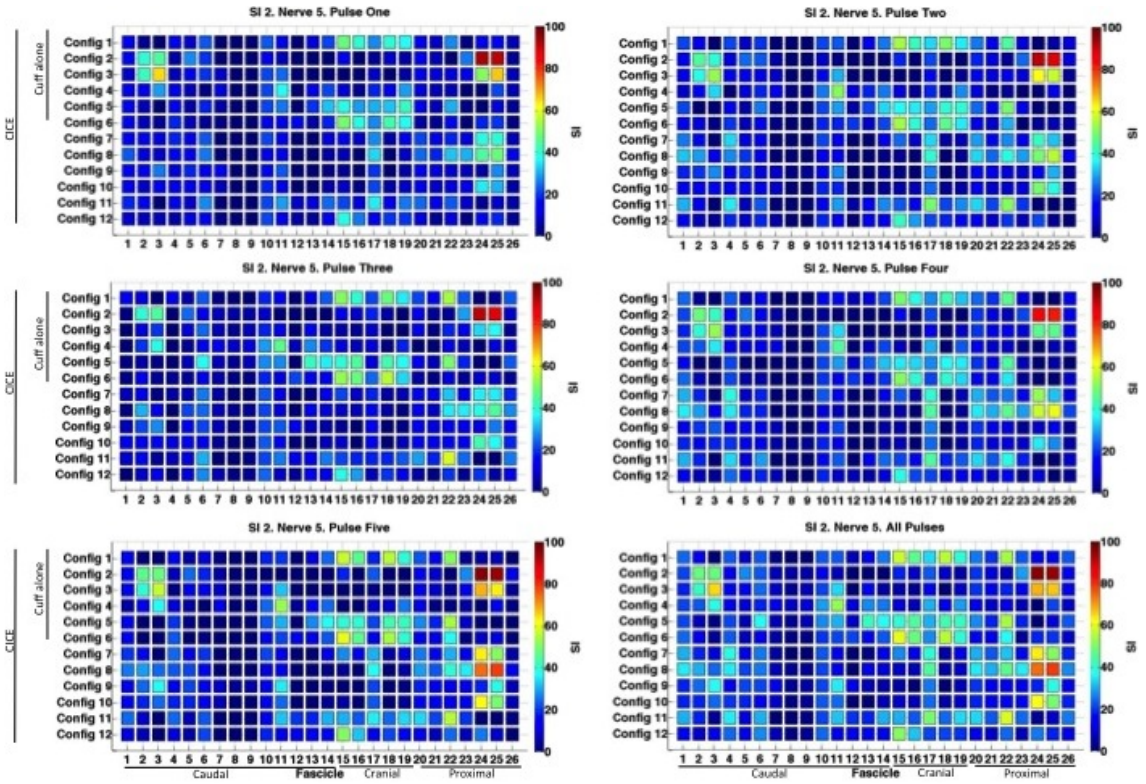

Figure 10: *Illustrates a color plot of SI2 for nerve 5, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. The SI for all five pulses was seen in the cranial and proximal branches. Besides the the tendency was the same for all five pulses.*

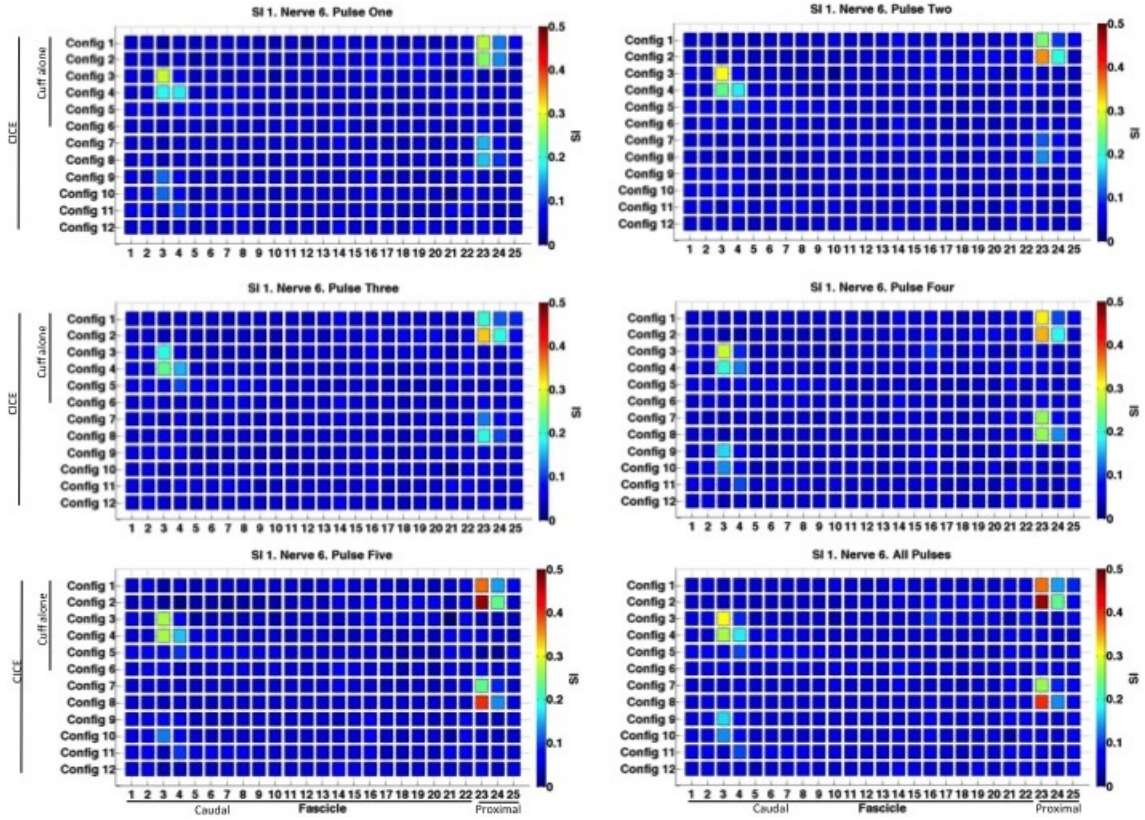

Figure 11: *Shows a color plot of SI1 for nerve 6, with all five pulses used during stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. A relatively low SI was seen in the five pulses expect in facile 3, 4, 23, and 24.*

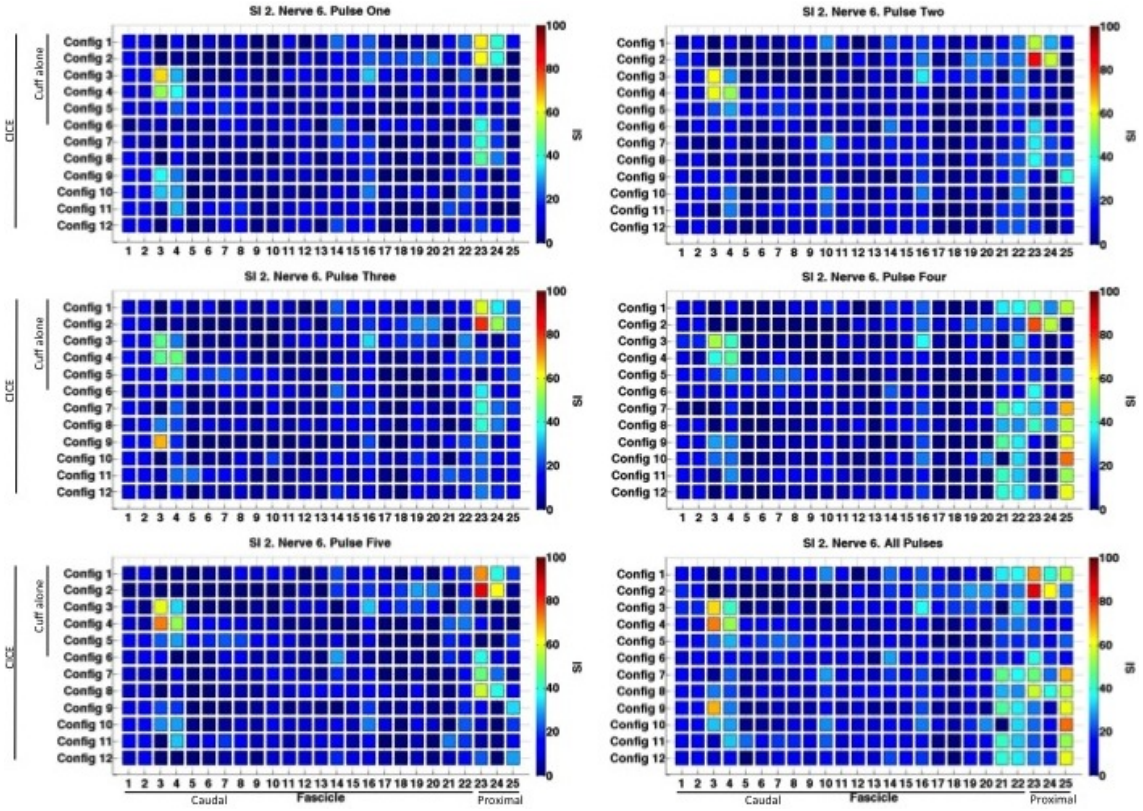

Figure 12: *Illustrates a color plot of SI 2 for nerve 6, with all five pulses used during the stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. Pulse four seemed to have a higher SI in more fascicles than the four other pulses.*

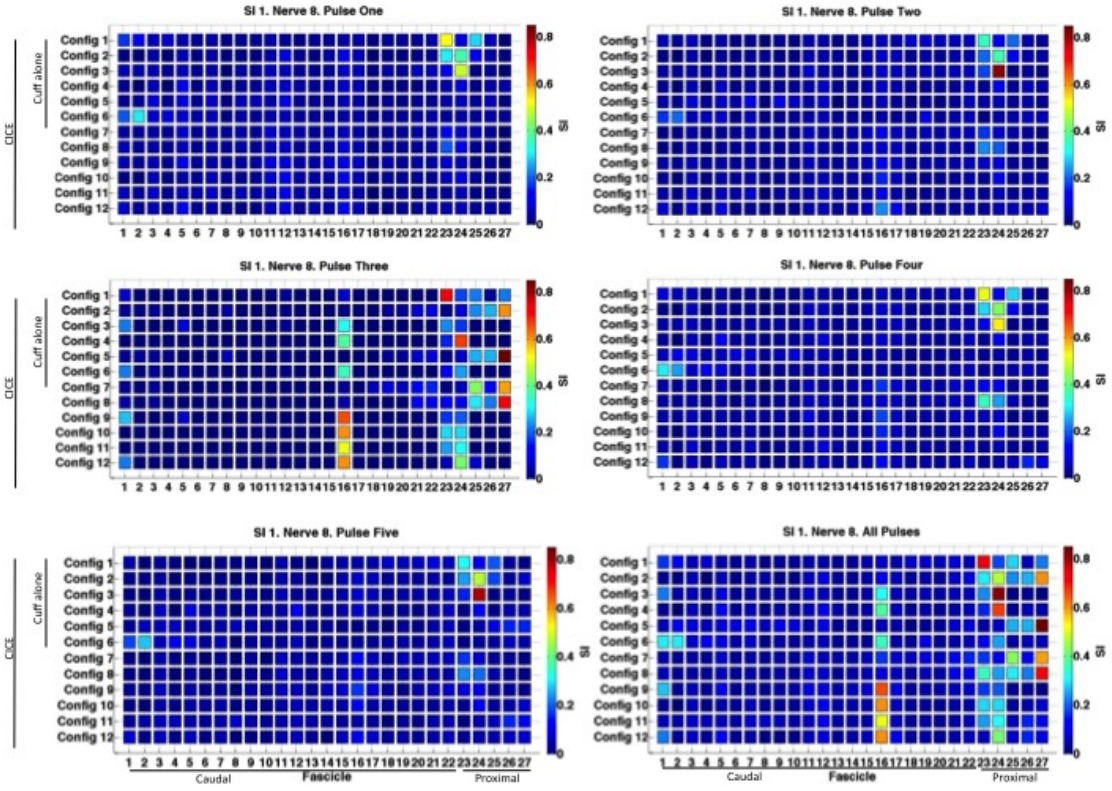

Figure 13: *Illustrates a color plot of SI1 for nerve 8, with all five pulses used during stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. Pulses three has the highest SI for the proximal branch were there is also seen a high selectivity in fascicle 16.*

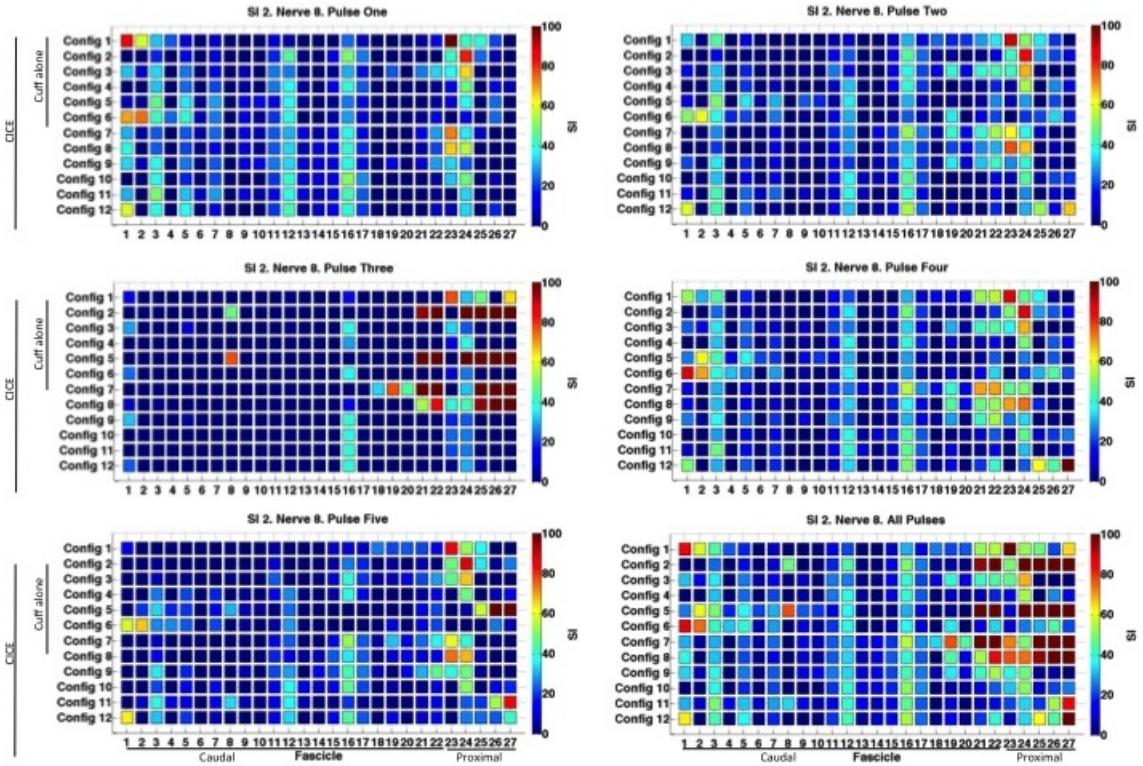

Figure 14: *Illustrates a color plot of SI2 for nerve 8, with all five pulses used during stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. The high SI was spread out trough all the fascicles where pulse one, two, four, and five has the same tendency.*

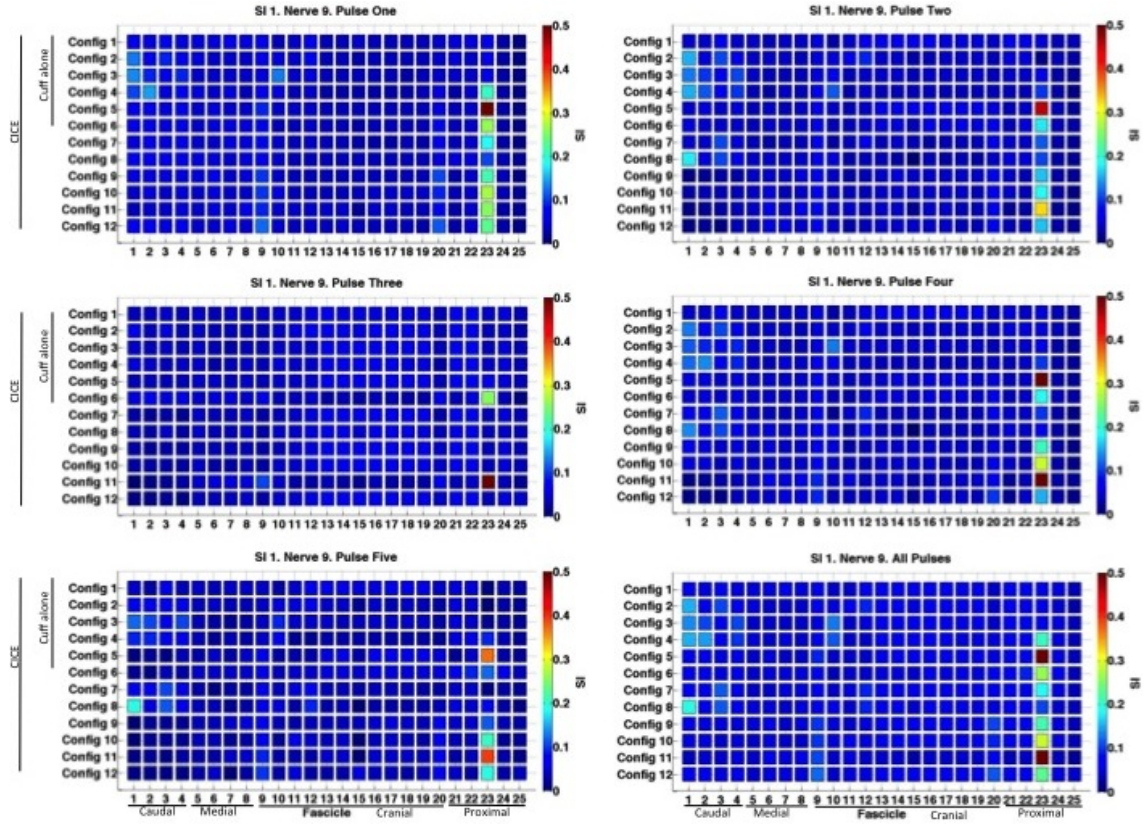

Figure 15: *Illustrates a color plot of SI1 for nerve 9, with all five pulses used during stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. The only fascicle with a relatively high SI was fascicle 23 the rest had a low SI for all the five pulses.*

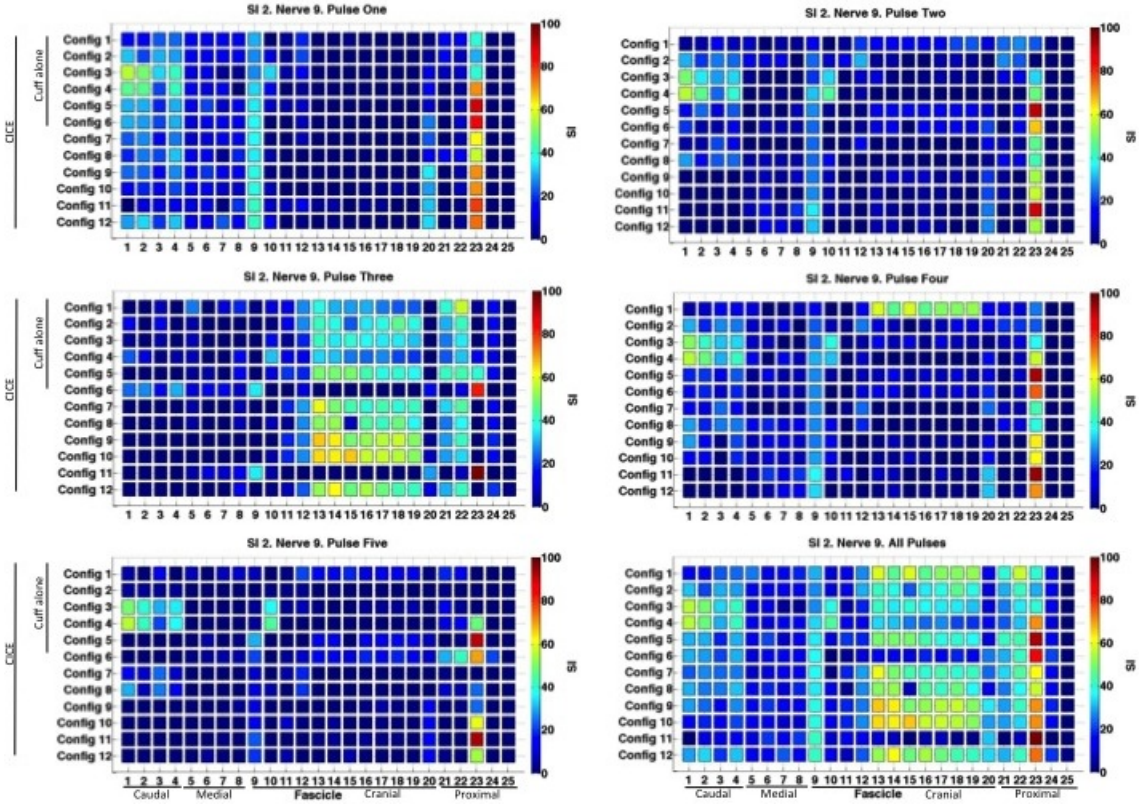

Figure 16: *Illustrates a color plot of SI2 for nerve 9, with all five pulses used during stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. Pulse one, two, four, and five has a high SI in fascicle 23 and in the caudal branch whereas pulse three has a high SI in the cranial branch.*

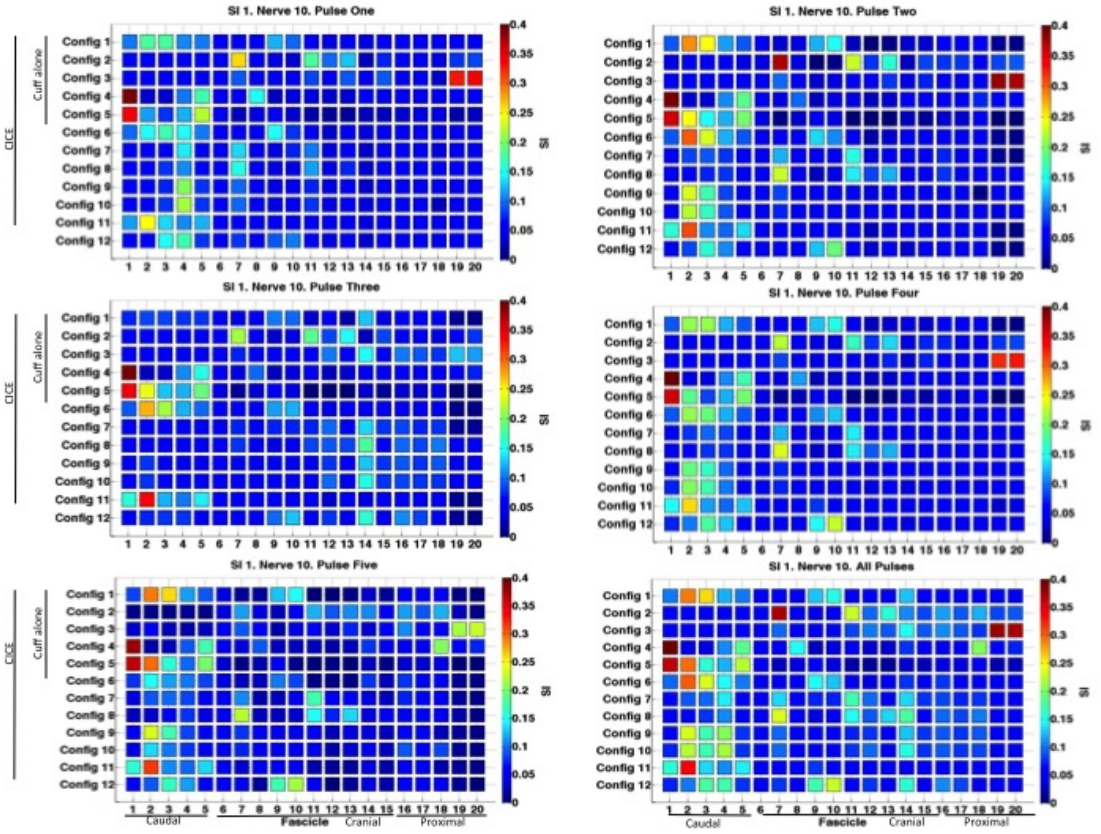

Figure 17: *Illustrates a color plot of SI1 for nerve 10, with all five pulses used during stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. A high SI is seen in the caudal branch and for the rest of the nerve it was spread out.*

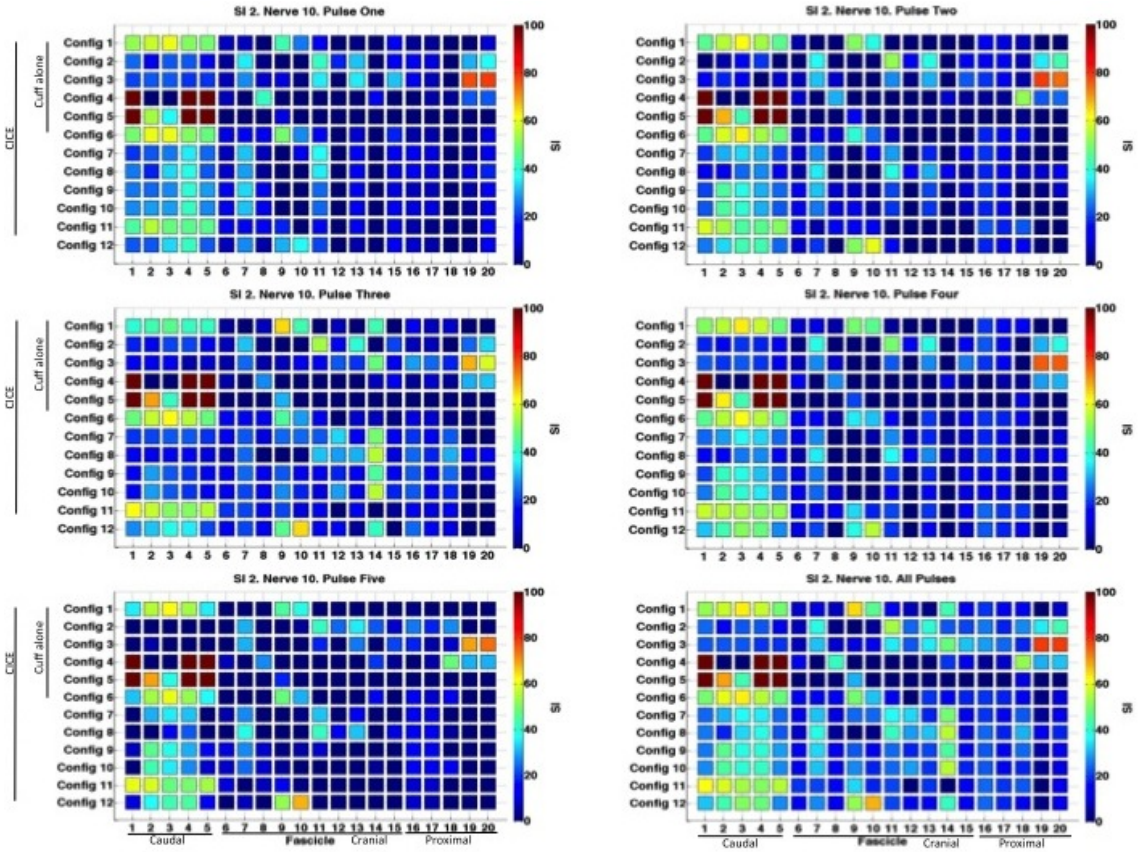

Figure 18: *Illustrates a color plot of SI2 for nerve 10, with all five pulses used during stimulation. On the x-axis the fascicles is shown and on the y-axis the 12 configurations were listed with the CICE configuration 1-12 and the cuff configurations 1-6. The colors indicate the level of selectivity reached in the individual fascicle. A more red color indicates higher selectivity, while a more blue color indicates lower selectivity. The caudal branch showed the highest SI for all the five pulses, which all had the same tendencies.*

B. Optimal Stimulation Appendix

Fascicle	SI 1	Configuration	Pulse	Intensity [μA]
1	0.148	8	1	1584
2	0.164	2	5	1307
3	0.121	1	1	1501
4	0.0691	8	1	1834
5	0.428	11	1	785
6	0.113	11	2	961
7	0.0835	11	1	1263
8	0.347	1	1	501
9	0.0610	6	2	1751
10	0.0679	5	5	4000
11	0.0738	5	5	3751
12	0.0967	5	5	3751
13	0.0597	7	4	4080
14	0.113	6	4	2845
15	0.103	5	5	1789
16	0.0633	2	4	3755
17	0.525	3	5	692
18	0.454	3	4	990
19	0.198	5	3	206
20	0.134	5	3	309
21	0.167	5	3	251

Fascicle	SI 2	Configuration	Pulse	Intensity [μA]
1	60.1	8	5	3251
2	82.3	12	1	4000
3	43.9	1	1	2501
4	22.1	5	1	4000
5	75.4	5	2	2251
6	47.8	5	2	4000
7	41.7	5	4	15005
8	52.2	1	1	1501
9	19.2	9	4	20000
10	21.3	5	4	10005
11	25.5	5	5	4000
12	46.0	5	5	4000
13	30.5	12	4	20000
14	47.2	6	5	3251
15	18.8	12	1	4000
16	28.7	3	3	3501
17	106	3	3	1251
18	101	3	3	1251
19	98.3	11	3	4000
20	61.5	11	3	4000
21	82.9	1	4	20000

Table 1: Shows the maximum SI for each fascicle for nerve 1. Besides this it also shows the optimal configuration, pulse and intensity to activate the individual fascicle as selective as possible.

Fascicle	SI 1	Configuration	Pulse	Intensity [μA]
1	0.178	2	5	934
2	0.100	6	1	402
3	0.0544	6	1	585
4	0.0883	11	5	2397
5	0.316	11	5	1803
6	0.0515	7	4	19995
7	0.0502	1	3	371
8	0.0459	5	3	3751
9	0.0527	11	1	4000
10	0.0715	5	1	1303
11	0.0725	11	5	2120
12	0.0864	5	1	484
13	0.176	10	2	751
14	0.0459	10	2	1118
15	0.0468	6	5	1004
16	0.0497	4	4	5475
17	0.0504	12	4	2855
18	0.0431	6	5	1058
19	0.0856	4	3	345
20	0.564	10	2	461
21	0.130	11	3	1251
22	0.123	11	3	1251
23	0.106	11	3	1001
24	0.0913	11	3	1251
25	0.279	9	1	1251

Fascicle	SI 2	Configuration	Pulse	Intensity [μA]
1	40.9	2	1	1251
2	36.9	6	1	1001
3	14.5	6	2	1251
4	40.3	11	4	11255
5	55.8	11	2	2001
6	28.8	7	4	20000
7	10.7	1	3	501
8	20.2	5	3	3751
9	39.6	11	1	4000
10	34.1	5	3	4000
11	32.6	11	1	3751
12	30.1	5	1	1001
13	9.72	6	3	4000
14	15.9	12	1	4000
15	12.1	5	5	4000
16	17.1	5	5	3751
17	13.6	1	3	3001
18	11.1	1	3	3751
19	42.3	3	3	1251
20	99.0	3	3	1251
21	80.8	3	3	1251
22	79.4	3	3	1251
23	60.8	3	3	1251
24	46.6	1	4	20000
25	131	9	1	3251

Table 2: Shows the maximum SI for each fascicle for nerve 2. Besides this it also shows the optimal configuration, pulse and intensity to activate the individual fascicle as selective as possible.

Fascicle	SI 1	Configuration	Pulse	Intensity [μA]
1	0.173	2	5	705
2	0.0644	2	3	391
3	0.0817	1	3	175
4	0.106	6	5	534
5	0.0599	2	3	416
6	0.0469	7	2	395
7	0.0871	2	2	751
8	0.0679	8	5	967
9	0.0444	11	2	1251
10	0.134	3	5	1001
11	0.0787	8	5	897
12	0.0626	9	1	394
13	0.0940	1	1	292
14	0.0902	6	5	601
15	0.0632	1	5	522
16	0.0544	9	1	427
17	0.0487	10	5	2251
18	0.0455	4	3	1501
19	0.0894	4	3	298
20	0.139	6	2	243
21	0.105	11	5	911
22	0.151	5	5	664
23	0.0657	4	3	392
24	0.103	3	5	953
25	0.298	3	5	548
26	0.0768	3	3	659
27	0.178	3	5	751

Fascicle	SI 2	Configuration	Pulse	Intensity [μA]
1	52.4	1	1	1251
2	32.0	1	4	20000
3	43.7	1	3	1001
4	56.4	1	1	1501
5	24.8	1	3	4000
6	18.4	1	2	4000
7	45.9	2	5	2001
8	31.0	2	2	1501
9	19.5	1	4	20000
10	46.9	4	5	1751
11	34.8	8	5	1501
12	31.4	9	3	1501
13	53.5	1	1	1001
14	68.3	1	1	1751
15	25.9	1	1	1001
16	18.0	10	5	2251
17	32.6	1	1	3751
18	27.1	1	1	4000
19	64.5	11	3	3751
20	48.0	6	5	1251
21	39.2	11	5	1751
22	49.6	5	5	1501
23	29.2	1	4	20000
24	22.6	6	4	20000
25	82.2	3	5	1501
26	25.2	3	3	1251
27	54.3	3	3	1001

Table 3: Shows the maximum SI for each fascicle for nerve 3. Besides this it also shows the optimal configuration, pulse and intensity to activate the individual fascicle as selective as possible.

Fascicle	SI 1	Configuration	Pulse	Intensity [μA]
1	0.145	2	5	561
2	0.0813	2	1	265
3	0.0988	2	5	730
4	0.104	3	5	773
5	0.106	3	3	532
6	0.0544	5	3	501
7	0.0793	3	5	900
8	0.0593	12	5	2077
9	0.131	2	5	599
10	0.0941	1	3	441
11	0.268	6	5	833
12	0.199	7	5	448
13	0.116	7	3	751
14	0.0907	12	4	7505
15	0.110	6	4	2460
16	0.103	4	1	364
17	0.101	12	5	2251
18	0.141	4	1	311
19	0.0746	5	3	501
20	0.0688	5	3	319
21	0.788	11	5	501
22	0.177	4	2	335
23	0.0790	5	3	280
24	0.289	4	2	226
25	0.244	4	2	263

Fascicle	SI 2	Configuration	Pulse	Intensity [μA]
1	56.6	10	1	4000
2	76.5	8	1	4000
3	42.0	2	5	2001
4	41.0	2	5	1751
5	59.6	3	4	6255
6	37.8	2	3	4000
7	37.7	3	3	2001
8	46.4	11	3	4000
9	43.9	2	5	1501
10	53.9	7	4	11255
11	67.8	7	5	2001
12	48.8	7	5	2001
13	63.5	7	3	1751
14	53.6	12	4	11255
15	59.2	12	4	11255
16	52.3	11	2	3251
17	83.4	5	3	2001
18	63.5	4	1	1001
19	100	1	4	20000
20	59.8	1	4	20000
21	145	11	3	1001
22	58.2	4	3	1251
23	82.3	1	4	20000
24	72.3	4	2	1001
25	68.1	4	2	1001

Table 4: Shows the maximum SI for each fascicle for nerve 4. Besides this it also shows the optimal configuration, pulse and intensity to activate the individual fascicle as selective as possible.

Fascicle	SI 1	Configuration	Pulse	Intensity [μA]
1	0.0497	8	4	4000
2	0.104	3	1	501
3	0.158	3	1	286
4	0.0519	7	4	4000
5	0.0688	2	5	2251
6	0.0637	2	5	2251
7	0.0544	4	2	870
8	0.0450	4	3	721
9	0.0485	4	3	677
10	0.0598	4	2	1001
11	0.146	9	5	769
12	0.0694	6	4	242
13	0.0639	5	3	341
14	0.0793	5	5	528
15	0.190	6	2	229
16	0.0940	12	5	711
17	0.0575	1	2	492
18	0.0873	5	1	219
19	0.101	5	2	299
20	0.0500	8	4	4000
21	0.0482	8	4	4000
22	0.0600	11	3	1251
23	0.125	2	5	692
24	0.490	2	5	277
25	0.359	2	1	111
26	0.0500	2	5	2501

Fascicle	SI 2	Configuration	Pulse	Intensity [μA]
1	35.0	8	4	20000
2	48.0	2	4	7505
3	65.6	3	1	1001
4	38.1	7	4	20000
5	28.1	2	1	2251
6	37.0	5	3	4000
7	13.3	4	3	1251
8	8.2	4	3	1001
9	10.7	4	3	1001
10	29.1	4	3	1751
11	52.6	4	5	2251
12	21.4	6	3	1001
13	40.8	5	3	1501
14	36.1	5	5	1501
15	59.7	6	5	1251
16	47.6	6	3	1251
17	49.5	11	2	4000
18	55.1	1	5	2251
19	41.9	5	5	1251
20	35.8	8	4	20000
21	31.0	8	4	20000
22	59.1	11	3	1501
23	35.8	8	3	4000
24	95.9	2	5	1251
25	94.0	2	5	1251
26	27.7	8	3	4000

Table 5: Shows the maximum SI for each fascicle for nerve 5. Besides this it also shows the optimal configuration, pulse and intensity to activate the individual fascicle as selective as possible.

Fascicle	SI 1	Configuration	Pulse	Intensity [μA]
1	0.0574	11	1	677
2	0.0602	11	1	656
3	0.308	3	2	362
4	0.177	4	1	235
5	0.0596	5	3	546
6	0.0632	5	5	870
7	0.0682	5	3	483
8	0.0681	5	5	824
9	0.0505	5	3	751
10	0.0499	7	2	4000
11	0.0470	6	1	575
12	0.0458	6	5	1534
13	0.0449	12	4	488
14	0.0829	6	5	1037
15	0.0486	6	5	1471
16	0.0877	3	4	533
17	0.0537	2	1	1501
18	0.0620	2	5	2501
19	0.0672	2	5	3001
20	0.0692	2	1	1001
21	0.0704	11	5	845
22	0.0797	3	4	578
23	0.472	2	5	568
24	0.234	2	5	1250
25	0.0876	1	3	573

Fascicle	SI 2	Configuration	Pulse	Intensity [μA]
1	18.7	3	4	13755
2	18.6	3	4	13755
3	72.1	4	5	1501
4	52.6	4	5	2001
5	23.8	11	3	4000
6	19.5	5	3	1251
7	23.9	5	4	5005
8	22.1	5	4	5005
9	14.9	5	3	1251
10	29.1	7	2	4000
11	13.0	3	1	2751
12	13.5	8	5	2251
13	12.7	8	5	2251
14	29.3	6	5	2001
15	12.8	1	1	4000
16	37.2	3	4	8755
17	19.4	2	1	1751
18	20.9	2	1	1501
19	28.3	2	5	3501
20	27.7	2	1	1501
21	46.0	7	4	20000
22	37.4	1	4	20000
23	88.9	2	5	2001
24	62.2	2	5	2001
25	73.7	10	4	20000

Table 6: Shows the maximum SI for each fascicle for nerve 6. Besides this it also shows the optimal configuration, pulse and intensity to activate the individual fascicle as selective as possible.

Fascicle	SI 1	Configuration	Pulse	Intensity [μA]
1	0.296	6	4	160
2	0.296	6	1	146
3	0.0862	5	4	501
4	0.116	6	4	249
5	0.114	12	1	311
6	0.0880	5	4	416
7	0.0973	5	2	742
8	0.0743	11	5	651
9	0.103	5	2	728
10	0.0845	5	4	428
11	0.0893	9	1	407
12	0.107	10	1	501
13	0.0505	9	1	543
14	0.0571	9	5	1018
15	0.0686	9	5	939
16	0.658	9	3	1047
17	0.0943	12	2	466
18	0.0712	1	2	1051
19	0.0984	6	2	551
20	0.0664	1	5	1451
21	0.145	7	3	601
22	0.157	7	3	396
23	0.736	1	3	488
24	0.822	3	2	333
25	0.433	7	3	207
26	0.257	2	3	251
27	0.905	5	3	82

Fascicle	SI 2	Configuration	Pulse	Intensity [μA]
1	88.7	6	4	1755
2	73.5	6	1	351
3	49.1	5	2	2000
4	32.5	6	4	1255
5	39.5	6	1	901
6	21.1	5	4	2250
7	29.9	5	2	1101
8	77.8	5	3	2000
9	22.9	5	2	901
10	19.3	5	4	2255
11	26.3	9	1	851
12	44.8	2	1	1601
13	12.2	10	5	1801
14	14.2	9	5	1151
15	23.4	8	5	2000
16	55.4	7	4	3005
17	28.0	7	4	3755
18	33.7	7	3	751
19	77.1	7	3	751
20	44.8	7	3	751
21	170	7	3	751
22	174	7	3	1001
23	99.0	1	1	701
24	132	2	3	1301
25	243	5	3	1301
26	527	5	3	1101
27	536	5	3	901

Table 7: Shows the maximum SI for each fascicle for nerve 8. Besides this it also shows the optimal configuration, pulse and intensity to activate the individual fascicle as selective as possible.

Fascicle	SI 1	Configuration	Pulse	Intensity [μA]
1	0.194	8	5	753
2	0.143	4	1	377
3	0.113	8	5	963
4	0.103	3	2	501
5	0.0536	1	1	1101
6	0.0571	9	1	901
7	0.0558	1	1	1101
8	0.0593	11	2	2000
9	0.118	12	1	639
10	0.131	3	4	273
11	0.0612	2	2	651
12	0.0930	2	2	419
13	0.0682	10	3	335
14	0.0670	12	3	551
15	0.0675	10	3	351
16	0.0635	10	3	651
17	0.0633	10	3	551
18	0.0654	5	5	1551
19	0.0605	10	3	601
20	0.113	12	1	646
21	0.0729	3	3	306
22	0.0868	6	5	2000
23	0.594	11	3	753
24	0.0637	6	5	1751
25	0.0393	12	3	407

Fascicle	SI 2	Configuration	Pulse	Intensity [μA]
1	56.0	4	2	1101
2	48.4	3	1	651
3	33.3	3	1	651
4	42.1	4	1	851
5	25.5	1	3	2000
6	21.6	5	1	1451
7	22.4	12	1	1851
8	20.0	11	1	2000
9	42.1	12	1	1301
10	45.0	4	2	1101
11	20.3	5	3	1451
12	30.2	2	2	1151
13	66.8	9	3	1151
14	64.0	10	3	1251
15	66.3	10	3	901
16	57.2	10	3	801
17	57.2	10	3	901
18	57.3	9	3	1101
19	51.8	1	4	10000
20	35.9	9	1	1151
21	41.6	5	3	651
22	56.5	1	3	2000
23	97.1	11	3	901
24	21.7	6	5	1751
25	1.68	12	3	407

Table 8: Shows the maximum SI for each fascicle for nerve 9. Besides this it also shows the optimal configuration, pulse and intensity to activate the individual fascicle as selective as possible.

Fascicle	SI 1	Configuration	Pulse	Intensity [μA]
1	0.416	4	4	345
2	0.339	11	3	365
3	0.259	1	5	523
4	0.214	10	1	519
5	0.219	5	1	146
6	0.0643	9	1	701
7	0.371	2	2	619
8	0.144	4	1	239
9	0.172	12	5	1201
10	0.226	12	4	1755
11	0.231	2	2	951
12	0.103	3	3	501
13	0.159	2	2	1002
14	0.179	8	3	372
15	0.0883	3	1	651
16	0.117	2	5	651
17	0.0937	3	3	551
18	0.204	4	5	351
19	0.378	3	2	651
20	0.372	3	2	651

Fascicle	SI 2	Configuration	Pulse	Intensity [μA]
1	607	5	2	2000
2	69.6	5	5	551
3	63.7	6	2	1151
4	232	5	4	10000
5	321	5	1	1151
6	20.0	11	3	1551
7	37.6	2	4	3505
8	41.2	4	1	451
9	64.7	1	3	1501
10	67.3	12	5	1401
11	54.4	2	3	1301
12	33.7	7	3	851
13	39.1	2	3	1251
14	55.6	8	3	701
15	32.5	3	1	701
16	29.6	3	3	651
17	23.6	3	3	651
18	52.4	4	2	1301
19	79.2	3	2	751
20	78.7	3	1	401

Table 9: Shows the maximum SI for each fascicle for nerve 10. Besides this it also shows the optimal configuration, pulse and intensity to activate the individual fascicle as selective as possible.

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