Exploring Neurophysiological and Sensory Alterations Induced by Functional Electrical Stimulation in a Model of Upper Limb Hemiparesis

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Group 10411

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Synopsis:

A potential method for restoring motor function in hemiparetic stroke patients is functional electrical stimulation (FES). However, only limited knowledge exists regarding the sensory alterations induced by FES, even though hemiparetic limbs are often affected by loss of feeling as well. Therefore, the current study examines the possible proprioceptive and tactile alterations which a single session of FES-assisted task-oriented therapy (TOT) can induce in a model based on upper limb immobilization of healthy subjects. Short term upper limb immobilization has shown to induce a temporary neurological state similar to hemiparesis. To examine the effects of FES-assisted TOT, multiple neurophysiological and clinical measurements were performed including median nerve somatosensory evoked potentials (SEPs), joint position sense (JPS) testing, quantitative sensory testing (QST) and the modified Jebsen-Taylor hand function (JTHF) test. These measurements were performed before and after 24 hours of upper limb immobilization and after a TOT session with or without FES assistance. Seven subjects were included in the study. The obtained and preprocessed results from the measurements were statistically analyzed and compared. The immobilization procedure induced motor but not sensory alterations. Additionally, no significant difference was found for any of the alterations induced by FES-assisted TOT versus TOT without FES assistance. Thus, the sensory impact of FES-assisted TOT in hemiparetic stroke patients is not clear based on the current study. However, further studies should be conducted and include a larger sample population.

Preface

This master's thesis has been produced during the 4th semester master in Biomedical Engineering and informatics at Aalborg University. The project period was from the 4th of February 2019 to the 6th of June 2019. In the thesis, the possible sensory and neurophysiological alterations induced by functional electrical stimulation (FES) in the rehabilitation of stroke patients with upper limb hemiparesis were investigated. This involved an experiment, in which neurophysiological and clinical measurements were performed before and after task-oriented therapy (TOT) with or without FES assistance. Subsequently, preprocessing and analysis of the experimental data was performed. The experiment was performed with healthy subjects, who underwent 24-hour upper limb immobilization procedures prior to two of the experimental sessions. The immobilization was performed to induce a short lasting condition similar to the hemiparesis, which is experienced by many stroke patients. Therefore, the clinical and neurophysiological measurements were also performed before the immobilization to obtain a baseline measurement for each subject.
This thesis can be read by anyone with a particular interest in stroke rehabilitation in general or more specifically FES. This encompasses e.g. biomedical engineering students, therapists and researchers.
The authors would like to thank the subjects, who participated in the experiment. Furthermore, the authors would like to thank main supervisor Erika G. Spaich and co-supervisor Federico Arguissain for their supervision and feedback throughout the project period.

Reading guide

The thesis is divided into six chapters followed by a bibliography and nine appendices.
Chapter 1 contains the introduction and an initiating problem which forms the basis for the background in chapter 2. In the background chapter, relevant aspects of the initiating problem are analyzed and finally the problem statement is presented. Chapter 3 presents how the chosen experimental measurements will be conducted and how the collected data will be processed and analyzed. Chapter 4 includes the experimental procedures for the pilot trial and experiment, while the results are presented in chapter 5. Finally, the synthesis is found in chapter 6. This includes a discussion and conclusion. The appendices include a detailed description of different types of hemiparesis, descriptions of relevant anatomical aspects, interview material, a detailed protocol over the pilot trial and experiment, different information material provided for the included subjects and lastly all the results from the experiment.
The chapters are subdivided into sections, which are numbered as the chapter number followed by the section number. For example, the first section in chapter 2 is section 2.1. Subsections are numbered following the same principle. Additionally, the figures of the thesis are numbered according to their appurtenant chapter. Appendices are indicated with letters.
Abbreviations are used for abbreviations and explaining terms. In regards of the abbreviations, the full word is written first followed by the abbreviation in parentheses, which will be used subsequently throughout the thesis. Harvard referencing is used in the thesis for stating the references, by which they are presented in square brackets with the author and year. If the reference is placed before the period in a sentence, the stated knowledge in that

sentence is obtained from this reference. If the reference is placed after the period, all the previous sentences until the last mentioned reference are based on this stated reference. In cases, where a specific study is described in more detail, the study will be introduced in the beginning of the sentence and elaborated afterwards.

Contents

Introduction 1

Stroke is currently the second leading cause of death and the third leading cause of lost disability-adjusted life-years (DALYs) worldwide [Hankey, 2017]. Stroke is defined as a sudden loss of neurological functions caused by a lack of blood, and thereby oxygen, to a part of the brain. After just 3-4 minutes, the neurons in the brain start to die. After 10 minutes, the brain damage can become severe. [Muir, 2008; Hankey, 2017; Markus, 2012] Despite the stable incidence rates and decreasing mortality rate over the past couple of decades, the number of stroke incidents, DALYs lost due to stroke and stroke-related deaths are still increasing worldwide [Hankey, 2017].

Approximately 80-90% of all stroke patients experience motor symptoms. Among these patients, at least two-thirds experience hemiparesis affecting the upper and lower limbs uniformly. [Caplan and van Gijn, 2012] Hemiparesis is a weakening of one side of the body, and the hemiparetic limbs are often also affected by loss of feeling [Caplan, 2006]. This is especially seen in stroke patients with more severe types of hemiparesis affecting one entire side of the body [Caplan and van Gijn, 2012]. The neurophysiological basis for the different types of hemiparesis is further elaborated in appendix A.

When rehabilitating the motor function in stroke patients, it is important to incorporate the capacity of neuron assemblies and spared neural networks for the patient to regain skills and thereby the use of the affected limbs. It is highly relevant to use well-defined training methods to induce neural adaptions, which can lead to behavioral improvements. Most approaches for upper limb rehabilitation are focused on practice of performing correct submovements and training sequences, which help the patient perform movements involving activation of multiple joints. [Dobkin, 2004] For stroke rehabilitation in the Western world, the Bobath Concept is the most popular treatment approach [Kollen et al., 2009]. This concept contains theoretical assumptions stating e.g. that sensory inputs are beneficial during motor rehabilitation, as sensorimotor integration is crucial in motor modulation [Chen et al., 2018]. Thereby, most rehabilitative techniques in the Western World might have a sensory aspect incorporated in the exercise, even though the focus is on motor rehabilitation. An example of a rehabilitation method is task-oriented therapy (TOT), which is considered to be a good approach in the rehabilitation of motor function, as it involves repetitive motor tasks with aspects of real-world activities [Langhorne et al., 2011; Dobkin, 2004]. Other rehabilitation methods include constraint-induced movement therapy (CIMT), during which the stroke patient is forced to use the affected upper limb by restraining the unaffected upper limb, and treadmill training with some body weight support. Both methods could include sensory aspects, as touching hot or cold items when performing exercises. Nonetheless, only 60% of the stroke patients with hemiparesis achieve functional independence in simple activities of daily living (ADLs) after six months of rehabilitation. [Dobkin, 2004]

In general, poor long-term motor recovery following stroke is a comprehensive problem, and the options for the patients with a minimal recovery after rehabilitation are very limited [Sterr and Conforto, 2012]. One of the requirements, which new rehabilitation strategies must fulfill, is that they must not depend on the residual function of the single patients. Thereby, the strategies are more likely to be suitable for all stroke patients. To examine such

rehabilitation strategies, it will be beneficial to use a model which resembles low-functioning hemiparesis. [Furlan et al., 2016] Furthermore, it can be assumed that a model can help reducing possible inter-subject variabilities found in stroke patients.
According to multiple studies, a possible assisting technique for restoration of motor function is functional electrical stimulation (FES) [Belagaje, 2017; Sharif et al., 2017; Jonsdottir et al., 2017]. A study by Jonsdottir et al. [2017], examining the use of FES along with conventional rehabilitation, found a trend indicating that the proportion of patients improving their condition was larger in the group receiving FES than the group receiving only conventional rehabilitation. The groups consisted of chronic stroke patients, among which one group received upper limb FES during TOT and the other group received conventional therapy including TOT. [Jonsdottir et al., 2017] Therefore, it is presumed that FES can contribute to a better motor rehabilitation of stroke patients when used along with standard rehabilitation techniques. However, it is not specified how FES neurophysiologically impacts hemiparetic stroke patients and how this can be evaluated. Additionally, it could be interesting to examine if the impairment induced by hemiparesis can be expressed through a hemiparetic stroke model. Thereby, the impact of FES could be assessed in this model, to accommodate the issues with the limited focus on stroke patients with a poor rehabilitation outcome. This leads to the following initiating problem:

Which impact does functional electrical stimulation have on neurophysiological alterations related to hemiparesis in stroke patients and how can this be evaluated in a hemiparetic stroke model?

Background 2

In this chapter, the principles of FES and how it affects motor and sensory function in hemiparetic stroke patients are introduced. It also includes points from an interview performed on a neurorehabilitation center, in which it is further clarified how FES might impact the sensory function of this patient group. Finally, methods for exploring neurophysiological and clinical alterations are introduced and compared.

2.1 Rehabilitation with Functional Electrical Stimulation

It is hypothesized that when stroke patients suffer from hemiparesis, the size and excitability of the cortical tissue surrounding the stroke decrease. This principle is illustrated in figure 2.1. [Furlan et al., 2016]

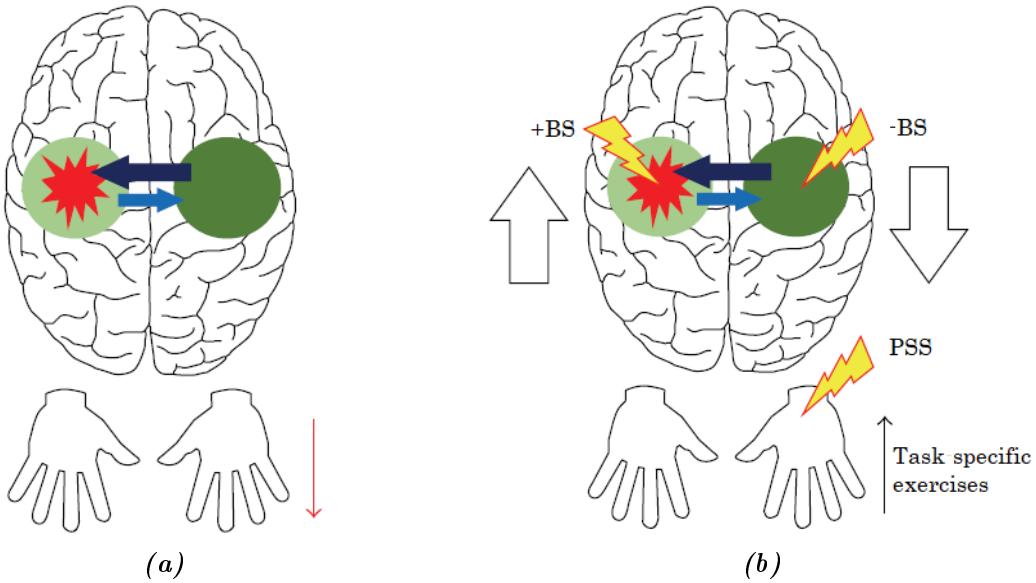


Figure 2.1: *The cortical alterations associated with hemiparesis (a). The light green circle with a red shape indicates a decreased excitability or size of the areas surrounding the stroke, whereas the darker green circle in the unaffected hemisphere indicates increased activity. The arrows between the hemispheres indicate a decreased inhibition from the injured hemisphere, which is then increased from the unaffected hemisphere. The red arrow pointing downwards indicates a decreased activity of the limb contralateral to the injured hemisphere. Methods including brain stimulation and peripheral somatosensory stimulation are believed to be useful in the rehabilitation of the imbalance between the hemispheres (b). +BS and -BS are excited and inhibited brain stimulation respectively, while PSS is peripheral somatosensory stimulation. The white arrows show how the activity is increased in the injured hemisphere while decreased in the unaffected hemisphere. The black arrow pointing upwards illustrate increased use of the affected limb during rehabilitation as e.g. task specific exercises. Modified from Furlan et al. [2016].*

As illustrated in figure 2.1, the excitability and size of the areas surrounding the stroke in the injured hemisphere can be decreased. The activity of the inhibitory circuits from the injured

to the unaffected hemisphere is reduced from the injured hemisphere. This causes an increased inhibitory activity from the unaffected hemisphere and thereby an increased activity in the motor areas of that hemisphere as well. By inducing an increased activity in the affected areas of the injured hemisphere, the imbalance between the hemispheres can potentially be reduced. To boost the effect of rehabilitation strategies as e.g. physiotherapy sessions, methods as brain stimulation and peripheral somatosensory stimulation can be used in combination with task specific exercises of the affected limb to rehabilitate the stroke patients. [Furlan et al., 2016]

FES can be used for inducing contractions in paretic muscles of stroke patients. The basic principle for FES is that neurons, i.e. excitable cells of the nervous system [Augustine, 2017], are more likely to be activated by electrical stimulation than muscles. This is due to a lower threshold for generating action potentials. When electrical stimulation is applied to nerves, action potentials can be generated, which leads to an activation of the nervous system and motor function. [Peckham and Knutson, 2005] The anatomy of a neuron and its physiological basis for generation of action potentials is further described in appendix B.
The nervous system includes the central nervous system (CNS) consisting of the brain and spinal cord, and the peripheral nervous system (PNS) consisting of the cranial, autonomic and spinal nerves and their appurtenant ganglia along with the motor and sensory neurons. Overall, the nervous system receives inputs from the surroundings and from internal structures, which are processed and sometimes utilized for planning a motor response following the inputs. [Augustine, 2017]
FES can be used on patients, when the lower motor neurons are not damaged and the synapse is intact [Peckham and Knutson, 2005]. The FES systems can be used during training sessions as done in several studies [Wilkins et al., 2017; Bustamante et al., 2016; Palmer et al., 2017], but other systems also aim to support the patients at home during ADLs [Peckham and Knutson, 2005].

2.1.1 Electrode Application and Stimulation Parameters

When FES is used for activating neuromuscular tissue, two or more electrodes are needed. These can be placed in either a monopolar or bipolar configuration. The monopolar application involves one active electrode, which is applied close to the specific nerve of the PNS, and a reference, which is applied over e.g. tendon. For the bipolar application, the active electrode is applied similarly to the active electrode in the monopolar application, but instead the reference is placed close to the active electrode. [Peckham and Knutson, 2005]
In healthy subjects, signals from the brain for activation of the skeletal muscles are transmitted through the nervous system to the motor neurons. A motor neuron and the innervated muscle fiber constitute a motor unit. The number of motor units along with the corresponding number of muscle fibers determines how accurately the muscle can be controlled. Motor neurons must transmit a series of impulses to the muscle fibers in order to cause a lasting contraction of a muscle. The natural activation of muscle fibers is usually performed with a frequency of 6-8 Hz. Thereby, the motor unit is not relaxed before it receives a new impulse. The activation of motor units follows an asynchronous pattern, by which muscles are not fatigued too fast. This principle is illustrated in figure 2.2. [Lynch and Popovic, 2008]

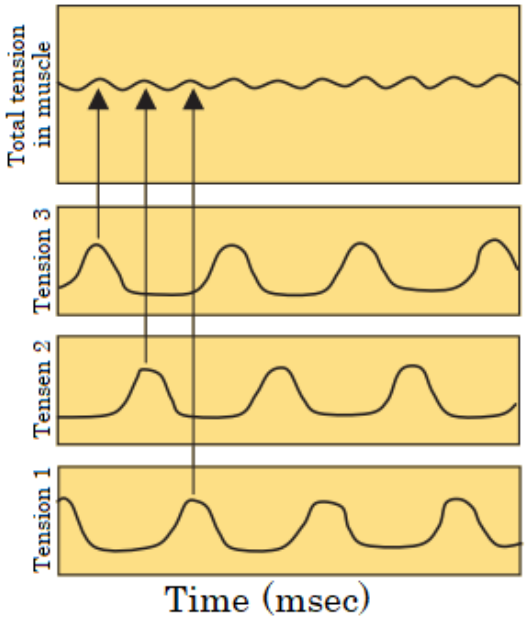


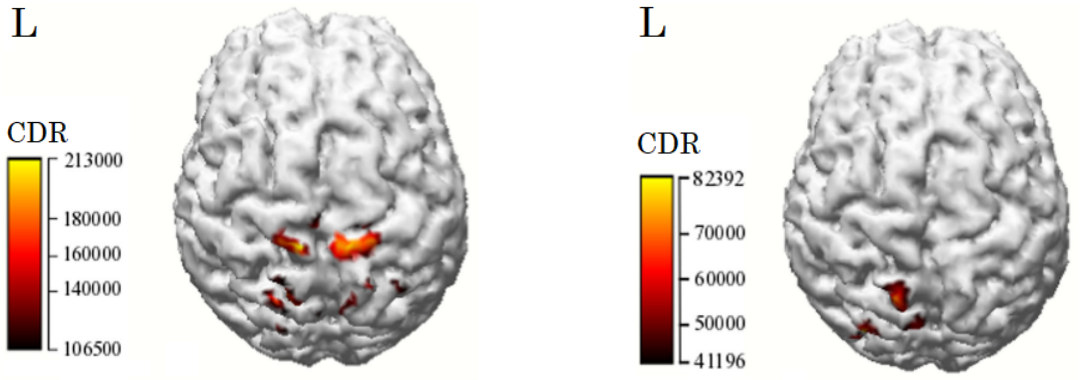
Figure 2.2: *The principle for normal activation of skeletal muscles following an asynchonous pattern. The sum of motor unit tensions constitutes the total activation of the muscle. Modified from Lynch and Popovic [2008].*

When using FES, the motor unit recruitment follows a synchronous pattern unlike for natural activation of muscles. The waveforms applied to induce muscle contractions during FES are defined from their frequency, amplitude and duration. When the frequency is not sufficiently high, the muscle will perform twitches. When the frequency is increased, temporal summation will cause a smooth muscle contraction rather than the twitches. Increasing the amplitude or duration of the waveform can cause a stronger muscle contraction due to spatial summation, which is activation of more axons and motor units. [Peckham and Knutson, 2005] Therefore, a high frequency for the FES stimulation is required compared to natural muscle contractions and a high degree of muscle fatigue is therefore associated with this method. [Lynch and Popovic, 2008]

2.1.2 Motor Alterations Induced by Functional Electrical Stimulation

As previously described in chapter 1, FES has shown to improve motor function in hemiparetic stroke patients. Other studies have focused specifically on the impact of FES on neuroplastic motor alterations. These studies demonstrate that FES can induce neuroplastic alterations in the CNS both after repetitive training over a longer period or even after a single training session. [Wilkins et al., 2017; Palmer et al., 2017] Neuroplasticity is elaborated further in appendix C.
In a study by Wilkins et al. [2017] it was found that sensorimotor cortical activity during a task involving hand opening assisted by EMG-FES was shifted to the ipsilesional cortex in six out of eight hemiparetic stroke patients with moderate to severe impairment following a seven-week training program. This alteration suggest that the patients increasingly rely on ipsilesional activation during the hand opening. This is considered to be a positive alteration, since the patients furthermore showed improvements in clinical assessments, i.e. the Box and Blocks Test (BBT) and active range of motion (AROM). Furthermore, the findings of the study indicated that the density of the ipsilesional primary motor and sensory cortex

was increased, while a decrease was found in the contralateral site. This might suggest that new synapses are formed and dendrites grow in the ipsilesional side. An example of how the activity from the sensorimotor cortex was shifted is illustrated in figure 2.3. [Wilkins et al., 2017]



(a) *(b)*

Figure 2.3: *An example of the cortical activity during the hand-opening task before (a) and after (b) the EMG-FES training intervention in a patient with a lesion in the left hemisphere. The activity in the contralesional cortex decreased after the intervention. The color bars indicate the current density reconstruction (CDR). Modified from Wilkins et al. [2017].*

Positive alterations can be seen even after a single session of FES. This is demonstrated in a study by Palmer et al. [2017], which included patients with lower limb hemiparesis. A significant increase of the corticomotor symmetry was detected before versus after intervention when receiving FES during one single walking session. There were no significant alterations, when the patients did not receive FES during the session. Furthermore, when receiving FES, the patients showed a significant increase in the ankle movement symmetry before versus after the intervention. This was not seen without FES. The walking sessions were conducted approximately one week apart. [Palmer et al., 2017]

The Importance of Voluntary Effort and Skill Training

Several studies have suggested that voluntary effort and skill training are important factors in order to obtain neuroplastic alterations from FES training [Khaslavskaia and Sinkjaer, 2005; Barsi et al., 2008; Perez et al., 2004].
In a study by Khaslavskaia and Sinkjaer [2005], it was found that voluntary cortical drive is involved in the modulation of the effect obtained from repetitive electrical stimulation. This was based on recording of motor evoked potentials (MEPs) in healthy subjects before and after different exercise sessions. Additionally, the impact of the applied stimulation was even larger when an agonistic voluntary effort from the subject was present. Finally, it was found that an antagonistic voluntary effort caused a decrease in the MEPs. [Khaslavskaia and Sinkjaer, 2005] The finding regarding the impact of voluntary cortical drive on the effect from electrical stimulation is further supported in a study by Barsi et al. [2008]. The study concluded that the volitional movements combined with FES increased the excitability for both relaxed and contracted finger flexors, while only FES or volitional movement alone did not [Barsi et al., 2008]. Thus, the findings obtained by Khaslavskaia and Sinkjaer [2005] and Barsi et al. [2008] suggest that FES combined with agonistic voluntary effort from the subject

induce the strongest effect from the treatment.
A study by Perez et al. [2004] found that skill training of ankle muscles significantly increased tibialis anterior MEPs, while non-skill training and passive movements did not. This adds to the findings by Khaslavskaia and Sinkjaer [2005] and Barsi et al. [2008] that training a skill during the FES rehabilitation increases the effect of the treatment with respect to cortical excitability. [Perez et al., 2004]

2.1.3 Sensory Alterations Induced by Functional Electrical Stimulation

To the knowledge of the authors, only limited knowledge exists regarding whether FES contributes to sensory rehabilitation. As mentioned in chapter 1, sensory deficits are often seen in hemiparetic stroke patients [Caplan, 2006]. One important point from the study by Wilkins et al. [2017] was, that despite the focus of the study was on motor aspects, an increase in the gray matter density within cortical sensory areas and the thalamus was found. The thalamus is an important structure in the somatosensory pathway [Augustine, 2017] as explained in appendix C. Thus, due to the augmented afferent feedback from the EMG-FES system used in the study, it is possible that sensory alterations might occur as well. [Wilkins et al., 2017]
Some other studies examining mechanisms affected by FES detected alterations in the activity within sensory areas of the brain or pointed out sensory aspects, that are relevant for the functional recovery [Iftime-Nielsen et al., 2012; Hara et al., 2008]. For example, a study by Iftime-Nielsen et al. [2012] found a decreased activation in the secondary somatosensory cortex in healthy subjects, when FES was combined with voluntary effort compared to when FES was applied alone. Overall this finding suggest that the combination of FES and voluntary movement makes the movement more predictable, which means that the movement to a larger extent belongs to the subject. [Iftime-Nielsen et al., 2012] Another study by Hara et al. [2008] found that daily EMG-FES therapy performed at home enhanced the recovery process of the hand and shoulder in stroke patients. In the study, it was pointed out that proprioceptive sensory feedback is potentially an important factor for the outcome of the therapy. [Hara et al., 2008] Thereby, these studies suggest that FES causes some alterations in cortical areas which are also related to sensory processing, but the focus of the studies were still on the motor or functional recovery and thereby not directly on the sensory aspects of rehabilitation.

In a case report by Bustamante et al. [2016], one of the aims was to examine the impact of FES on proprioception. Proprioception is the ability to sense the position and movement of the body. The cells involved in proprioception are mechanosensory neurons among others, which are located in the muscles, joints and tendons of the entire body. These are also known as proprioceptors. Other sensory neurons can also provide information about the body position, e.g. visual and tactile information. Examples of proprioceptors are muscle spindles, which contain structures sensitive to stretch and changes in the length of the muscles. Additionally, proprioceptive Golgi tendon organs are placed between the muscles and tendons, which encode the force produced by specific muscles and different types of receptors detecting when joints are moved to a specific threshold. Thus, proprioception helps stabilizing the body and performing movements. [Tuthill and Azim, 2018]
The case report by Bustamante et al. [2016] examined the impact of FES on proprioception in one hemiparetic stroke patient. The patient underwent training of the upper limb during five sessions a week for two weeks. During the training sessions, an FES device assisted the

movements. Before and after the training period, proprioceptive tests were performed as illustrated in figure 2.4. [Bustamante et al., 2016]

Figure 2.4: *The joint position sense (JPS) test in which the examiner supported the hemiparetic upper limb and placed it in a specific position. The examiner then asked the patient to place the unaffected limb similarly while keeping the eyes closed. [Bustamante et al., 2016]*

The results of the case report revealed no particular alterations in the proprioceptive sense for the shoulder and elbow, but a remarkable improvement was found for the wrist. The proprioceptive tests included angular measurements for examining joint position sense (JPS) and the time used for positioning the unaffected limb. This can be used for assessing the ability to place the unaffected upper limb similarly to the affected limb. [Bustamante et al., 2016]
Based on the findings from the case report by Bustamante et al. [2016] and the fact that the previously described studies are mainly focused on motor rehabilitation, it is clear that there is a need for further research within the sensory alterations induced by FES. The findings by Wilkins et al. [2017], suggesting that FES induces sensory alterations as well, further motivates a more detailed examination of this.

2.2 Clinical View of Functional Electrical Stimulation

Since only limited research exists regarding the sensory alterations induced by FES, the authors of the current thesis performed an interview with a development therapist who works with rehabilitation of hemiparetic stroke patients. This was done to include a clinical perspective on how the patients respond to FES. Furthermore, the interview contributed to examine whether therapists observe sensory alterations following the FES sessions. The development therapist had asked three colleagues to answer the interview questions as well. In appendix D, the danish interview guide and the part of the transcription focusing specifically on motor and sensory alterations are presented. The points and knowledge presented in this section are obtained from the interview. The interview included questions regarding the time of the rehabilitation process and the sensory and the motor aspects of FES therapy. However, this section is focused on the sensory aspects as this is the area which requires more research. Even though one of the colleagues reported that the hemiparetic stroke patients only experience small sensory alterations, which might not be due to FES, the other two colleagues reported that some patients tend to become more aware of the hemiparetic limb. One of these

colleagues stated that the increased awareness of the limbs makes the stroke patients more inclined to implicate the hemiparetic limb in different functions afterwards. The colleagues emphasized that the effect of FES is very different amongst the stroke patients dependent on e.g. the degree of hemiparesis and cognitive impairment. Additionally, it is also individual how many FES sessions the patients need to obtain an effect. The development therapist reported:

"Nogen de reagerer med det samme, andre nogen dage efter, og nogen patienter reagerer først efter uger af behandlingen. Igen afhænger det af skadens omfang og lokalisering, jo mindre skade, siger de (red: Kollegaerne), jo hurtigere effekt. Det er også helt afgørende at FES er placeret på den rigtige måde. Husk, at FES bare et et supplement."

Thus, some stroke patients react right away on the FES therapy, whereas others do not experience any progress for weeks. The effect of FES is dependent on the impairment of the stroke patient and how the technology is used.
The development therapist reported that the focus on rehabilitation of body sensation is generally increasing. For example, if the stroke patient has a rehabilitation session and is instructed to touch and lift a cup, the therapist makes sure to pour e.g. hot water into the cup. Thereby, the session is not only about the motor control of an upper limb, but also about receiving an increased sensory input when touching the cup. This statement is supported in a study by Chen et al. [2018], which concluded that sensory inputs are highly important for the patient, also for the motor rehabilitation, since the function of the sensory systems can impact motor function as well. This might be because the brain integrates sensory information to be able to produce suitable motor outputs. Thus, it will probably be beneficial to focus on both sensory and motor function in the rehabilitation of the patients. [Chen et al., 2018] However, the development therapist reported that the therapists do not have any rehabilitation strategies directly aimed on regaining body sensation. It is experienced as a secondary outcome from the motor rehabilitation. This is problematic, since e.g. a study by Doyle et al. [2014] which includes interviews with stroke patients with sensory impairments describes how the patients with decreased sensation in the affected limbs tend to use these limbs less. The patients in the study also reported that they had problems with performing ADLs due to their sensory impairments. Another point from the study was that the included participants felt like stimulation and increased movement of affected limb had a positive impact on the return of sensation. [Doyle et al., 2014] These points support the findings from the interview performed in the current study.
Thus, the points from the performed interview regarding potential sensory recovery following FES and the studies described in the current section further confirms the relevance of investigating the impact of FES on sensory function, mainly proprioception. These findings indicate that the processing of other types of sensory information is relevant to examine, e.g. the ability to feel tactile stimuli applied to the hemiparetic limb. The following section addresses some of the possible challenges associated with performing these investigations directly in hemiparetic stroke patients and furthermore proposes an alternative based on healthy subjects.

2.3 A Stroke Model for Exploring Neurophysiological and Sensory Alterations

Almost one third of all stroke patients experience a significant degree of cognitive impairment lasting several months after the stroke occurred [Gorelick and Nyenhuis, 2015]. Therefore, it can potentially be challenging to perform studies in a stroke population. Furthermore, the severity and experienced dysfunction following a stroke depends on the affected brain area [Caplan, 2006], by which it can be challenging to recruit a homogeneous group of patients. A study by Stinear [2010] concluded that inter-individual variabilities can be present among stroke patients between the initial impairment following the stroke and the subsequent functional recovery. To reduce these potential variabilities, an option would be to include subjects who, in theory, start out from a similar functional and cognitive basis.
According to a study by Furlan et al. [2016], a model that closely resembles the condition of low-functioning upper limb hemiparesis is upper limb immobilization of healthy subjects. Previous studies have examined the effect of upper limb immobilization of healthy subjects [Huber et al., 2006; Moisello et al., 2008; Bassolino et al., 2012]. An example of how upper limb immobilizations can be performed is illustrated in figure 2.5.

Figure 2.5: *Immobilization of the upper limb by putting cotton gauze between the fingers and wrap the hand and wrist in bandage as illustrated to the left on the figure. Additionally, the hand was fixed to a rigid support with tape. The upper limb was immobilized by putting it in a cotton sling to keep the elbow in a 90° angle as illustrated to the right. Modified from Bassolino et al. [2012].*

A study by Huber et al. [2006] examined the effect of 12 hours of upper limb immobilization. It was found that the motor performance deteriorated and both somatosensory evoked potentials (SEPs) and MEPs recorded immediately after the immobilization had decreased. [Huber et al., 2006] Another study by Moisello et al. [2008] found that after 12 hours of upper limb immobilization, the motor control of the immobilized limb was impaired. This was expressed through abnormalities in both hand trajectories and inter-joint coordination during reaching movements. This is also present in patients with proprioceptive deficits. This result was not detected after only six hours of upper limb immobilization. It was assumed that the detected alterations were related to cortical plastic alterations and occurred due to alterations of proprioceptive memory. The study furthermore points out that these alterations are less

severe following upper limb immobilization of healthy subject than in patients with actual proprioceptive deficits, since the healthy subjects do have intact feedback and feedforward systems. [Moisello et al., 2008] A study by Bassolino et al. [2012] investigated the kinematic effects after 10 hours of upper limb immobilization. The results showed a slower reaching movement, but an interesting result was that the initial effects decreased quickly trial-by-trial as illustrated in figure 2.6.

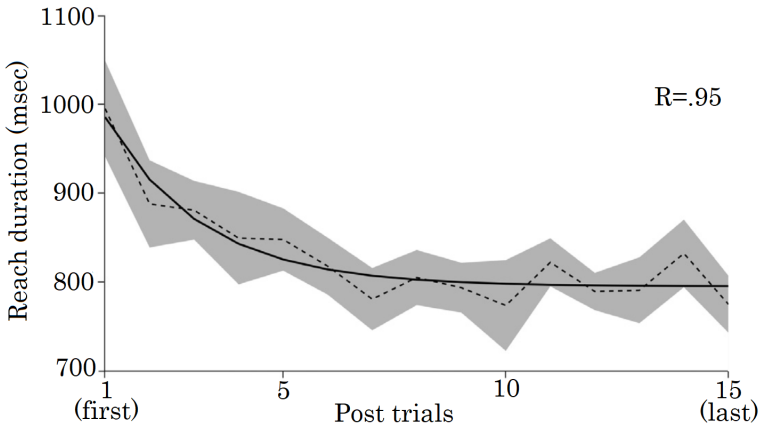


Figure 2.6: *The dashed line represent the mean trial-by-trial recovery curve of the reach duration ± standard deviation (SD) following the immobilization among all subjects. The solid line is fitted by an exponential function (R=.95). Modified from [Bassolino et al., 2012].*

As illustrated in figure 2.6, the initial effect of the immobilization was seen as an increased reach duration. However, the reach duration time consistently decreased trial-by-trial until it reached baseline values in the last repetitions. Thus, the effect from the immobilization rapidly vanished and returned to baseline. This study thereby support previous results regarding upper limb immobilization, but also points out that the effect fades rapidly due to the sensory inputs and motor outputs associated with the repetition of movements. [Bassolino et al., 2012] A strategy based on upper limb immobilization is not influenced by the level of residual function for the individual stroke patients [Furlan et al., 2016]. Thereby, by using this model in a study, it should contribute to the most optimal settings for examining the neurophysiology of stroke recovery in a population of healthy subjects. It should be noted that the healthy subjects should be immobilized for at least 10 hours to induce a significant effect, and these effects return to baseline rapidly when motor and sensory tasks are performed. [Furlan et al., 2016; Huber et al., 2006; Moisello et al., 2008; Bassolino et al., 2012]
Thus, upper limb immobilization can be used for inducing a condition in healthy subjects similar to hemiparesis, by which a relatively homogeneous group of subjects can be obtained. Therefore, this approach might be relevant for examining sensory alterations associated with FES. Suitable methods for assessing the sensory impact of FES should be chosen in order to perform a comprehensive examination. Therefore, in the following section, multiple methods for assessing different aspects of neurophysiology will be described.

2.4 Evaluation of Neurophysiological and Sensory Alterations

To evaluate neurophysiological and clinical alterations induced by FES, several methods can be utilized. Multiple studies included in the study by Furlan et al. [2016] have used

Transcranial Magnetic Stimulation (TMS) to assess neurophysiological alterations caused by e.g. neuroplasticity related to immobilization. The study by Wilkins et al. [2017] also examined neurophysiological alterations induced during rehabilitation of hemiparetic stroke patients with electromyography-FES (EMG-FES). However, electroencephalography (EEG) was used in that study. Therefore, TMS and EEG will be introduced in the following as relevant methods for examining neurophysiological alterations.
To further evaluate the sensory and motor alterations induced by FES, behavioral measurements and tests regarding perception of tactile and kinetic sensory inputs should be included. Thereby, if neurophysiological alterations can be observed with TMS or EEG, the effect in regards of how the body reacts to this can be examined. This type of measurements will furthermore be introduced.

2.4.1 Transcranial Magnetic Stimulation

TMS is based on electromagnetism, by which cortical areas can be stimulated through signals produced by magnetic coils. During TMS, the response to the stimulation can be recorded from the spinal cord or from the muscles with surface electromyography (EMG). There are different types of coils, each of which are suitable for different purposes. [Knotkova and Rasche, 2015] During single-pulse TMS, isolated pulses are transmitted to a specific cortical area. This type of pulses can be used for exploring the response to each individual pulse. When applied to the primary motor cortex, it causes activity in specific contralateral muscles that can then be recorded. The EMG activity is recorded as MEPs. Paired-pulse TMS is based on the application of two pulses, either to the same area of the cortex or to different areas. Thus, connections between areas can be examined. This paradigm can be used for assessing the ratio of excitation and inhibition in subjects. However, both types of TMS (single and paired-pulse) focuses on the motor response of a stimulus applied to the motor cortex. [Rotenberg et al., 2014] As stated in previous sections, there is a need for further research within the sensory alterations induced during rehabilitation of hemiparetic stroke patients. Particularly, proprioception and the ability to feel stimuli applied to the hemiparetic limb is relevant to examine. Therefore, TMS might not be the optimal method.

2.4.2 Electroencephalography

EEG is a technique for recording the electric activity in the brain. The recording is often performed from electrodes placed on the scalp, which detect generated voltage potentials from the neurons. [Biasiucci et al., 2019] EEG records the postsynaptic potentials [Biasiucci et al., 2019], which include excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) [Kirschstein and Köhling, 2009]. These potentials and the generation of an action potential are elaborated in appendix B. EPSPs are involved in the generation of action potentials, as described in the appendix. Thereby, the recorded EEG signal expresses the alterations of the resting membrane potentials. IPSPs can also be involved in the alteration of the resting membrane potential. The postsynaptic potentials are considerably longer than the action potentials (around 10 msec versus 1 msec), and can therefore be summed. This leads to potentials that are sufficiently large to be recorded from the EEG electrodes on the scalp. [Kirschstein and Köhling, 2009] One type of EEG examination is event-related potentials (ERPs), which will be further elaborated in the following.

Event-Related Potentials

ERPs are recordings of the brain's response to different events. The ERPs are relatively small signals, by which approximately 1000-2000 ERPs are often recorded and averaged to cancel out background brain activity and other types of disturbances as e.g. 50 Hz noise or movement of the cables. The structure of ERP signals are transient and do thereby not follow the cyclic structure. [Sanei and Chambers, 2007; Nuwer et al., 1994] The main characteristics of the ERP signals include the amplitude, latency and distribution of a given component. The amplitude informs about the extent of the generated brain activity, the latency informs about when these specific activities occur and the distribution can be used for examining the voltage gradient pattern of specific components. The ERP components are denoted with either a P, indicating a positive component or N, indicating a negative component. The letter is followed by a number which indicates how many milliseconds it takes from the stimulus is applied until the component occurs in the EEG signal. [Sanei and Chambers, 2007] One way of identifying an ERP component is by defining a time window covering an area which the component is likely to be in, and then finding the maximum peak within the window. This approach ensures that the highest possible value in the time window is not defined as the component of interest, as it might not be a component but part of a larger waveform continuing outside the window. Figure 2.7 illustrates a situation, in which this problem would occur if only focusing on the maximum voltage within the time window. [Luck, 2014]

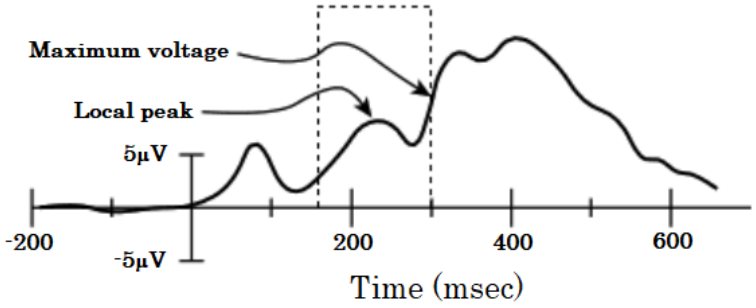


Figure 2.7: *The detection of an event-related potential (ERP) component within a time window. By defining the component as the maximum peak value and not the maximum voltage, this ensures that the highest maximum voltage is not defined as the component of interest. Modified from Luck [2014]*

As previously mentioned, event-related potentials can be recorded based on different types of sensory input. To examine the possible sensory alterations induced during rehabilitation of hemiparetic stroke patients with FES, examination of the somatosensory pathways could be evident, by which SEPs can be used [Daube and Rubin, 2009].

Somatosensory Evoked Potentials

The function of the somatosensory pathways and the processing of proprioceptive information along the peripheral nerve and the spinal-cortical pathways are reflected from SEPs [Huber et al., 2006]. These potentials can be recorded from electrodes placed on the limbs, from relevant locations on the spine and from the scalp. The recordings are performed while stimulation is applied to a peripheral nerve. Electrical stimulation is commonly used, since it causes a higher amplitude of the SEP components compared to e.g. finger taps. The electrical stimulation is most commonly applied to the wrist or the ankle of the subject. In general,

SEPs can be used for assessing features of the central somatosensory pathway. [Daube and Rubin, 2009]
Multiple studies have examined the impact of stroke on SEPs and how rehabilitation affects the components. Stroke can lead to abnormal components expressed as a decreased amplitude and increased latency. Some stroke patients can also lose one or more components completely. [Macdonell et al., 1990; Yoon et al., 2018] According to a study by Macdonell et al. [1990], absent SEP components are normalized during rehabilitation of stroke patients. According to the study by Huber et al. [2006], only 12 hours of upper-limb immobilization can result in SEP component alterations.

2.4.3 Clinical Outcome Measurements

To clinically evaluate the possible sensory and motor alterations caused by FES, different types of measurements can be performed. For example, behavioral assessments can be included. These will be further elaborated in the following. Additionally, different kinds of tests can be performed to examine the clinical effect from FES on sensory parameters. These tests will also be introduced.

Behavioral Assessment

As indicated in section 2.1, several types of clinical outcome measures can be used for behavioral assessment of upper limb hemiparetic stroke patients. A study by Murphy et al. [2015] synthesized the current knowledge regarding different outcome measures used for assessing stroke patients specifically focusing on the psychometric properties and clinical utility. Among the identified outcome measures, six specific tests were found to have a high measurement quality and clinical utility. These tests were the Fugl-Meyer Assessment (FMA), Action Research Arm Test (ARAT), Box and Block Test, Chedoke Arm and Hand Activity Inventory, Wolf Motor Function Test and ABILHAND, since they can be used for assessing many different behavioral aspects. Thus, they are recommended for evaluation of the upper limb function for both research and clinical examinations. [Murphy et al., 2015] The FMA and ARAT were used in some of the studies described in subsection 2.1. The FMA is a cumulative numerical score system developed for evaluating factors related to motor function and balance, sensation and joint mobility in patients with hemiparesis or hemiparalysis. The test consists of several subtests within each of the different parameters. [Fugl-Meyer et al., 1975] The ARAT is a test based on observations which can be used for evaluating upper limb function. The test requires a relatively large amount of equipment, e.g. wood blocks, stones and tubes. The test consists of different subtests aiming to assess grasp, grip, upper limb movements and pinch. The performance in the different subtests is evaluated on a point scale. [McDonnell, 2008]
Another test is the Jebsen-Taylor Hand Function (JTHF) test which includes seven motor tasks that are similar to different ADLs. It is used for assessing fine motor skills and thereby indirectly sensorimotor integration. [Allgöwer and Hermsdörfer, 2017] A study by Allgöwer and Hermsdörfer [2017] revealed that hemiparetic stroke patients were significantly slower than healthy subjects in the performance of the JTHF test. In a study by Bovend'Eerdt et al. [2004], a modified version of the JTHF test (consisting of three of the subtests from the original test) was tested on patients with neurological disorders to find out if this test could be used for examining gross functional dexterity of the upper limbs. The tests included:

Flipping five cards, stacking four cones and placing five kidney beans in a bowl with a spoon. In the study, the validity of the modified JTHF test was compared to the Nine Hole Peg Test (NHPT) and the grip strength of the subjects. The tests were performed on the subjects over two different sessions. Some of the findings of the study included a good correlation between the results of the modified JTHF test and the NHPT (r=0.86 for the first session and r=0.88 for the second session) and lower for the modified JTHF test and the grip strength (r=0.53 for the first session and r=0.44 for the second session). The test-retest reliability for the modified JTHF test was good (r=0.95) which was also seen for the NHPT (r=0.98) and moderate for the grip strength (r=0.72). Thus, the modified JTHF test has shown high reliability and validity. Furthermore, the study points out the importance of comparing the affected and unaffected upper limb (a ratio) when using this type of tests. [Bovend'Eerdt et al., 2004] Thus, these findings show that reliable results regarding the fine motor skills, and thereby motor functions, can be obtained based on the modified JTHF test.

Sensory Assessment

As previously elaborated, FES tends to make the hemiparetic stroke patients more aware of their hemiparetic limb and its placement in space. Thereby, it is assumed that the stroke patient might improve the proprioception and tactile sensation, by which these parameters are relevant to examine further. One of these aspects is kinesthetic sensation and proprioception, which is the ability to perceive the position of the body and how it moves in the space. In a review by Adams et al. [2015], different methods for assessing proprioception were compared. The methods were chosen based on which methods, that had been reported as the most evident in the literature. These include evaluation of the threshold to detection of passive motion (TTDPM), joint position reproduction (JPR) and active movement extent discrimination assessment (AMEDA), focusing on the ankle, knee and shoulder. In the TTDPM test, the limb of a blinded subject is passively moved, and then the subject stops the passive movement when the actual movement and direction of it is detected. It is classified as imposed proprioception, which is the opposite to obtained proprioception, that arises from active, voluntary movements. There are multiple forms of the JPR test, but in most scenarios, the subject performs active, voluntary movements. This is done by passively placing e.g. one upper limb in a specific position and then ask the subject to actively place the other upper limb in the same position. For the AMEDA test, the subject is blinded and asked to actively perform movements, recognize the different movements and discriminate between them. Thus, for this test, the memory of a specific proprioceptive aspect is recognized and repeated. [Adams et al., 2015]
According to Adams et al. [2015], there are pros and cons for all three tests in regards of applicability and validity, but the JPR test is described as efficient and enables exploration of hemispheric asymmetries in sensorimotor abilities. The case report by Bustamante et al. [2016] also examined proprioception. Here, the examination involved the JPS test, because the magnitude of the end-position errors has been thought to be a good indicator of acuity in motion and position sense. The JPR and JPS test are very similar, the only difference can be what kind of proprioceptive sensation they obtain, e.g. replication of a passive movement or performance of two similar movements after each other. This case report by Bustamante et al. [2016] emphasizes that the JPS test has been used multiple times by researchers to assess proprioception. Based on the findings in this case report, the JPS test seems to be a relevant method for evaluating proprioception.

As previously described in section 2.2, another sensory aspect which is possibly relevant to examine following FES in hemiparetic stroke patients, is the tactile sensation for the hemiparetic limb. One possible method, which can be used for this, is quantitative sensory testing (QST).
QST is a method used for identifying the absolute sensory threshold of a subject. The procedure for QST is simple and noninvasive. Another advantage of QST is that the tests can be standardized. [Kahn, 1992] QST can be used to quantify both detection and pain thresholds. One of the subtests is thermal testing. Another test is an identification of the mechanical detection threshold, which can be done based on Von Frey hairs. Furthermore, a vibration detection threshold can be identified based on e.g. a Rydell-Seiffer tuning fork. [Mücke et al., 2016] Therefore, QST can be beneficial to include in the test of sensory alterations induced by FES since different modalities can be tested and the included subtests are standardized.

2.5 Problem Statement

One possible supplementary technique to help restoring lost motor function in hemiparetic stroke patients is FES [You et al., 2014; Belagaje, 2017; Sharif et al., 2017; Jonsdottir et al., 2017]. However, only limited knowledge exists regarding the sensory alterations induced by FES, even though the hemiparetic limbs are often also affected by loss of feeling [Caplan, 2006]. The sensory alterations appear relevant to examine further, since alterations in proprioception for a single hemiparetic stroke patient has been demonstrated [Bustamante et al., 2016] and furthermore, alterations in the gray matter density within sensory cortical areas and the thalamus have been found following upper limb FES [Wilkins et al., 2017]. An interview with a development therapist supported these findings regarding proprioception along with the need for further examination of neurophysiological and sensory alterations, as an increased awareness of hemiparetic limbs is observed during and after rehabilitation sessions of the stroke patients. This can possibly indicate alterations of proprioception and tactile sensation following FES therapy. In general, an increased focus on sensory rehabilitation of stroke patients might be relevant. This is because stroke patients with sensory impairments in their limbs tend to use the affected limbs less and have problems with ADLs due to the sensory impairments [Doyle et al., 2014].
It has been suggested in the study by Furlan et al. [2016] that a model which closely resemble the condition of low-functioning upper limb hemiparesis is upper limb immobilization of healthy subjects. Other studies have furthermore confirmed the effect of upper limb immobilization [Huber et al., 2006; Moisello et al., 2008; Bassolino et al., 2012]. Therefore, it is potentially a suitable approach to examine the potential sensory alterations caused by FES described above, which may reflect the sensory alterations that would occur in hemiparetic stroke patients.
To focus on the neurophysiology of sensory recovery, an evident technique for examining the transmission of somatosensory information is the recording of SEPs [Daube and Rubin, 2009]. Previous studies have shown alterations of SEP components in hemiparetic stroke patients, including increased latency and decreased amplitude or even loss of components [Macdonell et al., 1990; Yoon et al., 2018]. To further evaluate these alterations, it is relevant to include behavioral measurements as the modified JTHF Test, which has proven to be valid and reliable [Bovend'Eerdt et al., 2004]. This is beneficial, since the effects of upper limb immobilization have shown to decrease rapidly [Bassolino et al., 2012]. Furthermore, clinical

tests of proprioception as JPS should be performed to see if findings similar to those of the case report by Bustamante et al. [2016] can be found. To further examine the alterations of tactile sensation, tests from QST can be beneficial to perform, as the included subtests are standardized [Kahn, 1992] and include different modalities [Mücke et al., 2016]. The association between the SEP recordings and the different clinical outcome measurements can provide information about the sensory state of the immobilized limb before and after FES-assisted TOT. It can furthermore contribute to understanding the possible alterations of the SEP components. This leads to the following problem statement:

How does a single session of functional electrical stimulation alter proprioception and tactile sensation in a hemiparetic stroke model based on upper limb immobilization and given these alterations, which associations can be found between the neurophysiological and clinical measurements of these?

Methodology 3

To introduce the selected interventions and measurements included in the experiment performed in this study, this chapter describes the procedures and parameter settings for the FES-assisted TOT, SEPs, JPS test, QST and the modified JTHF test.

In this study, an experiment will be performed to examine how a single session of TOT assisted by FES alters proprioception and tactile sensation in a hemiparetic stroke model based on upper limb immobilization, and which association that can be found between the neurophysiological and clinical measurements of these alterations. As described in section 2.5, multiple specific measurements makes it possible to examine proprioceptive and tactile sensation of the subjects, such as the JPS test and the QST. The neurophysiological parameters related to proprioception and tactile sensation will be examined with SEP recordings and analysis. Another clinical measurement is the modified JTHF test, which can clarify possible motor function alterations caused by FES during TOT and indirectly alterations related to sensorimotor integration [Allgöwer and Hermsdörfer, 2017].
The different measurements will be performed before and after 24 hours of immobilization of the non-dominant upper limb to induce a short lasting condition similar to hemiparesis [Furlan et al., 2016]. 24 hours of immobilization is chosen, since this is considered the most convenient time period for the subjects and the examiners. The measurements will additionally be performed after a TOT session, which either involves FES to assist the subject during the movements or no FES assistance. The non-dominant upper limb is chosen for immobilization since it is believed to cause the least inconvenience for the subjects. The pipeline for the experiment will be presented in chapter 4. In the following, the TOT session and the different measurements will be elaborated.

3.1 Task-Oriented Therapy Sessions

The TOT sessions in the current study involve a task in which the subject reaches out and grasps a bottle. There are two TOT sessions in the experiment, in which the subjects are assisted by FES in one of them. In the other part of the experiment, the TOT does not include FES assistance. This part without FES assistance will function as a control in order to examine the effects caused by FES. FES is more likely to cause neurophysiological alterations and result in recovery if the subject is actively moving the affected limb while receiving FES assistance. The different steps (a-h) of the exercise are illustrated in figure 3.1.

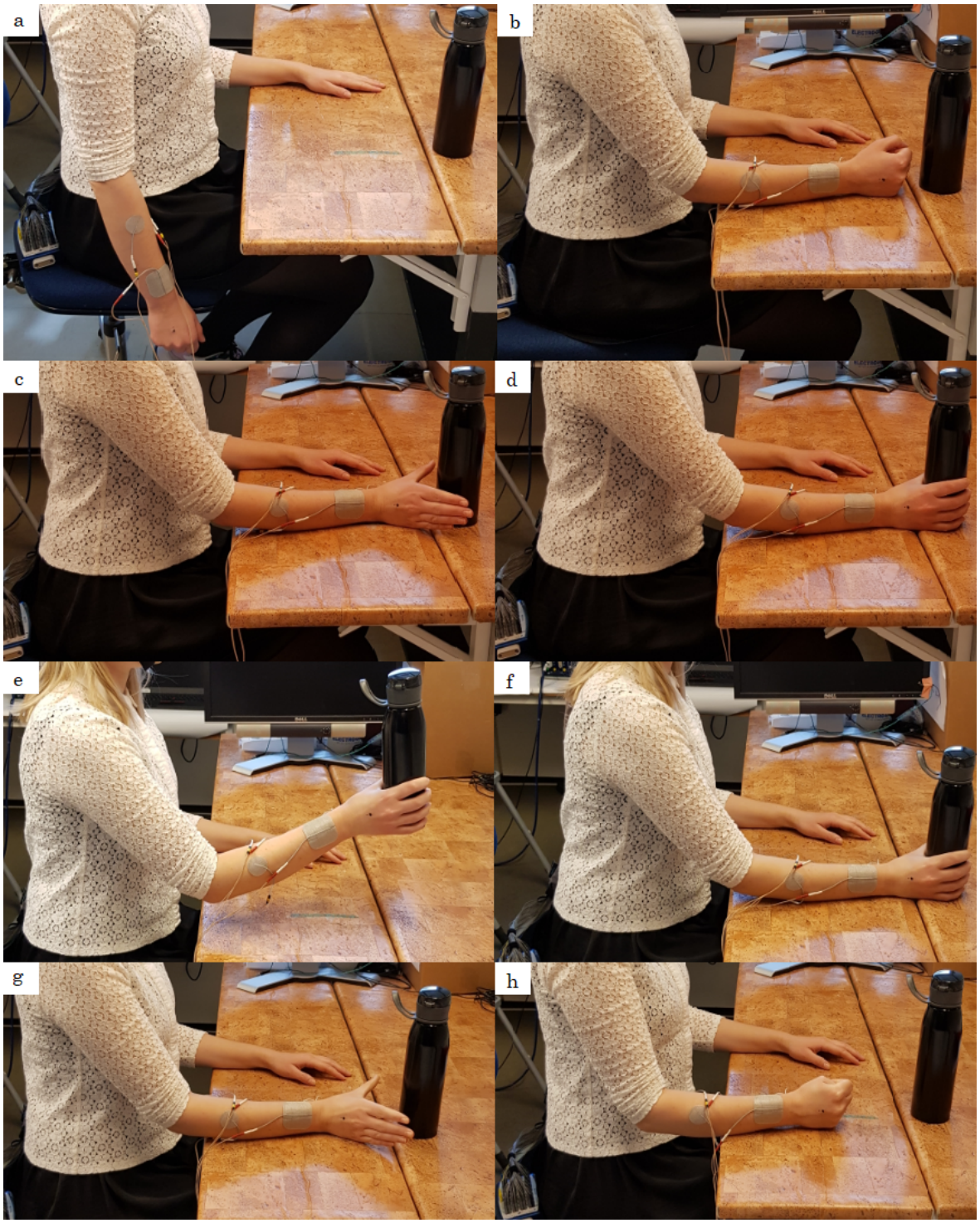


Figure 3.1: *The steps (a-h) of the grasping exercise performed repetitively during the task-oriented therapy (TOT). First, the subject has the affected upper limb resting alongside the body (a). The subject then moves the upper limb towards the bottle on the table (b) and opens the hand (c). Then the subject closes the hand around the bottle (d) and lifts it up (e). Subsequently, the subject puts the bottle back down (f), opens the hand (g) and withdraws the upper limb while closing the hand (h). For the next repetition, the subject again starts out from (a).*

During FES-assisted TOT, the stimulation is applied to the wrist flexors and extensors to specifically support the grasp and release of the bottle during the exercise. figure 3.2 illustrates

the stimulation pattern.

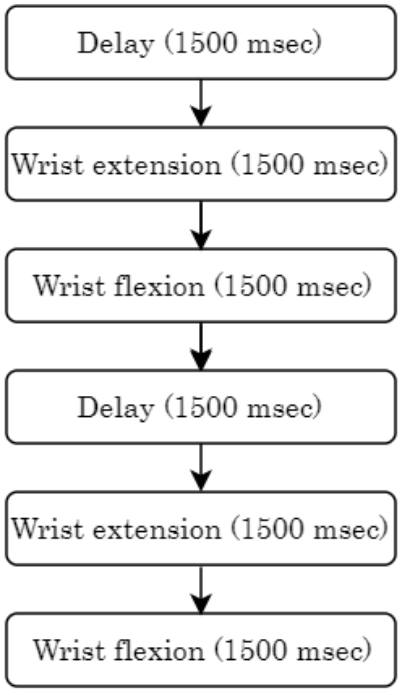


Figure 3.2: *The pattern for the stimuli applied during the FES-assisted task-oriented therapy (TOT) divided into steps showing the specific action and duration.*

When the exercise starts, the subject has 1500 msec to lift the upper limb from alongside the body and move it towards the bottle. The wrist extensors are then activated for 1500 msec to open the hand followed by a 1500 msec activation of the wrist flexors to close the hand around the bottle. The subject follows the hand opening and closing and actively moves the fingers during these movements. When the wrist flexion is supported by FES, the subject has to lift the bottle and keep it up until the wrist flexion assistance stops. Immediately after, the subject has to place the bottle on the table again and wait for the electrical stimulation to activate the wrist extension once more. When the 1500 msec wrist extension occurs again, the subject releases the bottle. During the 1500 msec wrist flexion, the hand is closed and the upper limb is withdrawn and placed alongside the body. The bottle is filled with cold water prior to the TOT to increase the sensory input to the subject as emphasized in section 2.2. The weight of the bottle is approximately 730 g.
This exercise is repeated 30 times. The choice of repetitions is based on the study by Wilkins et al. [2017], in which each training session consisted of 20-30 repetitions of the given exercise. Since the current study only includes a single training session, a number in the high end of this spectrum from the study by Wilkins et al. [2017] is chosen.

During the TOT session without FES assistance, the subjects perform exactly the same exercise 30 times. However, they must do it in their own pace.

3.1.1 Stimulation Parameters

The strength of the muscle contractions induced by FES can be controlled from recruitment (the number of activated nerve fibers) and temporal summation (the frequency of the stimulation) [Popovic and Sinkjaer, 2003]. Therefore, these parameters are relevant to consider.
A study by Behringer et al. [2016] examined the impact of stimulation parameters including frequency, pulse width and intensity on the development of muscle fatigue during neuromuscular electrical stimulation. It was found that the stimulation frequency was the only parameter which had an impact on the development of fatigue. The frequencies used in the study were 20 and 80 Hz respectively. [Behringer et al., 2016] The minimum frequency needed to obtain a smooth muscle activation in the upper limb is usually below 20 Hz [Popovic and Sinkjaer, 2003]. The study by Wilkins et al. [2017] and case report by Bustamante et al. [2016] directly found or indicated that sensory alterations might occur following upper limb FES in hemiparetic stroke patients. In these studies, the stimulation frequency was 50 Hz. Therefore, this frequency for FES is also used in the present study. The pulse width for the stimulation is 300 μseconds and the intensity will be individually set, so that it is sufficient enough to induce an optimal contraction of the muscle without causing any discomfort for the subject. [Wilkins et al., 2017; Bustamante et al., 2016] To ensure that the two electrodes are placed correctly to induce wrist extension and flexion, guidelines for the placement will be used [Baker et al., 2000]. The two electrodes are placed in a bipolar configuration [Peckham and Knutson, 2005].

3.2 Recording of Somatosensory Evoked Potentials

In the current study, SEPs are recorded during stimulation of the median nerve. It is a mixed nerve by which it contains both sensory and motor nerves. Additionally, the median nerve innervates the forearm and hand. [Martini et al., 2012] The stimulation will be applied to the non-dominant upper limb. The intensity of the stimulation should be high enough to induce a clearly detectable thumb twitch [Nuwer et al., 1994]. Multiple short latency SEPs (components which occur within 50 msec after the stimulation is applied [Nuwer et al., 1994]) are detected and analyzed, including the N20, N30 and P45 components. The long latency component N90 is also included in the detection and analysis.
When electrical stimulation is applied to the median nerve during SEPs, the nerve action volleys will be transmitted orthodromically through sensory fibers and antidromically through motor fibers. The components of interest in the current study are detectable from the contralateral side of the cortex in regards to the stimulated wrist. [Nuwer, 1998] Some of the components of interest are illustrated in figure 3.3.

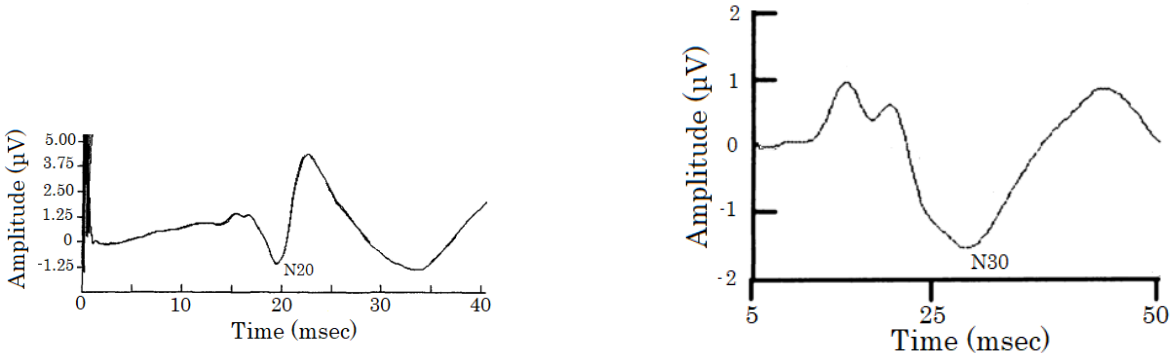


Figure 3.3: *Illustration of the N20 (a) and N30 component (b), which are two of the somatosensory evoked potential (SEP) components of interest in the current study. (a) is modified from Nuwer et al. [1994] and (b) is modified from Waberski et al. [1999].*

The N20 component is interesting for this study, as it emerges in the postcentral cortex which subserves sensory functions [Nuwer et al., 1994] and can in some patients decrease in amplitude as a result of sensory loss [Macdonell et al., 1990]. In patients with neurological disorders causing severe motor and sensory symptoms, the component might be absent [Tsuji et al., 1988]. If there are abnormalities for any other SEP components, it will most likely also be expressed through an alteration of the latency or amplitude of the N20 component [Zeman and Yiannikas, 1989]. In the current study, it is expected that the amplitude of the component will decrease following the immobilization session compared to the baseline recording. This could indicate that the effect of the immobilization is similar to what is experienced from a hemiparetic stroke patient. Moreover, it is expected that the amplitude of the component will increase as a result of FES-assisted TOT. This could potentially indicate that FES normalized the temporarily induced state caused by the upper limb immobilization, and improved the ability to detect tactile stimuli for the subjects. It is expected that the amplitude of the component will remain decreased as a result of TOT without FES assistance.
The latency of the N20 component is expected to increase following the immobilization compared to the baseline recording. This is based on the general abnormalities, which can be found for SEP components in stroke patients as described in subsection 2.4.2. After the TOT with FES assistance, it is expected to decrease again, which could potentially indicate an improvement in the ability to detect tactile stimuli. This could also be an indication of an improvement of the ability to detect tactile stimuli for the subject. It is expected that the latency of the component will remain increased as a result of TOT without FES assistance.
The N30 component is included, as it is believed to reflect sensorimotor integration [Lelic et al., 2016; Waberski et al., 1999]. It is mainly detectable in motor areas of the brain [Seiss et al., 2003]. The amplitude of the N30 component has been found to decrease in patients with Parkinson's disease, but to increase in patients with dystonia [Kanovsky et al., 2003]. Another study by Lelic et al. [2016] concluded that the amplitude of the component decreased as a result of a chiropractic spinal manipulation. Thereby, it is unknown what impact immobilization of the upper limb will have on the component. Based on the knowledge of what impact immobilization is hypothesized to have on the other components, the amplitude of the N30 component is expected to decrease following immobilization. Additionally, the amplitude is expected to increase following the FES-assisted TOT, and remain decreased following the TOT without FES assistance. The latency is expected to increase following the immobilization

session and then decrease following FES-assisted TOT. The latency of the N30 component is expected to remain increased following the TOT without FES assistance.
The P45 component represents proprioceptive information processing within sensorimotor cortical areas [Bassolino et al., 2012] and has shown to decrease in amplitude and increase in latency as a result from the neuroplastic alterations induced by upper limb immobilization [Huber et al., 2006]. Therefore, the amplitude of this component is also expected to decrease following immobilization and then increase following FES-assisted TOT. This again potentially indicates an improvement in the proprioceptive sense of the subject. The latency is expected to increase following the immobilization session, decrease following FES-assisted TOT and remain increased following the TOT without FES assistance.
According to a study by Seiss et al. [2003], the proprioception-related component N90 emerges in the precentral motor cortex mainly due to activity in muscle spindle afferents which is transmitted to the sensorimotor cortex. In the study, it was used for examining possible proprioceptive deficits in patients with Parkinson's and Huntington's disease. Therefore, it can potentially also be used for examining proprioceptive impairments in hemiparetic stroke patients. However, the N90 is elicited by passive movement of the finger in the study by Seiss et al. [2003]. Since the intensity of the electrical stimulation for SEPs in the current study will cause a thumb twitch, one could ague that this is a passive movement. The subject is not actively thinking about moving the finger and the muscle spindles will be activated regardless of movement type, i.e. passive or active. Since the study by Seiss et al. [2003] focused on patients with other neurological disorders, it is more uncertain which alterations that can be expected for the N90 component in the current study. However, it was found that one of the patient groups had a borderline significant reduced amplitude for the component. [Seiss et al., 2003] Therefore, it is possible that a reduced amplitude also can be found in the immobilized subjects. Since a normal latency was demonstrated for both patient groups in the study, the latency might not be altered in the subjects either, but the latency of the component will still be analyzed.

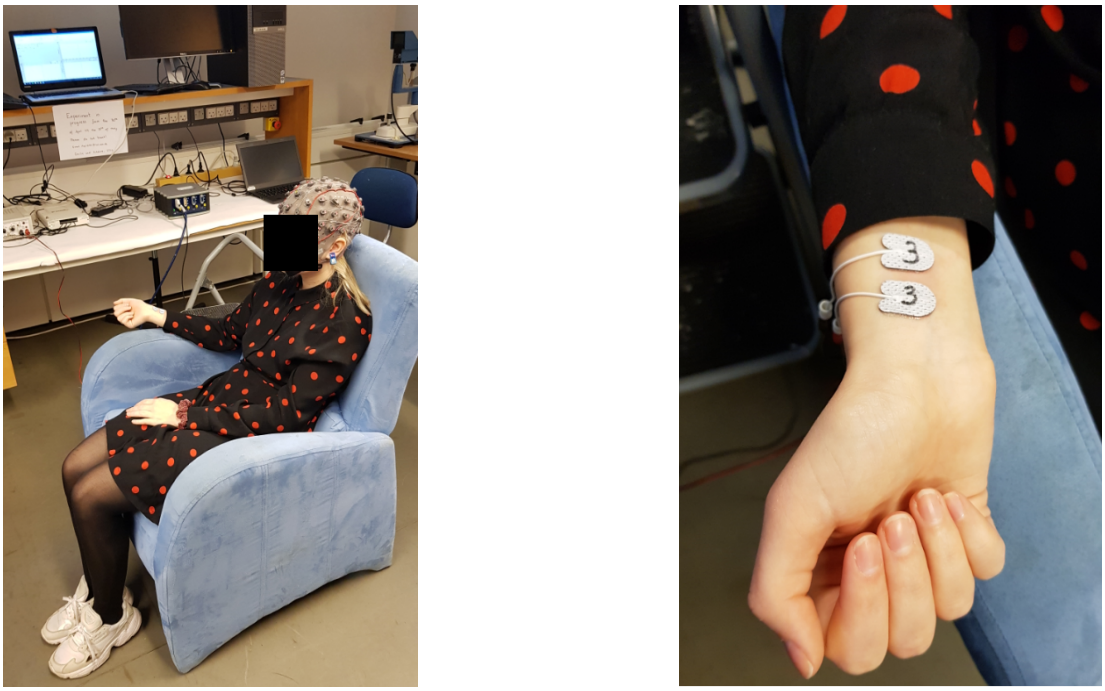
To sum up, the hypotheses in regards of the SEP components are that:

- The amplitudes of the four components are expected to:
  - Decrease after the 24 hours of immobilization.
  - Increase after the FES-assisted TOT.
  - Remain decreased after the TOT session without FES assistance.
- The latencies of the N20, N30 and P45 are expected to:
  - Increase after the 24 hours of immobilization.
  - Decrease after the FES-assisted TOT.
  - Remain increased after the TOT session without FES assistance.
- The latency of the N90 is not expected to change.

3.2.1 Recording Parameters

In the current study, the SEPs recording is based on the IFCN recommended standards for short latency SEPs [Nuwer et al., 1994]. Thus, the SEPs are recorded with EEG and the stimulation is applied to the median nerve as 200 μsec square pulses with a frequency of 2.85-3.33 Hz. By having a minor bandwidth for the frequency of the stimuli, the resulting response is less likely to attenuate [Passmore et al., 2014]. The intensity of the stimulation is 1 mA above the individual motor thresholds causing a visible thumb twitch. During the recording of SEPs, the subject sits comfortably in a chair with the lower arm and hand in a

supine position. This is illustrated in figure 3.4 along with the electrode placement over the wrist.



(a) *(b)*

Figure 3.4: *The experimental setup for recording of the somatosensory evoked potentials (SEPs). The subject sits in a chair while receiving electrical stimulation of the median nerve. Electroencephalography (EEG) is recorded with the cap on the head of the subject (a). The stimulation electrodes are placed over the crease of the wrist (b).*

The SEPs are recorded with EEG from 32 scalp electrodes [Nuwer et al., 1994], which are placed according to the standardized 10-20 system. The electrodes are named by their locations: Frontopolar (Fp), frontal (F), central (C), parietal (P), temporal (T) and occipital (O). The electrodes are also numbered with odd numbers for the left hemisphere, even numbers for the right hemisphere and z refers to the midline between the two hemispheres. The magnitude of the electrode number indicates the distance to the midline. Thus, F5 is located further from the midline compared to e.g. F1. [Blum and Rutkove, 2007; Luck, 2014] According to the IFCN recommended standards, 1000-2000 SEPs should be recorded [Nuwer et al., 1994]. The exact number of recorded SEPs will be decided based on a pilot trial described later.
The N20 component is most distinct at the C3/C4 or CP3/CP4 electrode site contralateral to the stimulation [Waberski et al., 1999]. The electrodes included for detecting the N30 component is F3/F4 contralateral to the stimulation [Lelic et al., 2016], but the component can also be clearly detectable at other electrode sites as FC1/FC2 or C1/C2 [Seiss et al., 2003]. The study by Lelic et al. [2016] chose F3 based on visual inspection as it revealed that the N30 component tended to have the largest amplitude at this electrode site. The P45 component can be detected at C1/C2, C3/C4 or CP1/CP2, CP3/CP4 and the N90 component is primary found at FC3/FC4 or C3/C4 contralateral to the stimulation [Seiss et al., 2003]. Thus, as multiple studies suggest different electrode sites for detection of the components, a visual inspection of the data is necessary in the current study to find the electrode site in which the specific component is most distinct. Therefore, all 32 electrode sites are included in the

recording of SEPs as illustrated in figure 3.5.

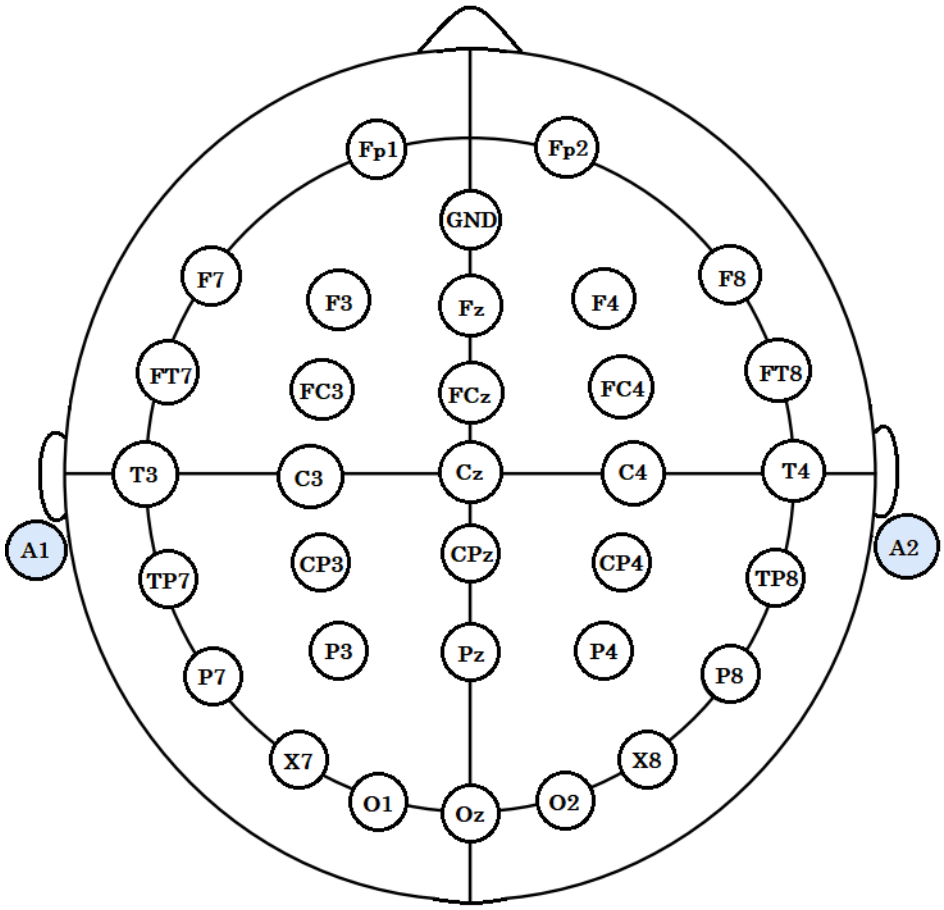


Figure 3.5: *The electrode sites included in the somatosensory evoked potential (SEP) recording of the current study. The references are marked with blue. The figure is based on knowledge gained from Luck [2014].*

The signal is sampled with a frequency of 9600 Hz.

3.2.2 Preprocessing and Detection

The pipeline for preprocessing of the recorded SEP data is illustrated in figure 3.6.

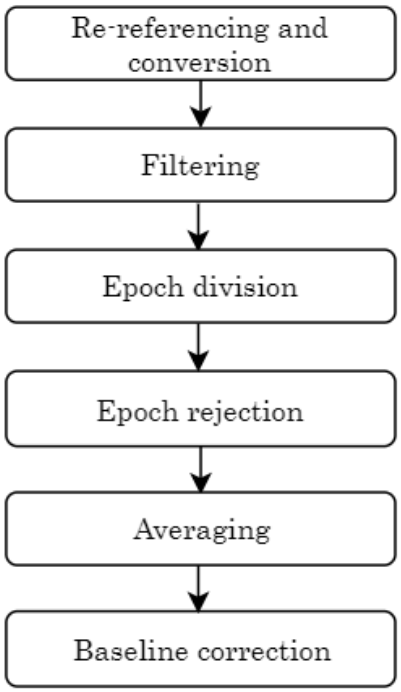


Figure 3.6: *The overall pipeline for the preprocessing of somatosensory evoked potential (SEP) data.*

First, the recorded data for each subject is re-referenced in EEGLAB to the electrode on the ipsilateral earlobe with respect to the stimulated wrist. EEGLAB is an interactive MATLAB toolbox for processing, analysis and visualization of EEG data [Neuroscience, 2019]. By using this program, the recorded data can be converted from .HDF5 files to .mat files which allows for further preprocessing in MATLAB.
Following the re-referencing and conversion of data, all electrode sites containing EEG data are loaded into MATLAB. According to the IFCN recommended standards, the cutoff frequency for the lowpass filter should be 3000 Hz when processing short-latency SEPs [Nuwer et al., 1994]. The exact cutoff frequency for the lowpass filter will be decided based on the pilot trial described later. The signals are highpass filtered with a cutoff frequency of 3 Hz [Nuwer et al., 1994]. Furthermore, the signals are filtered with a notch filter at 50 Hz. The offsets for the notch filter are -1 Hz and +1 Hz for the lowpass and highpass respectively. The notch filter is included to reject 50 Hz noise from the recordings [Sanei and Chambers, 2007]. The filters are all second order Butterworth filters.
After filtering, the data is split into epochs containing one SEP each. The single epochs are extracted from 150 msec pre- to 150 msec post-stimulus. By visual inspection of data from the electrode site Fp2, a threshold for eye blink artifacts is determined for each subject. This is done by looking into the individual datasets for the single subjects, in which the eye blinks appear as high peaks at the Fp2 electrode site. Thereby, an epoch recorded from another electrode site can be excluded, if an eye blink is detected in Fp2 at the specific epoch. Since the amplitude of eye blinks can potentially differ between the subjects, individual thresholds are determined. The threshold should be high enough to ensure that epochs which only contain SEP data are not excluded. Furthermore, the threshold should be sufficiently low to ensure that as many epochs as possible containing eye blinks are excluded. The remaining epochs

are used for calculating an average to cancel out noise as elaborated in subsection 2.4.2. The averaged epochs from each electrode are then further preprocessed, as the average of the pre-stimulation data (-150 msec to 0 msec) is subtracted from the signal [Luck, 2014]. Thereby, the data is corrected in regards of the baseline.

When the final electrode sites are selected based on data from the pilot trial, only data from these electrode sites is saved for component detection. In the component detection, a time window is defined and within this window, the most positive or negative peak is localized. The amplitude and latency of this component are then measured. The time window is set dependent on the specific component as illustrated in figure 2.7. [Luck, 2014] The window in the current study is set to be -8 msec and +8 msec with respect to the component of interest. Thus, the N20 component is localized between 12-28 msec after stimulus and so forth. In the current study, a graphical user interface (GUI) is developed and used for the detection based on the principles described above. Figure 3.7 illustrates the GUI.

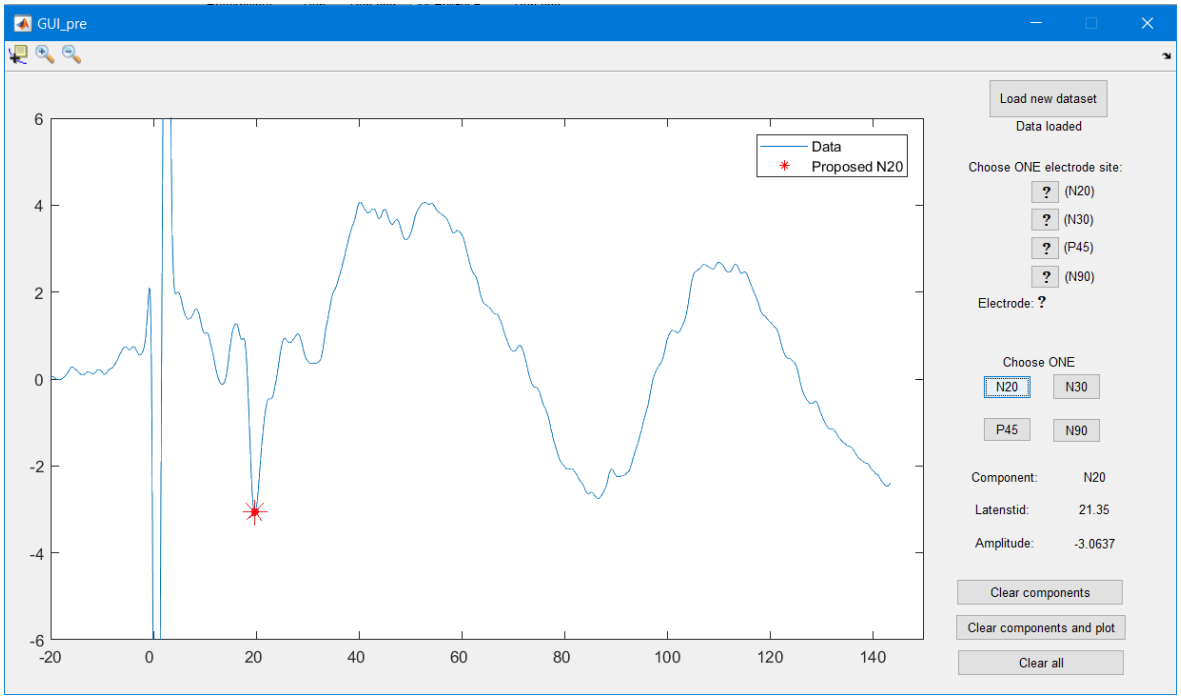


Figure 3.7: *An example of the somatosensory evoked potential (SEP) component detection in the current study. When data is loaded, the component of interest is marked on the plotted signal with a red whisker. The buttons with question marks indicate that the specific electrode sites for detection of each component are still unknown and will be defined based on data from the pilot trial. The amplitude and latency of the component can then be saved or the examiner can mark another peak instead, if it is evaluated that the graphical user interface (GUI) did not detect the correct component.*

The specific electrode sites for the single components are chosen based on the pilot trial described later. The component of interest is plotted in the signal as the largest positive or negative peak within the time window for the specific component. To verify the proposed components, an examiner performs a visual inspection of these. If the examiner agrees with the detection, the coordinates indicating the latency and amplitude of the components are saved. If the examiner disagrees with the detection, the data cursor can be activated and the examiner can then mark the presumed correct component instead. The evaluation is based

on the knowledge of the appearance of the component and its placement. This approach is chosen, since a complete manual detection of components might be subject to bias, as the examiner could perform the analysis based on informal rules [Luck, 2014]. Additionally, a complete automatic detection of components migh as well result in incorrect results. Thus, the role of the examiner in the component detection is to verify the already detected components, but also to correct a detection if it is found to be incorrect.

3.3 Joint Position Sense Test

For examining the proprioceptive sense of the subject, the JPS test is used and is one of the most reliable methods as described in subsection 2.4.3. The procedure is based on the case report by Bustamante et al. [2016].
The subject is asked to lie supine on a surface with the eyes closed and both upper limbs down by the torso while bending the knees. The examiner moves the non-dominant upper limb and positions it over the head of the subject. Then, the subject is asked to replicate this position with the dominant upper limb, still with the eyes closed. When the subject believes that the position of the dominant upper limb is similar to the non-dominant upper limb, the subject has to inform the examiner about this. Then a photo is taken of the subject's positioning of the upper limbs from a standardized point above the subject. The photo is then used for measuring the joint angles later. An example of the JPS test is illustrated in figure 3.8.

Figure 3.8: *The joint position sense (JPS) test during which the subject has to place the dominant upper limb similarly to the non-dominant upper limb placed by the examiner. The subject in this illustration is right handed, by which the examiner places and retains the left upper limb. A photo similar to this is saved for further analysis of the joint angles.*

The time it takes for the subject to place the dominant upper limb will also be measured as done in the case report by Bustamante et al. [2016]. Thereby, the results for proprioceptive sensation of the subject used in the further analysis are based on the angular difference for placement of the shoulder, elbow and wrist joint along with the time period for placement of the dominant upper limb.
It is expected that the subjects will have an impaired proprioceptive sense after the immobilization compared to the baseline, as the temporarily induced state is similar to low-

functioning hemiparesis [Furlan et al., 2016]. The case report by Bustamante et al. [2016] revealed no particular alterations in the proprioceptive sensitivity for the shoulder and elbow after training with FES, but a remarkable improvement was found for the proprioceptive sense of the wrist. Thereby, the subjects are expected to improve their proprioceptive sense of the affected wrist after the FES-assisted TOT. This can be expressed as a decreased angular difference between the placement of the dominant and non-dominant wrists after the FES-assisted TOT compared to the measurement obtained before the TOT. The interviewed development therapist also mentioned (appendix D) that the hemiparetic stroke patients tended to become more aware of the hemiparetic limb, which most likely indicates an improved proprioceptive sense.
Thus, the hypotheses in regards of the JPS test are that:

- The angular differences for the shoulder, elbow and wrist joint placements are expected to increase after the 24 hours of immobilization.
- The angular difference for the wrist joint is expected to:
  - Decrease after the FES-assisted TOT.
  - Remain increased after the TOT session without FES assistance.
- The angular differences for the shoulder and elbow joints are expected to remain increased after both TOT sessions.
- The time required to place the affected limb is expected to:
  - Increase after the 24 hours of immobilization.
  - Decrease after the FES-assisted TOT.
  - Remain increased after the TOT session without FES assistance.

3.4 Quantitative Sensory Testing

QST is a method used for identifying the absolute sensory threshold of a subject. It can be used for investigating the functional state of the somatosensory system. [Mücke et al., 2016; Kahn, 1992] However, since the original test battery is time consuming, only single subtests are included in the current study to evaluate the tactile sensitivity of the subjects.
To identify the detection thresholds for tactile stimulation, Von Frey hairs are utilized along with electrical stimulation. The Von Frey hairs are depicted in figure 3.9.

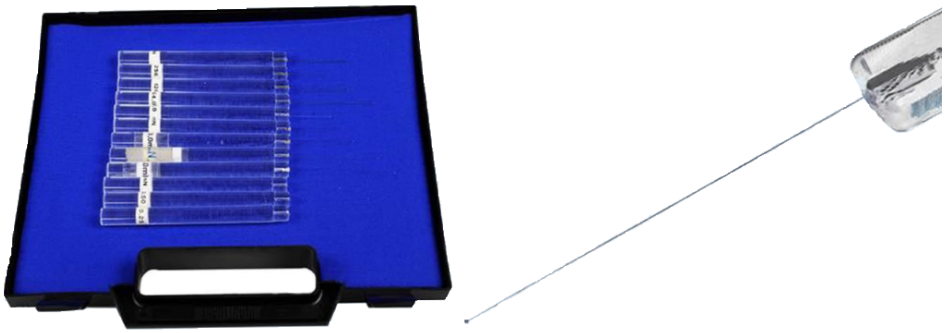


Figure 3.9: *The different Von Frey hairs to the left along with one of the Von Frey hairs up close to the right. Modified from Mücke et al. [2016].*

Both electrical, mechanical and thermal stimulation methods can be utilized for analysis of tactile sensation and pain [Wong et al., 2010]. Thereby, as electrical stimulation is already included in the study for SEP recording, this type of stimulation is included for sensory testing

along with the mechanical stimulation from the Von Frey Hairs.
The Von Frey hairs are made of glass fiber and have different diameters and length [Mücke et al., 2016]. The different hairs have numbers from 1 to 20. The Von Frey hair with the number 1 is the smallest. The numbers indicate the applied force in gram-force (gf) which is a unit numerically equal to the weight in grams. The SI derived unit for force is Newton, by which 1 gf is approximately 9.8 mN. [de Sousa et al., 2014] Table 3.1 depicts how many mN each of the Von Frey hairs applies.

SI Units for Von Frey Hairs ([gf] = [mN])										
gf	1	2	3	4	5	6	7	8	9	10
mN	9.8	19.6	29.4	39.2	49	58.8	68.6	78.5	88.3	98.1
gf	11	12	13	14	15	16	17	18	19	20
mN	107.9	117.7	127.5	137.3	147.1	157	166.7	176.5	186.3	196.1

Table 3.1: *The force applied by each of the Von Frey hairs from the standardized set in gram-force (gf) and millinewton (mN) respectively.*

When conducting the test, the hair with the smallest length and diameter is used first. In order to standardize the amount of force used for finding the mechanical detection threshold, the hairs must touch the skin and be forced down until they bend in an s-shape. The contact time with the skin should be approximately two seconds. [Mücke et al., 2016] To find the mechanical detection threshold, the examiner applies the Von Frey hairs in an increasing order until the subject is able to feel the applied hair (upper threshold). Then, the examiner applies the Von Frey hairs in a decreasing order, starting from the one the subject is able to feel, until the subject is unable to feel the applied hair (lower threshold). The application of a Von Frey hair is illustrated in figure 3.10.

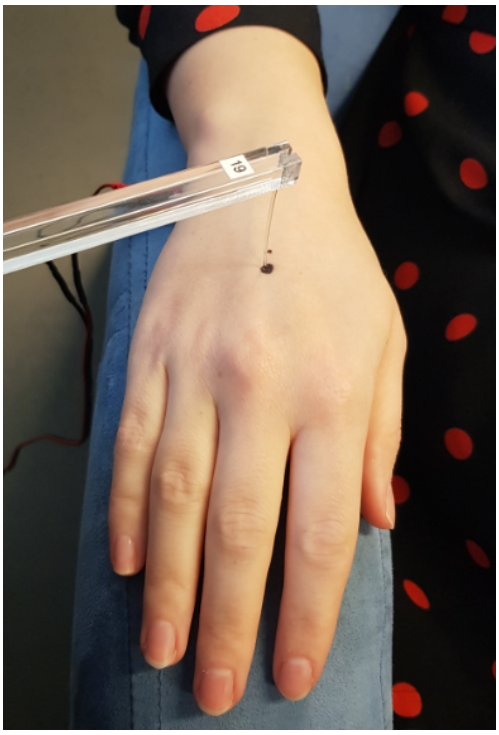


Figure 3.10: *The application of a Von Frey hair to the center of the back of the hand.*

This procedure is repeated five times, and a geometric mean is calculated and defined as the final threshold as suggested by Rolke et al. [2006] and used in the data analysis. The subjects keep their eyes closed during the procedure.

The detection threshold for electrical stimulation is identified by increasing the intensity from 0.2 mA with steps of 0.1 mA at a time to find the threshold for when the subject is able to feel the stimulation (upper threshold) and then decreasing the intensity until the subject is unable to feel the stimulation again (lower threshold). Thus, this subtest follows the procedure for the Von Frey hairs. This is repeated three times, and a geometric mean is calculated and defined as the final threshold used in the analysis [Rolke et al., 2006]. The stimulation electrodes from the SEP recording are used for this purpose.
It is expected, that the subjects will have an impaired tactile sensation after the immobilization compared to the baseline, as the temporary induced state of function will be similar to low-functioning hemiparetic stroke patients [Furlan et al., 2016]. This patient group is additionally often affected by sensory loss [Caplan and van Gijn, 2012]. Thereby, the subject will presumably need to be exposed for thicker Von Frey hairs before it is detectable. An increased detection threshold is also expected for the electrical stimulation after immobilization. Based on the rehabilitative effects of FES for stroke patients described in section 2.1, it is expected, that the subjects will gain a better tactile sensitivity after the FES-assisted TOT but not after the TOT without FES assistance. Thus, the hypotheses in regards of the QST are that:

- The thresholds for detection of Von Frey hairs and electrical stimulation are expected to:
  - Increase after the 24 hours of immobilization.
  - Decrease after the FES-assisted TOT.
  - Remain increased after the TOT without FES assistance.

3.5 Modified Jebsen-Taylor Hand Function Test

To examine the motor control of the immobilized upper limb and thereby indirectly sensorimotor integration [Allgöwer and Hermsdörfer, 2017], the modified JTHF test is performed. The three subtasks included in the test are conducted in the following order: Flipping over five cards, spooning five kidney beans into a bowl and stacking four checkers. The modified JTHF test is performed with the Jebsen Test of Hand Function test kit from Patterson Medical Ltd similar to the one used in a study by Simonsen et al. [2017]. The order and setup is furthermore based on this study. The objects are placed on a horizontal board at table height and are always placed in a standardized manner. The setup is illustrated in figure 3.11.

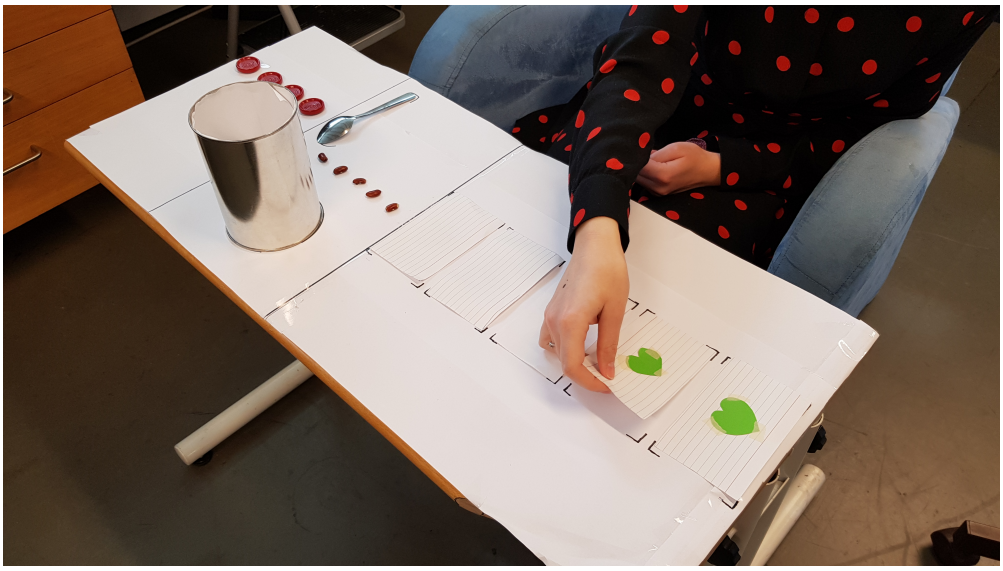


Figure 3.11: *The board with the objects for the different tasks in the modified Jebsen-Taylor Hand Function (JTHF) test. The subject conducts each subtask from left to right.*

The subject conducts the test three times with each hand starting with the dominant hand. The time it takes for the subject to perform the task with one hand is recorded each time, after which all objects are placed in the standardized manner before conducting the task again. An average is calculated for the time is took with each upper limb followed by the ratio. This is done because it has been found that it is important to use the ratio when analyzing the results of this test. [Bovend'Eerdt et al., 2004]
It is expected that the subjects will have an impaired motor control after the immobilization compared to the baseline, as the temporarily induced functional state will be similar to hemiparetic stroke patients [Furlan et al., 2016]. Additionally, a study by Allgöwer and Hermsdörfer [2017] found that the fine motor skills were impaired and the time needed to perform the original JTHF test was increased for post-stroke patients compared to healthy controls. Thereby, the subject is expected to perform the modified JTHF test slower after the 24 hours of immobilization. The time period for the unaffected upper limb should remain approximately the same during all tests. However, the unaffected upper limb might be faster after the immobilization compared to baseline if the cortical alterations associated with hemiparesis are taken into consideration.
Based on the rehabilitative effects of FES for stroke patients described in section 2.1, it is expected that the subjects will gain a better motor control after the FES-assisted TOT. Thereby, the needed time to perform the test should decrease after the FES-assisted TOT. The motor control of the subjects is expected to remain the same before and after the TOT without FES assistance.
The hypotheses in regards of the modified JTHF test are that:

- The time needed to perform the test for the affected hand is expected to:
  - Increase after the 24 hours of immobilization.
  - Decrease after FES-assisted TOT.
  - Remain increased after TOT without FES assistance.
- The time needed to perform the test for the unaffected hand is expected to remain unchanged.

Experimental Procedure and Analysis 4

This chapter introduces the procedure for the experiment and the procedure and results of the pilot trial. The experiment and pilot trial are performed based on the methods described in the previous chapter.

To answer the problem statement stated in section 2.5 and to test the hypotheses stated throughout chapter 3, an experiment was performed. The overall pipeline of the experimental procedure is illustrated in figure 4.1.

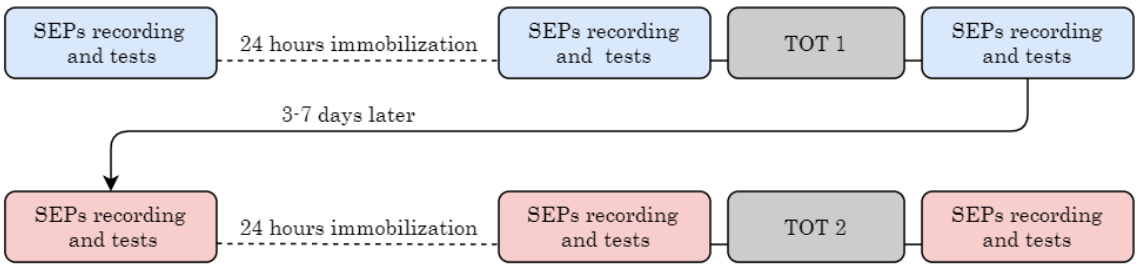


Figure 4.1: *The pipeline for the experiment which consisted of two different parts. The blue boxes stating "somatosensory evoked potentials (SEPs) recording and tests" indicate steps in the first part, while the red boxes indicate steps in the second part. The dashed lines indicate the immobilization sessions, while the solid line with and arrow indicates the time period between the two parts of the experiment. The grey boxes indicate the task-oriented therapy (TOT), which was either assisted by functional electrical stimulation (FES) or not. Thereby, if TOT 1 included FES assistance, TOT 2 did not and vice versa.*

The experiment followed a crossover design by which all subjects both underwent two immobilization sessions followed by either TOT with or without FES assistance. This design was chosen to be able to see whether potential neurophysiological and sensory alterations were caused by the FES assistance or just by the movement of the limb during the TOT.
The first step in both parts of the experiment involved baseline measurements after which the non-dominant upper limb of the subject was immobilized for 24 hours. Immediately after the immobilization, measurements were performed again to examine the effect of the immobilization. After the measurements, TOT either with or without FES assistance was performed. If the subject performed the TOT session with FES assistance in the first part of the experiment, TOT was performed without FES assistance in the following session and vice versa. Finally, measurements were performed again to examine the effect of the TOT session. Thus, each subject underwent two periods of 24 hours upper limb immobilization and six measurement sessions in total.
In the literature, only studies including a single immobilization session are found. Furthermore, the effect of the immobilization was measured immediately after the immobilization session stopped. [Huber et al., 2006; Moisello et al., 2008; Bassolino et al., 2012] Therefore, the optimal time period between the two immobilization sessions in the experiment was unknown. As elaborated in section 2.3, the effects induced by the

upper limb immobilization quickly fade and the functionality of the upper limb returns to normal [Bassolino et al., 2012]. Based on this, it was assumed that a relatively short washout period for the immobilization effect was needed. Among the different measurements and tests, the modified JTHF test was considered to be the one with the largest potential practice effect. This was considered because the subjects had to perform the different motor tasks repetitively. Therefore, the minimum time period required between the two parts of the experiment was decided based on this test. In the previously described study by Bovend'Eerdt et al. [2004], the mean time between the assessments with the modified JTHF test was 9.6 days (SD=7.1 days). The study revealed no significant difference in the performance between the assessments when using the ratio between the unaffected and affected hand. Thus, due to the time limit for the current study and the relatively large standard deviation in the study by Bovend'Eerdt et al. [2004], a range of 3-7 days between the first and second part of the experiment was chosen. This range was also preferable in order to provide some flexibility for the included subjects.

4.1 Pilot Trial

A pilot trial with the following aims was performed to identify unknown parameters:

- Identify the most optimal immobilization procedure for the experiment to ensure that it was comfortable for the subject and kept the potentially affected limb as steady as possible.
- Identify the most optimal order of the measurements during the experimental sessions to make the experimental procedure efficient, and retain the effect of the immobilization as long as possible.
- Identify a suitable cutoff frequency for the lowpass filter in the preprocessing of SEPs to obtain the most distinct components.
- Identify the minimum required number of SEPs to minimize the duration of the experiment.
- Verify the motor impact of short-term upper limb immobilization as previously described [Huber et al., 2006; Moisello et al., 2008; Bassolino et al., 2012].
- Explore the sensory impact of short-term upper limb immobilization.
- Examine if similar baselines were obtained from the two parts of the experiment to possibly exclude one of the baseline measurements in the experiment.
- Identify the most optimal electrode sites from which the single SEP components of interest should be analyzed.

As two different immobilization procedures were examined and baselines were compared, the two immobilization procedures were performed with three days between. The overall pipeline for the pilot trial is illustrated in figure 4.2.

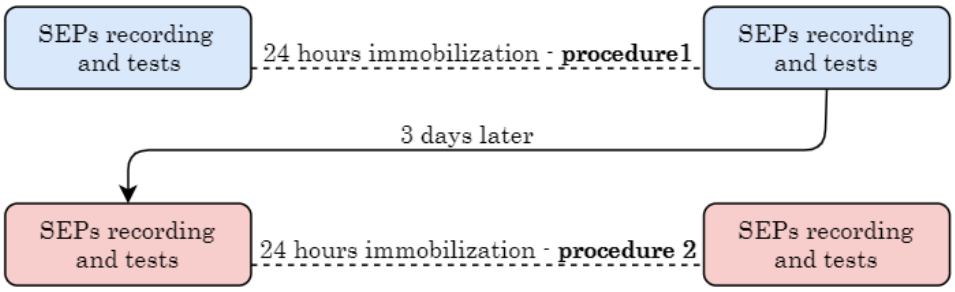


Figure 4.2: *The pipeline for the pilot trial. The blue boxes stating "somatosensory evoked potentials (SEPs) recording and tests" indicate steps in the first part, while the red boxes indicate steps in the second part. The dashed lines indicate a 24-hour immobilization session between the baseline and post-immobilization measurements in each part of the pilot trial. Procedure 1 and 2 indicate the two tested immobilization procedures. There were three days between the two parts of the trial.*

Measurements were performed before and after each immobilization procedure as illustrated in figure 4.2. This involved all the measurements described in chapter 3. Thereby, the results before and after immobilization could be compared to confirm the effect of the short-term upper limb immobilization found in previous studies [Huber et al., 2006; Bassolino et al., 2012; Moisello et al., 2008], and furthermore to explore the sensory impact as well. The baselines between the sessions could be compared to see if the measurements were normalized in the second part of the experiment.
The tests were performed in the following order:

1. SEP recording
2. JPS test
3. QST (Von Frey hairs followed by electrical stimulation)
4. Modified JTHF test

The modified JTHF test was performed last as this test was expected to involve the most active movement from the subject, which could potentially make the effect from the immobilization session fade more rapid. A detailed experimental protocol over the pilot trial can be found in appendix E.

4.1.1 Methods for the Pilot Trial

In the following, methods specifically used to fulfill the aims of the pilot trial are elaborated.

Immobilization Procedures

The overall principle for the immobilization procedures tested in the pilot trial is illustrated in figure 4.3 along with the additional step in immobilization procedure 1.

Figure 4.3: *The steps for both immobilization procedures which included application of a glove (a), covering the hand and wrist with gauze (b), covering the lower arm with gauze (c) and placing it in a sling (d).*

For immobilization procedure 1, an extra step was added between b and c in figure 4.3, i.e. the placement of a spoon on the hand and lower arm for stabilization. This was placed before applying the second roll of gauze. The placement is illustrated in figure 4.4.

Figure 4.4: *The spoon applied during immobilization procedure 1 between step b and c in figure 4.3.*

The most appropriate immobilization procedure was chosen based on which procedure that induced the most evident effects on the measured sensory and motor parameters. Furthermore, the subjects were asked which method they preferred in terms of comfort during daily life activities and sleep.

Measurement Order

To identify the order in which the measurements should be performed, it was considered throughout the pilot trial, whether it would be more efficient to perform certain measurements in a different order.

Recording and Preprocessing of Somatosensory Evoked Potentials

The maximum recommended number of SEPs is 2000 according to the IFCN recommended standards [Nuwer et al., 1994], by which 2000 SEPs were recorded in the pilot trial.
The EEG data was preprocessed following the overall procedure described in subsection 3.2.2 and with different cutoff frequencies and number of averaged epochs.

To determine the cutoff frequency of the lowpass filter for the experiment, five different cutoff frequencies were tested: 3000 Hz, 2000 Hz, 1000 Hz, 500 Hz and 200 Hz. To identify the number of epochs needed to obtain a clear signal from averaging, three different numbers of SEPs were averaged for each tested cutoff frequency: 2000, 1500 and 1000. To determine the optimal values for the cutoff frequency and number of epochs, data was visually inspected. It was desired to obtain a relatively smooth signal, by which the components of interest were clearly detectable.

Impact of Immobilization and Baseline Comparison

To verify the motor impact and further examine the sensory impact of short-term upper limb immobilization, the values before and after each of the two immobilization procedures were compared. The baselines were also compared. As only two subjects were included in the pilot trial, a statistical analysis was not possible to perform. Therefore, the comparison of the values was solely descriptive.

Electrode Sites

To select the electrode sites for identifying the single SEP components of interest, the four components were identified in the signal from the different electrode sites described in subsection 3.2.1, as previous studies have shown that the components are most clearly detectable from these sites. The most optimal electrode sites for the individual components were evaluated based on the amplitude and latency of the component, and whether the components had a clear structure in the signal.

4.1.2 Pilot Data Analysis

After the SEPs were obtained, they were preprocessed and electrode sites for the SEP components were selected in order to obtain detectable components. Subsequently, the data from the different measurements was analyzed.

Neurophysiological Impact of Immobilization

The signals with different cutoff frequencies for the lowpass filter and number of averaged epochs are illustrated in figure 4.5-4.9.

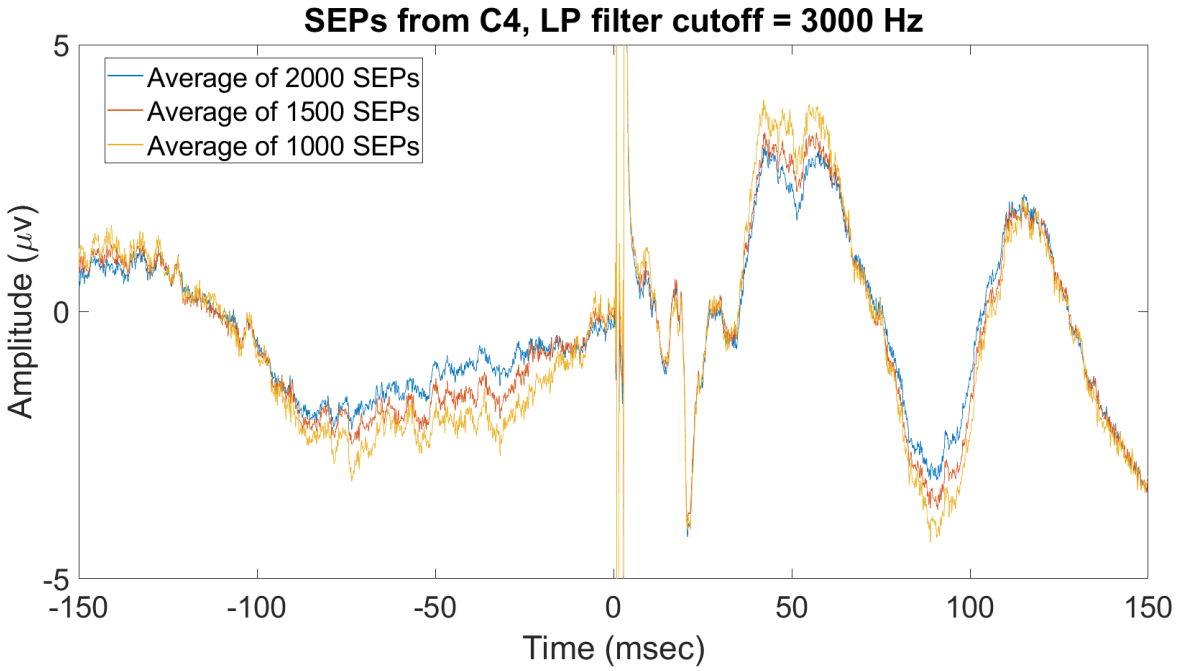


Figure 4.5: *2000, 1500 and 1000 averaged somatosensory evoked potentials (SEPs) respectively from electrode C4 from one subject in which the cutoff frequency for the lowpass (LP) filter was 3000 Hz.*

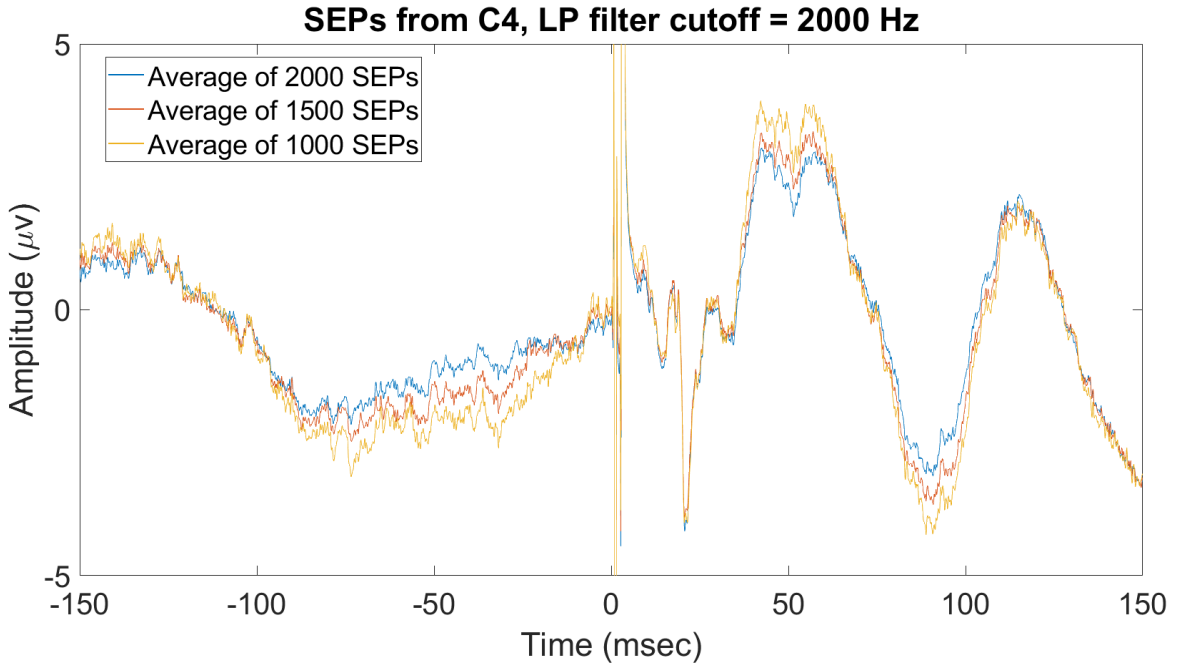


Figure 4.6: *2000, 1500 and 1000 averaged somatosensory evoked potentials (SEPs) respectively from electrode C4 from one subject in which the cutoff frequency for the lowpass (LP) filter was 1500 Hz.*

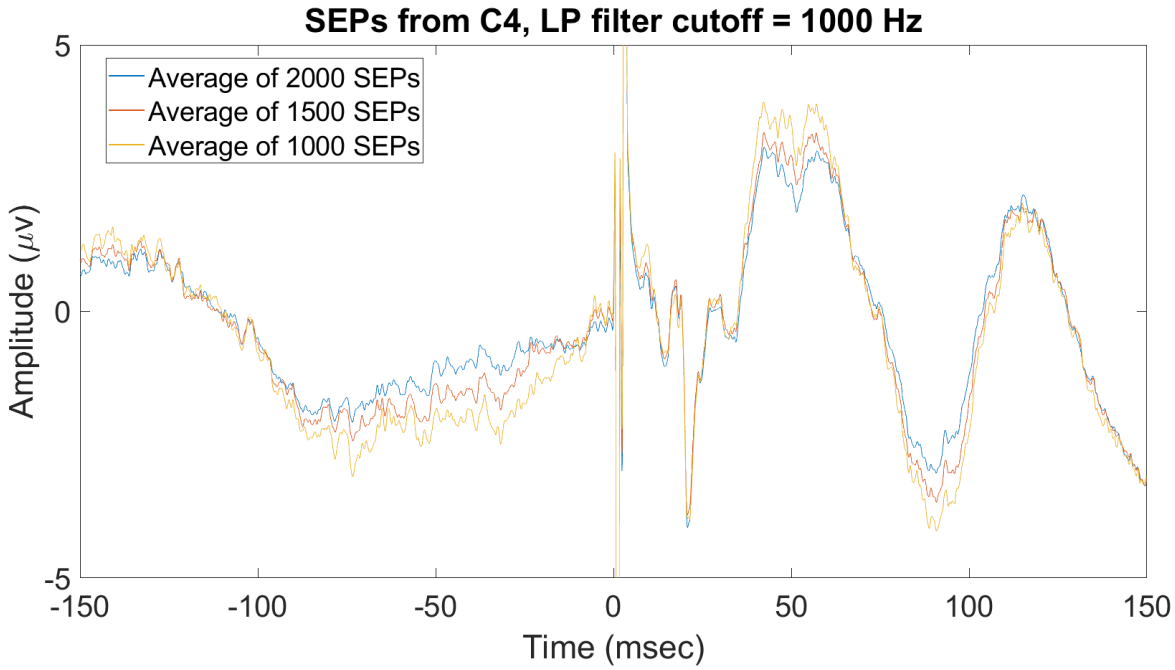


Figure 4.7: *2000, 1500 and 1000 averaged somatosensory evoked potentials (SEPs) respectively from electrode C4 from one subject in which the cutoff frequency for the lowpass (LP) filter was 1000 Hz.*

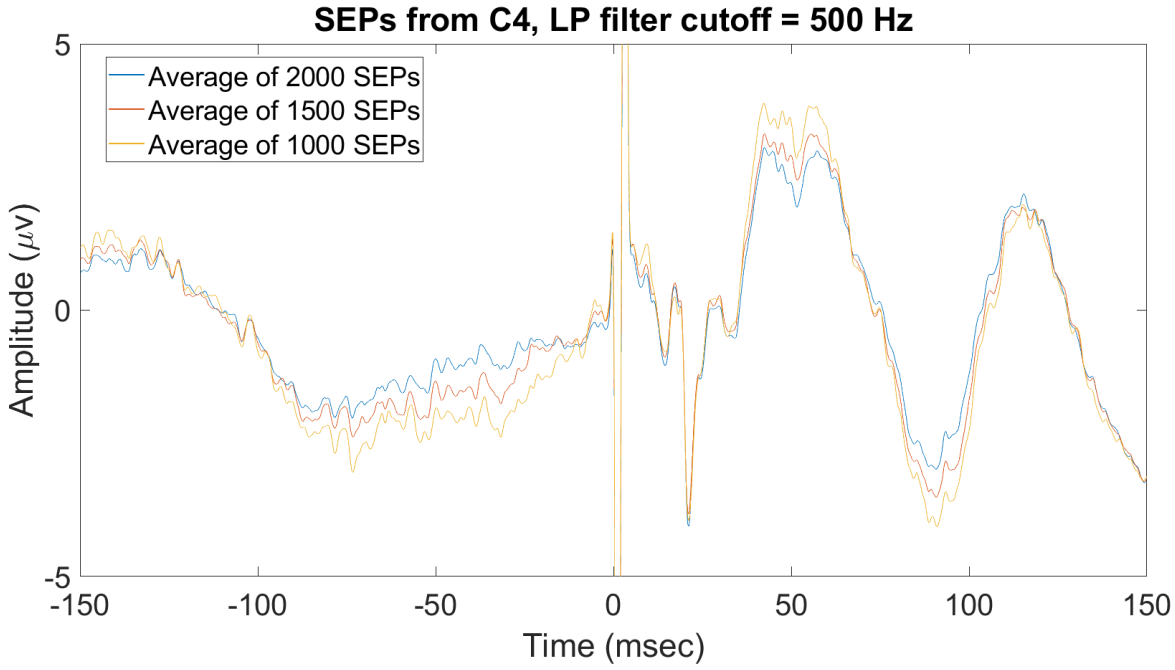


Figure 4.8: *2000, 1500 and 1000 averaged somatosensory evoked potentials (SEPs) respectively from electrode C4 from one subject in which the cutoff frequency for the lowpass (LP) filter was 500 Hz.*

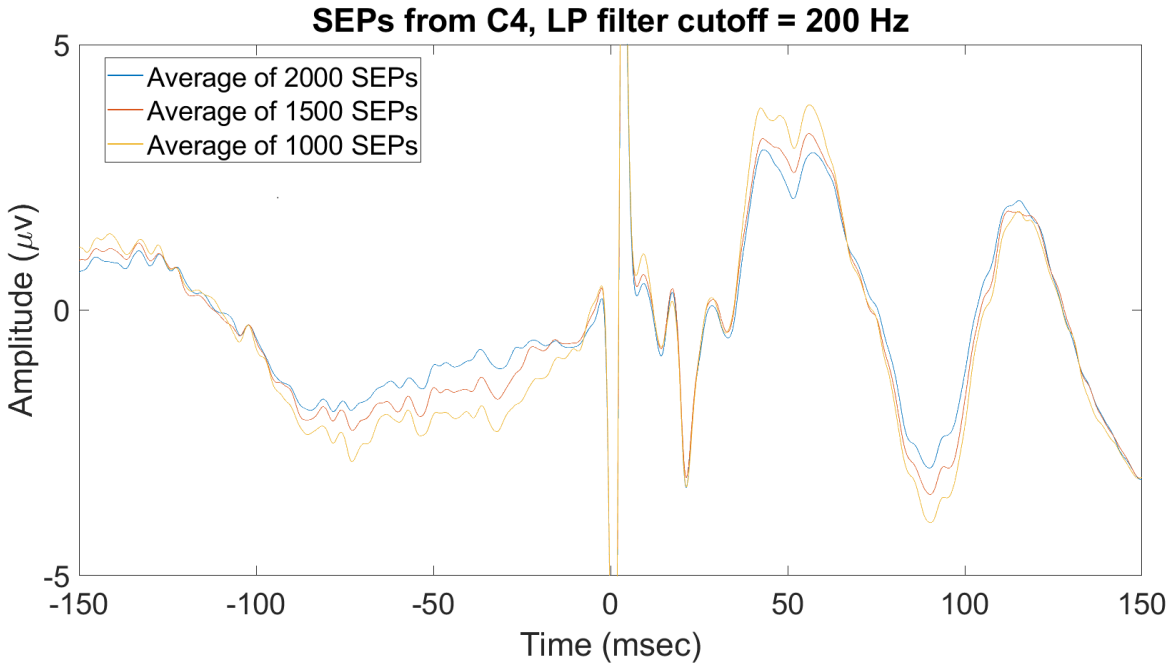


Figure 4.9: *2000, 1500 and 1000 averaged somatosensory evoked potentials (SEPs) respectively from electrode C4 from one subject in which the cutoff frequency for the lowpass (LP) filter was 200 Hz.*

Based on a visual inspection of the illustrated signals, it was evaluated that there were minor differences in the amplitude of the peaks between the averaging of 2000, 1500 and 1000 SEPs respectively. From the plotted data, it was decided that 1500 epochs were a sufficient number since the obtained signal was located approximately in between the signals obtained by averaging of 1000 and 2000 SEPs. Therefore, for the further analysis in the pilot trial and in the experiment, 1500 SEPs were evaluated to be sufficient to obtain a clear signal.
It was furthermore evaluated from visual inspection that a cutoff frequency of 3000 Hz for the lowpass filter was too high, since many high frequency components interfered with the desired signal. When inspecting the examples of SEPs in figure 3.3, the signals were smoother than the signals illustrated in figure 4.5. This might be due to a lower sample frequency or a lower cutoff frequency for the lowpass filter in the study [Nuwer et al., 1994; Waberski et al., 1999]. Therefore, it was evaluated from figure 4.6 and figure 4.7 that cutoff frequencies of 1500 and 1000 Hz respectively were still too high. However, when the cutoff frequency was set to 500 Hz, as illustrated in figure 4.8, the SEP signals were smoother. Thereby, the components were more evident. But as depicted in figure 4.9, a cutoff frequency of 200 Hz was too low and much of the signal was filtered out. This was expressed as e.g. small peaks, which could potentially indicate a component, were filtered out. An example of this is illustrated in figure 4.10.

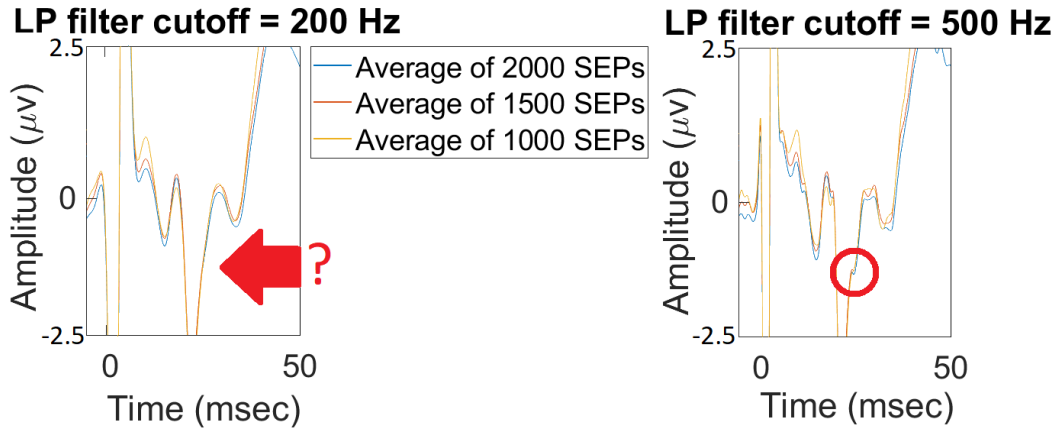


Figure 4.10: *2000, 1500 and 1000 averaged somatosensory evoked potentials (SEPs) respectively, in which the cutoff frequency for the lowpass (LP) filter was 200 Hz and 500 Hz in each of the plots. The small peak marked with a red circle in the signal to the right is not present in the signal to the left.*

Therefore, a cutoff frequency of 500 Hz for the lowpass filter was chosen for data analysis in the pilot trial and in the experiment. The sampling frequency remained the same (9600 Hz) for the experiment.
After this analysis, data from all electrode sites was preprocessed again with the chosen cutoff frequency of 500 Hz for the lowpass filter and averaging of 1500 epochs. The total minimum, maximum and average number of remaining epochs are depicted in table 4.1 for SEP measurements before and after the immobilization. The average number of epochs is presented as a mean value ± SD.

	Remaining Number of Epochs	
	pre	post
Total minimum	1388	1406
Total maximum	1486	1473
Mean	1425±46	1455±33

Table 4.1: *The total minimum, maximum and average number of epochs for all electrode sites after epoch rejection for the somatosensory evoked potential (SEP) measurement before (pre) and after (post) the immobilization. The mean number of epochs is indicated as mean±SD.*

Based on visual inspection as described in subsection 4.1.1, the electrode sites for detection of the N20 and N90 components were chosen as C3/C4. For detection of the N30 component, electrode sites FC3/FC4 were chosen and the electrode sites CP3/CP4 were chosen for detection of the P45 component. Therefore, only these six electrode sites were included for further analysis of the components. Whether the even- or odd-numbered electrode site was used depended on the dominant hand of the subject. The neurophysiological impact of the immobilization procedures was examined based on the amplitude and latency of the SEP components of interest. For the detection of the four SEP components, the GUI was used. The final look of the GUI is illustrated in figure 4.11, in which the electrode sites could be chosen by clicking on the specific buttons.

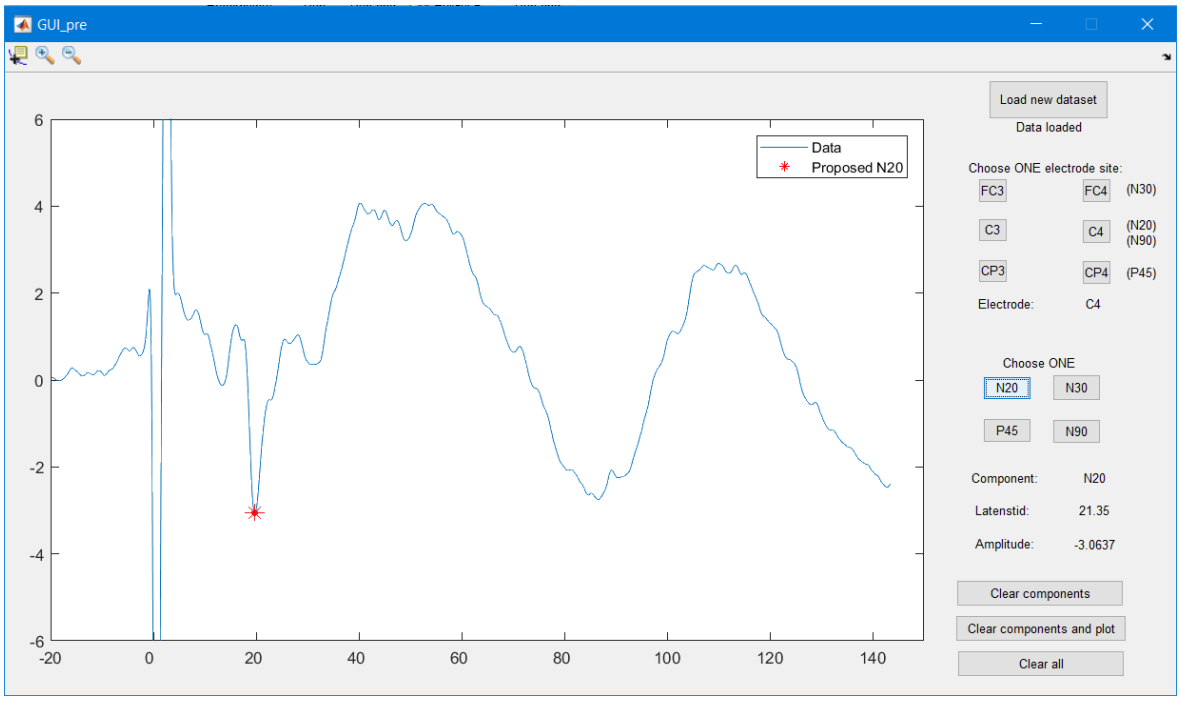


Figure 4.11: *The graphical user interface (GUI) used for detection of somatosensory evoked potentials (SEPs). The GUI now contained six buttons for selection of which electrode site that should be plotted in the graph. A text field under the buttons displayed the electrode site that was plotted.*

Sensory Impact of Immobilization

To examine the impact of the immobilization procedures on proprioception, the data before versus after the immobilization was compared for the two different immobilization procedures. For the JPS test, the angles of the shoulder, elbow and wrist were measured by use of the Kinovea (v. 0.8.15; Copyright 2006-2011, Joan Charmant & Contrib, `http://www.kinovea.org/`) software. Kinovea is a video player used for sport analysis, which provides measuring tools [Kinovea, 2019]. A study by El-Raheem et al. [2015] found that the Kinovea software had a high inter- and intra-rater reliability in the measurement of range of motion for the dominant wrist joint in healthy subjects. Thereby, the software was believed to be a valid tool for measuring the angles in the current study.
The angles of the shoulders, elbows and wrists were measured by calculating the angle between two vectors. The same examiner performed the angle measurements for all photos based on the following instructions:

- The angle of the shoulder was measured with one vector alongside the thorax of the subject with terminal point at the bottom of the picture near the ribs. The other vector was placed along the upper arm, i.e. along the humerus, with terminal point in the middle of the elbow joint. The initial points of both vectors were placed approximately in the beginning of the humerus near the shoulder.
- The angle of the elbow was measured by having one vector along the humerus, same as described in the previous point, with terminal point in the previous initial point (approximately at the beginning of the humerus near the shoulder). The other vector was placed along the forearm with terminal point in the middle of the wrist joint. The

initial points of both vectors were placed approximately in the middle of the elbow joint.

- The angle of the wrist was measured by having one vector along the forearm, same as described in the previous point, with terminal point in the previous initial point (approximately in the middle of the elbow joint). The other vector was placed along the side of the hand with terminal point at the beginning of the fifth digit. The initial points of both vectors were placed approximately in the middle of the wrist joint.

One angular measurement performed by the examiner based on these instructions is illustrated in figure 4.12.

Figure 4.12: *The angular measurements of the shoulder, wrist and elbow performed in Kinovea by the same examiner for all photos. The vectors which measured the angle of the elbows are marked with red, while the vectors which measured the angle of the shoulders and wrists are marked with green.*

The final angular differences for the subjects before and after the immobilization were compared for the two different immobilization procedures to see the impact of each procedure on the proprioceptive sense.

The impact of the two immobilization procedures on the tactile sensation was accessed based on the geometric means for the detection threshold for the Von Frey hairs and electrical stimulation respectively. These were also compared for the subjects before and after immobilization for the two different procedures.

Motor Impact of Immobilization

To examine the impact of the two immobilization procedures on the motor performance of the subject, and thereby sensorimotor integration [Allgöwer and Hermsdörfer, 2017], the ratio in time used for the test between the affected and unaffected hand was compared before versus after immobilization for both immobilization procedures for each subject.

Baseline Comparison

To examine whether the effect of the first immobilization session was washed out when the second immobilization session was initiated, the baseline data for the first and second part of the pilot trial were compared for each of the performed measurements for the single subjects.

4.1.3 Pilot Results

The results for the different measurements and tests of the pilot trial are presented in the following. Procedure 1 was the immobilization with the wooden spoon for stabilization of especially the wrist. Procedure 2 was the immobilization without the wooden spoon.

Somatosensory Evoked Potential Component Alterations

In table 4.2, the amplitudes of the components of interest for the single subjects before and after immobilization procedure 1 and 2 are displayed.

Somatosensory Evoked Potentials - Component Amplitudes [μV]					
		Subject 1		Subject 2	
		IM 1	IM 2	IM 1	IM 2
Pre-immoblization	N20	-3.69	-3.06	-1.60	-2.00
	N30	-2.49	-2.32	-3.32	-3.18
	P45	3.16	2.64	1.12	-0.37
	N90	-2.39	-2.75	-1.49	-1.77
Post-immoblization	N20	-4.97	-3.44	-2.25	-3.17
	N30	-2.33	-2.52	-3.30	-3.75
	P45	2.31	2.83	0.13	1.31
	N90	-3.64	-3.06	-1.65	-0.32

Table 4.2: *The amplitude of the somatosensory evoked potential (SEP) components of interest detected in the recorded and preprocessed data for each subject before (Pre-immobilization) and after (Post-immobilization) the two different immobilization procedures. IM 1 indicates immobilization procedure 1 and IM 2 indicates immobilization procedure 2. When a cell is green, it indicates that the amplitude after immobilization was increased compared to before. When a cell is red, it indicates that the amplitude after immobilization was decreased compared to before.*

As depicted in table 4.2, mixed results were seen for the alterations of the SEP component amplitudes for both immobilization procedures. This was expressed by the amplitude of some components being decreased following the immobilization, while others had increased. This was unlike the expectations described in subsection 3.2.

In table 4.3, the corresponding latencies of the components of interest are depicted.

Somatosensory Evoked Potentials - Component Latencies [msec]					
		Subject 1		Subject 2	
		IM 1	IM 2	IM 1	IM 2
Pre-immoblization	N20	21.77	21.35	22.60	22.50
	N30	28.65	30.52	32.29	33.65
	P45	44.06	42.60	60.00	61.04
	N90	90.31	91.04	80.94	85.73
Post-immoblization	N20	21.98	21.67	25.00	25.10
	N30	31.35	33.96	33.23	32.50
	P45	41.77	43.44	60.42	64.38
	N90	93.65	88.33	79.48	81.46

Table 4.3: *The latency of the somatosensory evoked potential (SEP) components of interest detected in the recorded and preprocessed data for each subject before (Pre-immobilization) and after (Post-immobilization) the two different immobilization procedures. IM 1 indicates immobilization procedure 1 and IM 2 indicates immobilization procedure 2. When a cell is green, it indicates that the latency after immobilization was increased compared to before. When a cell is red, it indicates that the latency after immobilization was decreased compared to before.*

As depicted in table 4.3, the results for the alterations in the latency of the components are also mixed. However, the N20 showed an increase for both subjects and both immobilization procedures after immobilization, even though it was minor for subject 1.

Thereby, the effect of the immobilization was not clear based on the latency and amplitude of the SEP components. This was due to a large amount of the components demonstrated an increased amplitude following the immobilization, unlike the expectations described in subsection 3.2. However, the N20 latency increased for both subjects and immobilization procedures as expected.
Differences were seen between the baseline values of the SEP amplitudes and latencies, ranging from minor to larger. For example, the amplitudes for the P45 component for subject 2 went from 1.12 μV to -0.37 μV.

Joint Position Sense Alterations

Table 4.4 depicts the difference between the angles of shoulders, elbows and wrist for both subjects before and after immobilization procedure 1 and 2.

Joint Position Sense Test - Difference between Left and Right Upper Limb [°]					
		Subject 1		Subject 2	
		IM 1	IM 2	IM 1	IM 2
Pre-immoblization	Shoulder	14	4	1	2
	Elbow	10	13	16	6
	Wrist	-18	8	-9	6
Post-immoblization	Shoulder	22	18	9	12
	Elbow	13	3	3	1
	Wrist	1	5	13	3

Table 4.4: *The angular difference between the dominant and non-dominant upper limb for the joint position sense (JPS) test for each subject before (Pre-immobilization) and after (Post-immobilization) the two different immobilization procedures. The angular differences are calculated by subtracting the angle of the joints. A negative value indicates that the angle of the dominant upper limb was larger than the angle of the non-dominant, immobilized upper limb and vice versa. IM 1 indicates immobilization procedure 1 and IM 2 indicates immobilization procedure 2. When a cell is green, it indicates that the angular difference after immobilization was increased compared to before. When a cell is red, it indicates that the angular difference after immobilization was decreased compared to before.*

An impaired JPS was associated with a larger difference between the angles of the joint for each upper limb, since this was used as an indicator for the ability to sense the placement of the upper limbs in the vase report by Bustamante et al. [2016]. The values in the table above indicate no clear trend for the effect of the two immobilization procedures, which was in accordance with the findings for the SEP components and was unlike the expectations described in section 3.3. However, the angular difference for the placement of the shoulder increased following immobilization for both subjects and procedures, which was expected, as it indicated an impaired proprioception after the immobilization.
When comparing the baselines, both subjects demonstrated difference for all joints, ranging from minor to larger. For example, for subject 2, the baseline went from an angular difference of 16° to 6°, which was considered to be a considerable alteration in the baseline.

Table 4.5 depicts the time it took for the subjects to place the dominant upper limb in a similar position as the non-dominant upper limb during the JPS tests.

Joint Position Sense Test - Time [sec]				
	Subject 1		Subject 2	
	IM 1	IM 2	IM 1	IM 2
Pre-immoblization	10.11	11.90	11.56	12.95
Post-immobilization	13.33	18.16	14.54	13.86

Table 4.5: *The time it took for the subjects to place the dominant upper limb during the joint position sense (JPS) test for each subject before (Pre-immobilization) and after (Post-immobilization) the two different immobilization procedures. IM 1 indicates immobilization procedure 1 and IM 2 indicates immobilization procedure 2. When a cell is green, it indicates that the time after immobilization was increased compared to before.*

The values in the table indicate a more clear trend than the angular difference measurement, as the post-immobilization time was increased compared to baseline for both subjects and both immobilization procedures. This result followed the expectations described in subsection 3.2.

When comparing the baselines between the procedures for both subjects, only minor alterations were found.

Quantitative Sensory Testing Alterations

Table 4.6 depicts the detection thresholds for the two subjects and the different immobilization procedures for the Von Frey hairs.

Detection Threshold for Von Frey Hairs [gf]				
	Subject 1		Subject 2	
	IM 1	IM 2	IM 1	IM 2
Pre-immoblization	6.33	7.16	5.48	5.58
Post-immobilization	6.92	7.48	5.95	5.65

Table 4.6: *The detection threshold for the Von Frey hairs for the subjects calculated as the geometric mean of the upper and lower thresholds for each subject before (Pre-immobilization) and after (Post-immobilization) the two different immobilization procedures. IM 1 indicates immobilization procedure 1 and IM 2 indicates immobilization procedure 2. When a cell is green, it indicates that the threshold after immobilization was increased compared to before.*

The values in the table indicate that the detection threshold for the Von Frey hairs was increased following the immobilization for both subjects and both procedures. This result followed the expectations described in section 3.4.
The baseline dectection threshold increased for immobilization procedure 2 for both subjects. For subject 2, this increase was only minor.

Table 4.7 depicts the detection thresholds for the two subjects for the different immobilization procedures for the electrical stimulation.

Detection Threshold for Electrical Stimulation [μA]				
	Subject 1		Subject 2	
	IM 1	IM 2	IM 1	IM 2
Pre-immoblization	2,060	4,300	3,460	2,210
Post-immobilization	4,360	4,100	3,270	4,160

Table 4.7: *The detection threshold for electrical stimulation for the subjects calculated as the geometric mean of the upper and lower thresholds for each subject before (Pre-immobilization) and after (Post-immobilization) the two different immobilization procedures. IM 1 indicates immobilization procedure 1 and IM 2 indicates immobilization procedure 2. When a cell is green, it indicates that the threshold after immobilization was increased compared to before. When a cell is red, it indicates that the threshold after immobilization was decreased compared to before.*

As indicated in table 4.7, there was not any clear trend in the data indicating the effect that the immobilization procedures had on the tactile sensation of the subject and which of the immobilization procedures that induced the most evident alterations. This result was unlike the expectations described in section 3.4.
The baseline showed a considerable increase for immobilization procedure 2 for subject 1 while a decrease was seen for subject 2.

Modified Jebsen-Taylor Hand Function Test Alterations

Table 4.8 depicts the results for the modified JTHF test as ratios between the dominant and non-dominant hand.

Modified JTHF Test Time Ratios (unaffected[sec]/affected[sec])				
	Subject 1		Subject 2	
	IM 1	IM 2	IM 1	IM 2
Pre-immoblization	0.75	0.81	1.02	0.99
Post-immobilization	0.66	0.79	0.88	0.96

Table 4.8: *The time for performing of the modified Jebsen-Taylor Hand Function (JTHF) test expressed as the ratio between the mean time for the unaffected (dominant) and affected (non-dominant) hand respectively, i.e. the mean time for the unaffected hand divided by the mean time for the affected hand. The more similar the performance of the hands was, the closer the value is to 1. A value below 1 indicates that the performance for the unaffected hand was faster than the affected. A value above 1 indicates that the performance for the affected hand was faster that the unaffected hand. This data for each subject before (Pre-immobilization) and after (Post-immobilization) the two different immobilization procedures. IM 1 indicates immobilization procedure 1 and IM 2 indicates immobilization procedure 2. When a cell is green, it indicates that the range relative to 1.00 had increased compared to the previous measurement.*

The values in the table show a trend which demonstrate that both immobilization procedures had an impact on the motor function of the subjects. This result followed the expectations described in section 3.5. The impact on the motor function was particularly seen for immobilization procedure 1.
When looking at the ratios for the baseline tests, small alterations were also seen for both subjects between the two immobilization procedures. To further compare the values, the mean values were also evaluated. These values are depicted in table 4.9.

Modified JTHF Test Time Ratios (unaffected[sec]:affected[sec])				
	Subject 1		Subject 2	
	IM 1	IM 2	IM 1	IM 2
Pre-immoblization	22.22 : 29.63	20.60 : 25.47	23.81 : 23.44	22.89 : 22.94
Post-immobilization	20.33 : 30.72	18.94 : 24.04	21.91 : 24.81	24.16 : 25.05

Table 4.9: *The ratio between the left and right hand of the subjects expressed as the two mean times separated by a colon for the modified Jebsen-Taylor Hand Function (JTHF) test for each subject before (Pre-immobilization) and after (Post-immobilization) the two different immobilization procedures. IM 1 indicates immobilization procedure 1 and IM 2 indicates immobilization procedure 2. When a cell is green, it indicates that the range relative to 1.00 had increased compared to the previous measurement.*

It was found that particularly the baseline for subject 1 for the affected hand had decreased at immobilization procedure 2.

Immobilization Procedure Evaluation

After the pilot trial, both subjects emphasized that immobilization procedure 2 was preferable since the spoon included in procedure 1 was uncomfortable. However, it helped the subjects

keeping the upper limb more stable. As there was no clear difference in the measurement results for the two immobilization procedures, the opinion of the subjects determined which procedure that was chosen, i.e. procedure 2.

4.1.4 Discussion and Conclusion

The overall assumption was that the immobilization procedures would cause a decreased amplitude and increased latency for the SEP components of interest, an increased angular difference and time period for the JPS test, an increased detection threshold for the QST tests and finally an increased time for the performance of the modified JTHF test.
As indicated by the values of the tables for the different tests above, both immobilization procedures had an impact on sensory and motor parameters for both subjects. Some of the results were unexpected and contradictory, but it is possible that a more clear trend can be found in a larger sample population. Based on the results from the pilot trial it was decided that:

- Immobilization procedure 2 was the most optimal procedure for the experiment since it was more comfortable for the subjects in the pilot trial.
- The most optimal order for the measurements was the following:
  - QST tests (Detection threshold for electrical stimulation followed by Von Frey hairs).
  - SEP recording.
  - JPS test.
  - Modified JTHF test.
- The cutoff frequency for the lowpass filter should be 500 Hz.
- The minimum required number of SEPs was 1500.
- The motor and sensory impact of short-term upper limb immobilization procedures were mixed and a larger study population might be necessary to identify any possible effect.
- The baseline measurements for the two immobilization sessions were generally not similar.
- The electrode sites which should be used for analyzing the SEP components of interest were the following:
  - N20: C3/C4.
  - N30: FC3/FC4.
  - P45: CP3/CP4.
  - N90: C3/C4.

4.2 Experiment

After the pilot trial, the experiment shown in figure 4.1 was performed. A detailed experimental protocol over the pilot trial can be found in appendix F.
In both parts of the experiment, the included measurements and tests were performed in the following order before immobilization, before TOT and after TOT:

- QST tests (Detection threshold for electrical stimulation and Von Frey hairs).
- SEP recording.
- JPS test.
- Modified JTHF test.

4.2.1 Subjects

For subject recruitment, inclusion criteria were that the subjects had to be studying at Aalborg University and be healthy. Exclusion criteria were any neurological, heart and lung deceases. Additionally, the subjects were required not to have a cardiac pacemaker, any implanted metal plates or be pregnant.
Seven subjects agreed to participate in the experiment. All subjects received an information sheet prior to the experiment as seen in appendix G. Additionally, the subjects were verbally informed about the procedure of the experiment, before they read the information sheet and gave their written informed consent to participate. The information sheet and consent form are illustrated in appendix H. The subjects included five females and two males with mean age ± SD of 23.7±1.5 years. Two subjects were left handed, by which the right upper limb was immobilized and vice versa. During the experiment, four of the subjects received FES assistance in the second TOT session. The remaining three subjects received FES assistance in the first TOT session.

4.2.2 Procedure and Analysis

The procedure for the measurements and preprocessing followed the descriptions in chapter 3. However, the number of recorded SEPs was adjusted to 1500 based on the pilot trial. Additionally, the cutoff frequency for the lowpass filter was adjusted to 500 Hz for filtering the SEP data.
The data analysis for the single measurements furthermore followed the procedure described in chapter 3. For the SEP analysis, the N20 was analyzed from electrode sites C3/C4, N30 from FC3/FC4, P45 from CP3/CP4 and N90 from C3/C4 dependent on the dominant upper limb of the subject.

4.3 Statistics

The presumed effects of the immobilization were initially validated by statistically examining if there was a significant difference in data between baseline and after the immobilization for each measurement. Next, the effects of FES were examined by calculating the difference for each measurement before and after TOT. The differences between the outcome measurements before and after TOT with FES assistance were statistically compared to the outcome measurements before and after TOT without FES assistance. This was done to examine if there was a significant difference when FES was incorporated in the grasping task. Lastly, based on the possible detected alterations caused by FES-assisted TOT, the association between the neurophysiological and clinical measurements was expected to be found. As the possible alterations are needed to be confirmed prior to examining the associations, the description of this procedure will be presented after the results. This description is therefore found in section 5.3.

4.3.1 The Effect of Upper Limb Immobilization

To examine if the immobilization procedure had a sensory and motor impact, the obtained values from all of the baseline measurements versus measurements before TOT were included in a statistical analysis. This involved the outcome measurements from the following:

- Threshold for thumb twitch.
- Detection threshold for Von Frey hairs and electrical stimulation.
- Amplitude and latency of the N20, N30, P45 and N90 components.
- Angular difference for the elbow, shoulder and wrist from the JPS test.
- Time used for the JPS test.
- Ratio between the time it took for the subjects to perform the modified JTHF test with the dominant and non-dominant limbs.

Comparisons were made for the two baseline versus the two measurements before the TOT for each subject. Prior to the comparisons of baseline versus measurements before the TOT, the distribution of all the data was tested using a Shapiro-Wilk test with a significance level of α=0.05. The results from these tests are found in appendix I. Thereby, if one of the datasets was not normally distributed, a non-parametric statistical test was used. On the other hand, if the data was normally distributed, a parametric test was used. For the normal distributed data, a paired sample t-test was used, while if the dataset was not normally distributed, a Wilcoxon signed-rank test was used.

4.3.2 Alteration of Proprioception and Tactile Sensation

To examine how a single session of FES-assisted TOT alters proprioception and tactile sensation, the difference for relevant outcome measurement before versus after TOT was calculated. Even though the modified JTHF test and the N30 component are related to motor function and not proprioception and tactile sensation, it was decided to include the outcome measurement from these in the statistical analysis anyhow. This was chosen, since these measurements can reflect aspects of sensorimotor integration [Allgöwer and Hermsdörfer, 2017; Lelic et al., 2016; Waberski et al., 1999; Kanovsky et al., 2003]. Furthermore, the threshold for thumb twitch was included as it might indirectly express alterations in sensorimotor integration as well.
The difference was calculated by subtracting the values from all the measurements performed after TOT from the values before TOT. For example, as illustrated in table I.1, the threshold for subject 1 in regards of thumb twitch was 5 mA before TOT and 3.2 mA after TOT in one session. Thereby, the difference is -1.80 mA for this subject.
Prior to the comparisons of differences in data for the TOT session with versus without FES assistance, the distribution of all the data was tested using a Shapiro-Wilk test with a significance level of α=0.05. Thereby, if the data was normally distributed, an unpaired sample t-test was used. If one of the datasets was not normally distributed, a Mann Whitney U test was used.

4.3.3 Association between Measurements

To examine if there was an association between the neurophysiological and clinical measurements, multiple comparisons could be made:

- The N20 component emerges in the postcentral cortex, which subserves sensory functions [Nuwer et al., 1994] and can decrease in amplitude as a result of sensory loss [Macdonell et al., 1990]. Therefore, this SEP component would be interesting to compare to the QST results, which clinically examines the functional state of the somatosensory system [Mücke et al., 2016; Kahn, 1992].

- The N30 component is thought to reflect sensorimotor integration [Lelic et al., 2016; Waberski et al., 1999]. Therefore, it would be relevant to investigate if there is an association between the amplitude and latency alterations of this neurophysiological component with the results from the modified JTHF test, which is a clinical measurement that examines the motor control of the subject and relies on sensorimotor integration [Bovend'Eerdt et al., 2004].
- The P45 and N90 components are related to proprioception, as mentioned in section 3.2. Thereby, these neurophysiological SEP components are interesting to compare with the clinical JPS test, which examine the proprioceptive sense of the subject [Bustamante et al., 2016].

The following chapter presents the results of the study.

Results 5

In the following chapter, the results of the current study are presented. The results include the effect of the immobilization procedure and the neurophysiological and sensory alterations obtained by FES-assisted TOT.

All of the obtained values from the different measurements and single subjects are found in appendix I.
To visualize an example of a dataset, the ratios in time for the Modified JTHF test depicted in table I.15 are illustrated in figure 5.1.

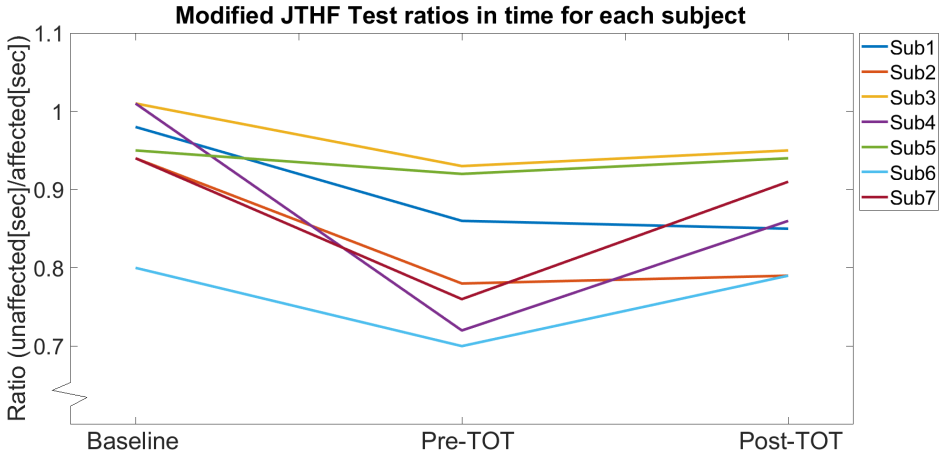


Figure 5.1: *The ratios in time for the Modified Jebsen-Taylor Hand Function (JTHF) test for each subject obtained at baseline, before TOT (Pre-TOT) and after TOT (Post-TOT) in the experimental session without functional electrical stimulation (FES) assistance.*

Another example of a dataset is illustrated in figure 5.2. This visualizes preprocessed SEP data for baseline, before TOT and after TOT for a single subject.

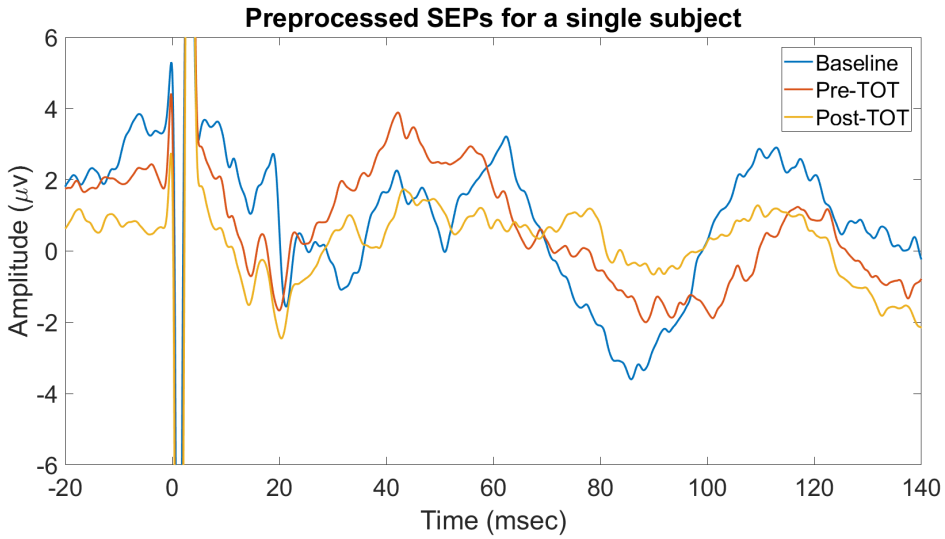


Figure 5.2: *Preprocessed somatosensory evoked potential (SEP) data from baseline, before TOT (Pre-TOT) and after TOT (Post-TOT) for a single subject at electrode site C4 in the experimental part without functional electrical stimulation (FES) assistance.*

5.1 The Effect of Upper Limb Immobilization

For examining the effect of upper limb immobilization, the methods followed the description in subsection 4.3.1.
Table 5.1 depicts the results for the comparison of baseline versus measurements before TOT for the session without FES assistance. Table 5.2 depicts the results for the comparison of baseline versus measurements before TOT for the session with FES assistance.

Comparison of Baseline versus Pre-TOT Data **without** FES Assistance		
Data	Statistical Values Baseline versus Pre-TOT	Result
Threshold for thumb twitch [mA]	t(6)=-2.437, P=0.051	Not significant
N20 amplitude [μV]	t(6)=1.814, P=0.120	Not significant
N20 latency [msec]	t(6)=1.856, P=0.222	Not significant
N30 amplitude [μV]	t(6)=0.745, P=0.485	Not significant
N30 latency [msec]	t(6)=-1.536, P=0.176	Not significant
P45 amplitude [μV]	t(6)=-1.236, P=0.263	Not significant
P45 latency [msec]	t(6)=-2.668, P=0.037	Significant
N90 amplitude [μV]	t(6)=-0.754, P=0.479	Not significant
N90 latency [msec]	Z = -0.676, P=0.499	Not significant
JPS - angular difference for shoulder [°]	t(6)=-1.626, P=0.155	Not significant
JPS - angular difference for elbow [°]	t(6)=1.697, P=0.141	Not significant
JPS - angular difference for wrist [°]	t(6)=-0.105, P=0.920	Not significant
JPS - time [sec]	t(6)=0.478, P=0.649	Not significant
QST - Electrical stimulation [μA]	Z=-0.730, P=0.465	Not significant
QST - Von Frey hairs [gf]	t(6)=-1.155, P=0.292	Not significant
Modified JTHF test (ratio)	t(6)=4.329, P=0.005	Significant

Table 5.1: *Comparison of the baseline versus data obtained before TOT (Pre-TOT) without functional electrical stimulation (FES) assistance calculated with either the paired sample t-test (indicated by the t-value) or the Wilcoxon signed-rank test (indicated by the Z-value). The significant results are written in red for clarity.*

Comparison of Baseline versus Pre-TOT Data **with** FES Assistance		
Data	Statistical Values Baseline versus Pre-TOT	Result
Threshold for thumb twitch [mA]	Z=-2.371, P=0.018	Significant
N20 amplitude [μV]	t(6)=1.408, P=0.209	Not significant
N20 latency [msec]	t(6)=-1.125, P=0.303	Not significant
N30 amplitude [μV]	t(6)=0.668, P=0.529	Not significant
N30 latency [msec]	t(6)=-2.219, P=0.068	Not significant
P45 amplitude [μV]	t(6)=0.268, P=0.798	Not significant
P45 latency [msec]	t(6)=-1.358, P=0.223	Not significant
N90 amplitude [μV]	t(6)=-0.976, P=0.367	Not significant
N90 latency [msec]	t(6)=-1.228, P=0.265	Not significant
JPS - angular difference for shoulder [°]	Z = -1.609, P=0.108	Not significant
JPS - angular difference for elbow [°]	t(6)=-2.111, P=0.079	Not significant
JPS - angular difference for wrist [°]	t(6)=-0.776, P=0.467	Not significant
JPS - time [sec]	t(6)=-1.091, P=0.317	Not significant
QST - Electrical stimulation [μA]	Z = -0.405, P=0.686	Not significant
QST - Von Frey hairs [gf]	t(6)=1.947, P=0.100	Not significant
Modified JTHF test (ratio)	Z = -2.371, P=0.018	Significant

Table 5.2: *Comparison of the baseline versus data obtained before TOT (Pre-TOT) with functional electrical stimulation (FES) assistance calculated with either the paired sample t-test (indicated by the t-value) or the Wilcoxon signed-rank test (indicated by the Z-value). The significant results are written in red for clarity.*

Based on the statistical tests, it was found that 4 of the 32 comparisons in table 5.1 and

table 5.2 showed a significant difference. Therefore, the majority of the hypotheses for each measurement regarding the effect of immobilization described in chapter 3 are rejected.

To visualize a dataset that was significantly different from baseline to before TOT, figure 5.3 illustrates the mean of the P45 latency for all subjects respectively at baseline and before TOT without FES assistance with a 95% upper and lower confidence interval. Figure 5.4 depicts the mean of the angular difference for the placement of the wrist for all subjects at baseline and before TOT without FES assistance with a 95% upper and lower confidence interval. This difference was not significant.

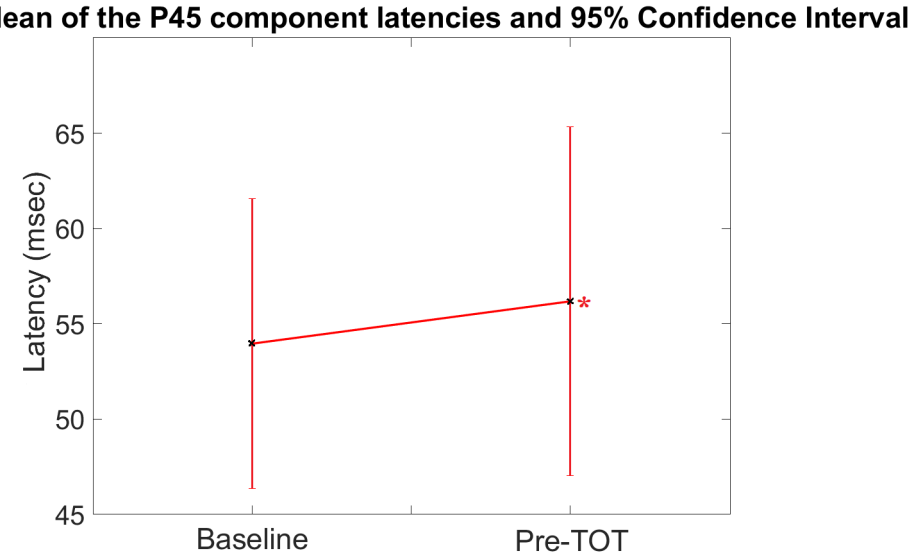


Figure 5.3: *Mean of the P45 latency for all subjects at baseline and before TOT (pre-TOT) without functional electrical stimulation (FES) with a 95% upper and lower confidence interval.*

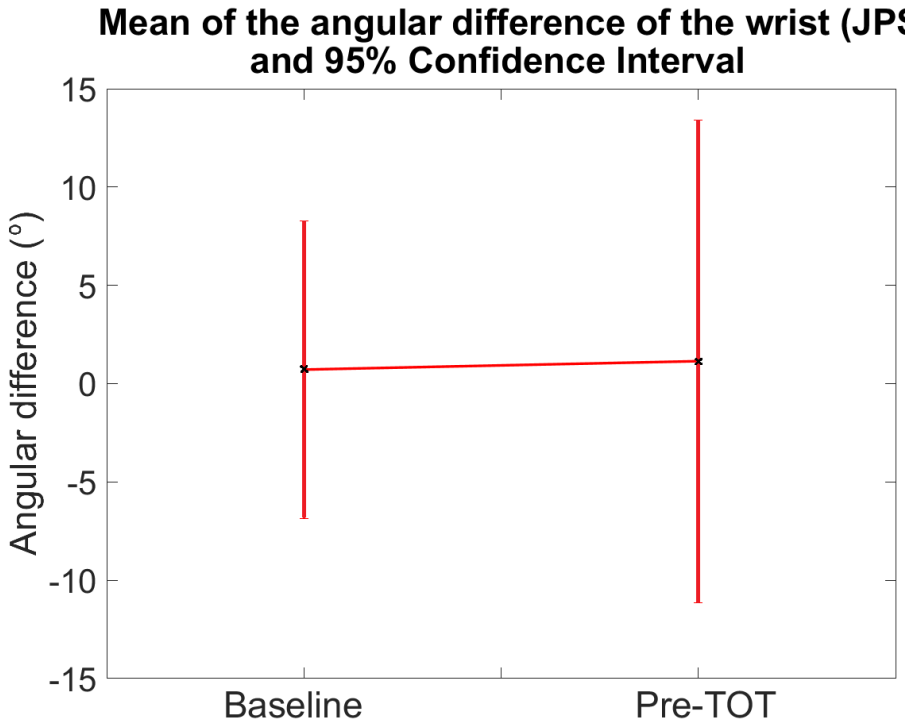


Figure 5.4: *Mean of the angular difference for the placement of the wrist in the Joint Position Sense (JPS) test for all subjects at baseline and data obtained before TOT (pre-TOT) without functional electrical stimulation (FES) assistance with a 95% upper and lower confidence interval.*

Figure 5.5 illustrates the mean of the averaged SEPs from electrode site C3/C4 with 95% upper and lower confidence interval.

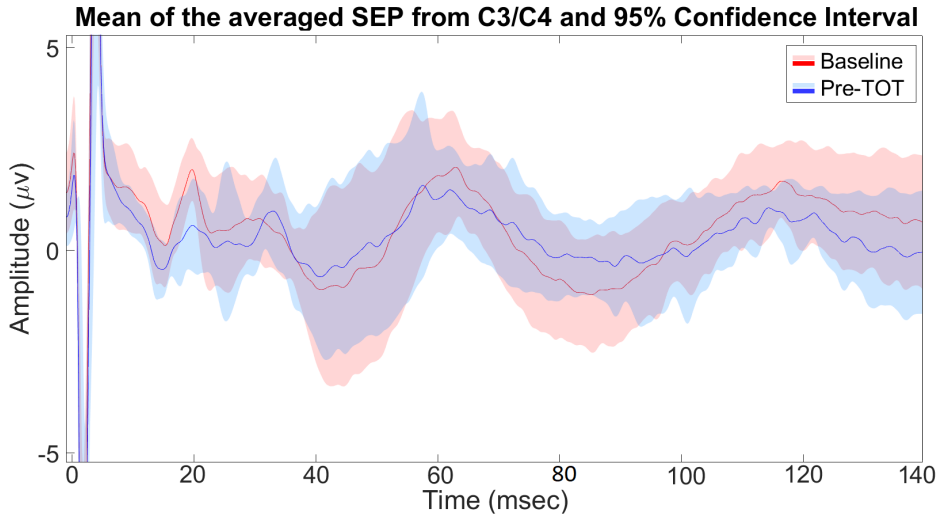


Figure 5.5: *Mean of the averaged somatosensory evoked potentials (SEPs) at baseline and before TOT (Pre-TOT) without functional electrical stimulation (FES) assistance from C3/C4 with a 95% upper and lower confidence interval. This electrode site was used for detection of the N20 and N90 component.*

Figure 5.5 illustrates that there was no clear trend in the data for the measurements. The confidence intervals overlap almost completely.

5.2 Alterations induced by Functional Electrical Stimulation

For examining how a single session of FES-assisted TOT alters neurophysiological and sensory function, the methods followed the description in subsection 4.3.2.
The results are presented in table 5.3.

Statistical Comparison of Difference Pre-TOT to Post-TOT for the Session with versus without FES Assistance		
Data	Statistical Values TOT with versus without FES	Result
Threshold for thumb twitch [mA]	t(12)=1.199, P=0.254	Not significant
N20 amplitude [μV]	t(12)=166, P=0.871	Not significant
N20 latency [msec]	U = -0.772, P=0.440	Not significant
N30 amplitude [μV]	t(12)=0.661, P=0.521	Not significant
N30 latency [msec]	t(12)=-0.287, P=0.776	Not significant
P45 amplitude [μV]	t(12)=-0.516, P=0.615	Not significant
P45 latency [msec]	t(12)=1.628, P=0.129	Not significant
N90 amplitude [μV]	t(12)=-0.960, P=0.356	Not significant
N90 latency [msec]	t(12)=0.88, P=0.931	Not significant
JPS - angular difference for shoulder [°]	t(12)=0.193, P=0.850	Not significant
JPS - angular difference for elbow [°]	t(12)=1.433, P=0.177	Not significant
JPS - angular difference for wrist [°]	t(12)=0.727, P=0.481	Not significant
JPS - time [sec]	t(12)=2.136, P=0.054	Not significant
QST - Electrical stimulation [μA]	t(12)=1.121, P=0.284	Not significant
QST - Von Frey hairs [gf]	U = -1.407, P=0.159	Not significant
Modified JTHF test (ratio)	U = -1.090, P=0.276	Not significant

Table 5.3: *Comparison of the difference from before TOT (pre-TOT) to after TOT (post-TOT) data with versus without functional electrical stimulation (FES) assistance. The comparison was analyzed by using either the unpaired sample t-test (indicated by a t-value) or the Mann Whitney U test (indicated by a U-value). The significant results are written in red for clarity.*

By using the unpaired sample t-test for normally distributed datasets and the Mann Whitney U test for non-normally distributed datasets, it was found that none the 16 comparisons illustrated in figure 5.3 showed a significant difference. Therefore, all of the hypotheses for each measurement regarding how a single session of FES alters proprioception and tactile sensation described in chapter 3 can be rejected.

5.3 Association between Measurements

For examining if associations can be found between the neurophysiological and clinical measurements, the methods were meant to follow the description in subsection 4.3.3.
As mentioned in the problem statement in section 2.5, the examination of any association should be based on the detected alterations, which the TOT sessions potentially would cause. There were no significant alterations in any of the measurements caused by the TOT session with FES compared to the control session. Therefore, associations were not examined.

Synthesis 6

The following chapter contains the synthesis of the study including a discussion and a conclusion based on the results, methodological considerations and future perspectives.

6.1 Discussion

In the current study, an experiment was performed to examine how FES-assisted TOT alters proprioception and tactile sensation in a hemiparetic stroke model based on upper limb immobilization of healthy subjects. Furthermore, the experiment included measurements of the motor function of the subjects to verify the motor impact of upper limb immobilization [Huber et al., 2006; Bassolino et al., 2012; Moisello et al., 2008; Furlan et al., 2016]. The measurements of the motor function contributed to assessment of alterations in sensorimotor integration [Allgöwer and Hermsdörfer, 2017]. All the measurements were performed before and after the 24-hour immobilization procedure, and were followed by a TOT session either with or without inclusion of FES assistance. After TOT, the measurements were performed again. To verify the effect of the immobilization procedure, statistical comparisons were made between the results of the baseline versus the results measured immediately after the immobilization. Additionally, the alterations of the outcome measurements from before to after the TOT were compared for the session with versus without FES assistance to examine whether the FES assistance improved the neurophysiological and sensory state of the subjects. The statistical analysis revealed a significant difference for the modified JTHF test from baseline to the measurements before the TOT for both parts of the experiment. This verifies a motor impact of the immobilization in the healthy subjects. When examining the effect of FES assistance, the statistical analysis no significant difference for any of the comparisons. Therefore, the results of the experiment suggest that the immobilization procedure had an impact on the motor performance of the subjects, but the neurophysiologic function, proprioception and tactile sensation were not affected. Furthermore, no difference between the alterations obtained from FES-assisted TOT versus TOT without FES assistance was detected for either motor or sensory performance. Therefore, the association between the neurophysiological and clinical measurements was not examined.

6.1.1 Study Design and Effect of Immobilization

As previously elaborated in section 2.3, the effect of short-term immobilization has mainly been documented for motor function [Huber et al., 2006; Moisello et al., 2008; Bassolino et al., 2012]. Therefore, it was uncertain how the immobilization in the current study would affect somatosensory function. Still, the study by Huber et al. [2006] showed alterations of the proprioception-related P45 component following immobilization. Short-term immobilization induces a temporary condition similar to low-functioning hemiparesis [Furlan et al., 2016] and sensory loss is mainly seen in patients with severe hemiparesis [Caplan and van Gijn, 2012]. Therefore, it was expected that the immobilization procedure would affect both motor and sensory function in the current study. However, the results revealed that the immobilization

did not have a significant impact on the majority of the performed measurements. A significant difference before versus after immobilization was found for the latency of the P45 component for one of the immobilization sessions. An increased latency following immobilization was expected for this component, since the study by Huber et al. [2006] also found this alteration following only 12 hours of upper limb immobilization. However, this difference was only seen for one immobilization session. Therefore, it might not actually express an alteration associated with the immobilization but rather a random finding. The study by Huber et al. [2006] also found a decreased amplitude of the P45 component, which was not found in the current study. Overall, the results therefore indicate that the immobilization procedure did not significantly impact the neurophysiological and sensory function of the subjects. It is possible, that a larger study population would show a more clear trend in the data, as the results in the current study are based on only seven subjects. Due to the relatively small sample population, the results are more likely to be affected by possible outliers.

Due to the lack of sensory and neurophysiological effect from the immobilization sessions, it is not surprising that there was no significant difference between the alterations obtained from FES-assisted TOT compared to TOT without FES assistance. If the immobilization procedure had led to impaired sensory function for the subjects, it was expected that FES-assisted TOT would improve the condition of the subject more than the TOT without FES assistance. This was because FES-assisted TOT has shown the potential to improve sensory function in hemiparetic stroke patients in previous studies [Wilkins et al., 2017; Bustamante et al., 2016].

It is uncertain why the immobilization procedure did not impact the sensory function of the subjects. Immobilization procedure 2 from the pilot trial was chosen, as it was the most comfortable for the subjects. However, it is possible that the lack of the extra stabilization (from the wooden spoon) has allowed for slight, unconscious movement of e.g the fingers. This might have reduced the immobilization effect. The studies by Bassolino et al. [2012] and Moisello et al. [2008] used an extra stabilizing element in the form of a splint or rigid support to ensure that the limb was kept completely still. Therefore, this might be an important factor for obtaining the desired effect. More studies should be performed to examine whether upper limb immobilization can actually impact the sensory function of healthy subjects, when an optimal immobilization method is found. Additionally, the duration of the immobilization period should be varied to see whether this is a critical factor for obtaining a potential sensory impact, as the effects obtained in the studies by Huber et al. [2006], Moisello et al. [2008] and Bassolino et al. [2012] are mainly examining the motor function.

It should be considered whether measurements involving less movement of the limbs could be used. As illustrated in figure 2.6, the immobilization effect fades rapidly during the performance of motor tasks [Bassolino et al., 2012]. Based on this, it could be considered to exclude the modified JTHF test in future studies as it involves the most motor performance amongst all the included measurements. Additionally, if future studies identifies a specific immobilization procedure that impacts the sensory function of the subject, it could be relevant to examine the potential for excluding the measurements before TOT. Thereby, this impact would not have to be confirmed together with the effect of FES assistance during TOT in the same study.

A significant difference was seen for the modified JTHF test before versus after immobilization for both immobilization sessions. This difference was expected, as stroke patients have shown to perform the JTHF test significantly slower than healthy subjects [Allgöwer and

Hermsdörfer, 2017] and the immobilization should induce a temporary condition similar to low-functioning hemiparesis [Furlan et al., 2016]. Therefore, it was expected that the subject would perform the modified JTHF test slower with the non-dominant hand following immobilization compared to baseline. This would lead to an increased ratio between the left and right hand for the time of the test. Thus, the immobilization procedure had an impact on the motor performance of the subjects as expected. This was also expressed through the altered threshold for thumb twitch before versus after the immobilization, which was borderline significant (P=0.051) for one session and significant for the other session (P=0.018).

6.1.2 Functional Electrical Stimulation and Task-Oriented Therapy

In the current study, the TOT session consisted of 30 repetitions of the grasping exercise. For the FES-assisted TOT, the stimuli controlled the pace of the upper limb movement, as the wrist extensors and flexors were activated at specific time points. Thereby, the FES-assisted TOT session lasted approximately the same amount of time for all subjects. Before the FES-assisted TOT could begin, the electrodes had to be placed so that they activated the nerves controlling the muscles for wrist extension and flexion. In some cases, the electrodes had to be moved around a couple of times prior to the FES-assisted TOT, either because some subjects felt that the electrical stimulation was uncomfortable or the wrist extensors and flexors were not activated. Thus, the amount of activation and stimulation of the affected upper limb might have varied between subjects for the FES-assisted TOT session. This could be problematic for the post-TOT measurement in regards of retaining the effects of the immobilization. Additionally, as the completion of the TOT session was based on number of repetitions, the time it took for the TOT without assistance of FES was not fixed. Another factor which could potentially be relevant for ensuring that the immobilization procedure was retained similarly among the subjects is the time for the TOT session. It might be better to fix the time rather than the number of repetitions as done in the current study. But as the subjects still have the opportunity to decide the pace themselves in the TOT session without FES assistance, this could result in that some subjects performs a considerably higher number of repetitions than others. Thereby, a similar issue arises. It is also unknown if time affects the immobilization effect but it is known that movement of the limb does affect it [Bassolino et al., 2012]. Therefore, a fixed number of repetitions was assumed to be the best option.
In the study by Wilkins et al. [2017], multiple variables were progressively altered during the grasping exercise to increase the level of difficulty for each subject. This procedure could also have been incorporated during the TOT in the current study by for example increasing the weight of the bottle or changing the orientation of it relative to the subject. However, the subjects who participated in the study by Wilkins et al. [2017] had chronic hemiparetic stroke and moderate to severe impairment. Therefore, a physical therapist determined when and how the variable should progressively be altered during the exercise. The examiners in the current study did not have the same experience with evaluating this, by which this procedure was not incorporated.
In the current study, the FES assistance was only applied to wrist extensors and flexors. As the entire non-dominant upper limb was immobilized, multiple other muscle groups could also be affected. It could have been relevant to apply FES to e.g. the elbow extensors and flexors to presumably allow the FES assistance to induce a greater neurophysiological impact. However, by only assisting two muscle groups with FES, the TOT became more simple for

the subject to perform and for the examiners to prepare for. If multiple muscle groups were to be assisted by FES, it is assumed that the preparation would be more time consuming, since more electrodes would have to be placed correctly. Because of the potentially increased preparation time and due to the FES assistance of even more muscle groups, the effect of the immobilization might fade even faster than if only the wrist extensors and flexors were assisted.

6.1.3 Relevance and Reliability of Measurements

It was chosen to include the proprioceptive N90 component in the SEPs recording and analysis, even though this component had previously been elicited by passive movement of the finger rather than electrical stimulation [Seiss et al., 2003]. Since the thumb twitch was passively induced during the SEPs recording, it was expected that this would have a similar effect as the movement described in section 3.2. However, in the study by Seiss et al. [2003] it is specified how the passive movement of the finger induces a rotation of a specific angle of approximately 9 °, a peak velocity of 230 mm/s and a movement duration of 100 msec. In the current study, it was not possible to control these parameters with the electrical stimulation. Thus, it is possible that this component might appear differently if the stimulation followed the protocol from the study by Seiss et al. [2003] more closely. Another study by Seiss et al. [2002] examined the impact of different movement characteristics on the appearance of the N90 component. An example is illustrated in figure 6.1. The signals in the figure were recorded from FC1, which is relatively close to the electrode sites used for detection of this component in the current study.

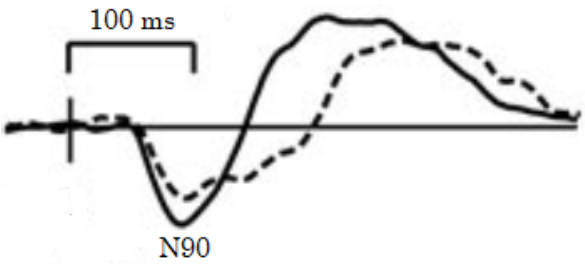


Figure 6.1: *The appearance of the N90 proprioceptive related component recorded at the FC1 electrode site following two different movement amplitudes. The solid line indicates a 15 mm movement of the right index finger while the dashed line indicates a 25 mm movement. Modified from Seiss et al. [2002].*

As illustrated in figure 6.1, the movement amplitudes (15 and 25 mm respectively) had a significant impact on the duration of the N90 component. However, the study found that the amplitude and latency of the component were not significantly impacted by the movement amplitude. [Seiss et al., 2002] Since the duration was affected, the appearance of the N90 component in the SEPs of the current study was uncertain. This could potentially have lead to challenges with identifying the correct component in the signal.
To completely follow the procedure used in the study by Seiss et al. [2003], it would require the inclusion of another stimulation protocol. This would make the experiment even more complex and time consuming, which was unwanted.

Most of the measurements included in the current study were chosen to examine the alterations of proprioception and tactile sensation. However, the reliability of some of these methods is

varying [Lönn et al., 2000; Tena et al., 2012]. For example, a study by Lönn et al. [2000] has shown that JPS testing systems have a moderate test-retest reliability at best, by which the potential of this type of test to detect proprioceptive alterations might be limited [Lönn et al., 2000]. Furthermore, only a fair to moderate test-retest reliability has been shown in a previous study for the Von Frey hairs. This is because the force of the application relies on the examiner. [Tena et al., 2012] In the current study, it was attempted to minimize this negative effect by letting the same examiner perform the Von Frey hair testing every time. Since identification of the detection threshold for electrical stimulation relied less on the examiner, it is expected that the test-retest reliability for this QST test is at least as good as for the Von Frey hairs and possibly better.
The Von Frey hairs used for the current study were from a standardized aesthesiometer. However, not all hairs were in a good condition. An example of this is illustrated in figure 6.2.

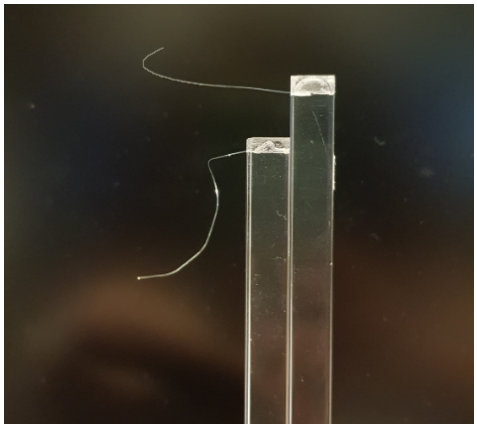


Figure 6.2: *Example of Von Frey hairs in a bad condition, which were used in the current study for assessing tactile sensation.*

Thus, it is possible that the bend on the hairs has made it challenging for the examiner to apply them similarly during every experimental session for all subjects. This could potentially have impacted the results.

As elaborated in the beginning of chapter 4, a time period of 3-7 days between the two parts of the experiment was chosen to reduce the potential practice effect associated with the modified JTHF test. However, as the test was performed before and after the 24-hour immobilization period and also after the TOT session (thus, the measurements before and after TOT were performed on the same day), there was a very short time period between these measurements in each of the experimental sessions anyhow. A study by Schaefer et al. [2018] showed that a group of healthy subjects significantly improved their performance after only 4 repetitions of the JTHF test when comparing to the time needed to conduct the test for the first repetition. The repetitions were separated by 60-90 seconds. Thus, it is possible that there was a practice effect impacting the measurements before and after TOT respectively. Thereby, the performance of the modified JTHF test for these measurements could partly be due to this practice effect, even though the exact duration of the practice effect is not known. The study by Bovend'Eerdt et al. [2004] found that the practice effect was not present between the sessions in which the modified JTHF test was performed. However, in that study, there were several days between the performance of the test (9.6 days, SD=7.1 days). Thus, it is not clear to what extent the practice effect has been present in the current study. One possible solution

to reduce the potential practice effect could be to let the subjects perform the test multiple times just before the measurements to obtain a stable performance [Schaefer et al., 2018; Boggio et al., 2006]. However, as the effect from the immobilization fades quickly [Bassolino et al., 2012], it is likely that this approach would make the effect fade even faster, since it requires more movement. When choosing the time period required between the immobilization sessions, it might as well be beneficial to include the other measurements in the considerations. The modified JTHF test was assumed to have the longest washout period due to the active involvement of the subject in motor tasks. However, the other measurements could have a considerable washout period as well. Though, it is still believed that the modified JTHF test is the most important to consider when choosing the time period. For example, a study by Xie and Urabe [2014] showed that for JPS tests, in which healthy subjects were allowed to visually inspect the position of the limbs (the non-dominant lower limb in the study), they were able to remember and reproduce the position five minutes after they saw the position. However, after 30 minutes, the subjects were not able to memorize the position anymore. This indicates that the subjects forgot about the position relatively quickly. Even though the subjects in the current study did not receive any visual information regarding the position of the limb, it is still possible that aspects of the test can be remembered. This could e.g. be the position of the unaffected limb with respect to the affected limb supported by the examiner.

6.1.4 Data Preprocessing

In the current study, the recorded SEPs data underwent filtering, epoch rejection based on a defined threshold for eye blink and finally averaging of the remaining epochs and baseline correction. The obtained signal was used for component detection and analysis. To further process the signal and suppress components that were not related to brain activity, approaches as independent component analysis or principal component analysis could be used. Thus, the data could be further processed based on scalp distributions of the different components in the signal. [Luck, 2014]

6.1.5 Statistical Analysis

In the statistical analysis, the paired t-test and Wilcoxon signed-rank test were included for examining the impact of immobilization. The unpaired t-tests and the Mann-Whitney U test were included for examining the impact of FES-assisted TOT.
Using the difference between the measurement values before TOT versus after TOT was considered a suitable approach, since the difference between FES-assisted TOT and TOT without FES assistance was of particular interest in the current study. It might, however, have been unnecessary for the examiners to calculate the difference and use this in the statistical analysis. Instead, an alternative approach could be to directly use the measurement values before and after TOT. Thus, the test would include two factors: Time (before/after TOT) and intervention (with/without FES assistance). A repeated-measures ANOVA (RMANOVA) would then be an appropriate statistical test, since it can handle a two-factor experiment with repeated measures on one factor. As the RMANOVA does not include information regarding where the significant differences between groups lie, a post hoc tests should be conducted because it can highlight exactly where these differences occur. [Zar, 2010].
Another possible alternative for comparison of the alterations obtained from FES-assisted TOT versus TOT without FES assistance would be to compare the alterations in percentage

calculated from the values of the measurements performed before TOT to the values after TOT. By using this method it would also be easier for the examiners to directly interpret the results from the two different TOT sessions. However, the use of percentage alterations might change the distribution of data by making it non-normally distributed. Furthermore, it has shown to have poorer statistical efficiency than e.g. using the alteration as done in the current study. [Vickers, 2001]
When interpreting the figures included in section 5.1, it is clear that the confidence interval of the three datasets in figure 5.3-5.5 overlap almost completely between the two compared groups. If the confidence intervals do not overlap, there will be a statistically significant difference between the means. However, the opposite is not necessarily true. [Zar, 2010] Therefore, by just focusing on the confidence intervals in the plots, it is not certain whether the means are statistically significant different. As the line between the mean values of the P45 latency from baseline to the measurements before TOT in figure 5.3 incline, it indicates that the latency increased. From the statistical analysis, it became clear that this difference was significant ($t(6)=-2.668$, $P=0.037$). This does not apply for the angular difference of the wrist as illustrated in figure 5.4, since the line is approximately horizontal by which there was almost no difference in the mean values from baseline to the measurements before the TOT. The statistical analysis also concluded, that there was no significant difference for this measurement ($t(6)=-0.105$, $P=0.920$). When interpreting the data in appendix I, there was no clear trend which might explain why only 4 of the datasets shows a significant difference from baseline compared to the measurements performed after the immobilization.

6.1.6 Future Perspectives

The model based on upper limb immobilization of healthy subjects used in the current study mainly showed an impact on the motor performance of the subjects while the neurophysiological and sensory function was not affected. Thus, it would be relevant in future studies to actually include hemiparetic stroke patients with sensory deficits to more clearly examine, whether FES-assisted TOT impacts the sensory function of this patient group.
Due to the uncertainty regarding the sensory impact of immobilization, it would be relevant for further studies to make more detailed examinations of this. This could potentially include more neurophysiological and clinical tests. Furthermore, it should be examined whether e.g. an increased immobilization period for the healthy subjects could induce a sensory impact.

6.2 Conclusion

In the current study, it was examined how a single session of FES-assisted TOT altered proprioception and tactile sensation in a hemiparetic stroke model based on upper limb immobilization of seven healthy subjects. The analysis of data before versus after immobilization revealed that there were no statistical significant differences for the neurophysiological and sensory measurements. Therefore, the immobilization procedure might not have induced alterations for these parameters. However, comparisons of the modified JTHF test results showed significant difference, which indicates that the immobilization procedure impacted the motor function of the subjects in accordance with previous studies. All the statistical comparisons between the alterations induced by FES-assisted TOT compared to TOT without FES assistance showed no significant difference. Thus, the sensory and neurophysiological impact of FES and the association between the alterations of these remain unclear.
Further studies should include a larger sample population. Additionally, the time period and method for immobilization should be reconsidered and examined in terms of the potential for generating sensory alterations based on upper limb immobilization in future studies.

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Hemiparesis A

Approximately 80-90% of all stroke patients experience motor symptoms. Among these patients, the incidence of hemiparesis is relatively high, since at least two-thirds experience this condition affecting the upper and lower limbs uniformly. Hemiparesis is defined as a weakness in one side of the body in the upper and lower limbs. This condition is therefore not equivalent to hemiplegia, which refers to a complete paralysis of the limbs in the affected side. [Caplan and van Gijn, 2012]
The hemiparetic limbs are often also affected by loss of feeling (tactile sensation). The severity of this sensory impairment range from inability to distinguish between a nickel and a dime applied to the hand to being completely unable to feel touch in some affected areas of the limb. The loss of feeling can furthermore involve inability to feel painful stimulation or temperature. [Caplan, 2006]
Hemiparesis can be divided into different subtypes. The type experienced by the patient depends on the brain areas affected by the stroke. Table A.1 presents different types of hemiparesis and the brain areas, which are typically involved in the stroke causing the condition. [Caplan and van Gijn, 2012]

Characteristics of Hemiparesis	Usual Location of Stroke
Severe impairment affecting the entire side uniformly.	The cerebrum, often involving the middle cerebral artery.
Impairment mainly in the distal areas.	The cerebrum, often involving the cerebral cortex.
Impairment mainly in the proximal areas.	The premotor cortex.
Pure motor impairment in the entire side.	Different possible areas: - The pons. - The corona radiata. - The posterior part of the internal capsule. - The medullary pyramid. - The mesencephalon.
Pure motor impairment in the arm and leg	The brain stem.
Pure motor impairment in one side of the face and the arm.	The middle cerebral artery and the cerebral cortex.

Table A.1: *The different types and characteristics of hemiparesis and the brain areas usually involved in the stroke when the given type of hemiparesis is present. Based on knowledge gained from Caplan and van Gijn [2012].*

Patients with a uniform weakness in the limbs on one side of the body often have more severe symptoms than other hemiparetic stroke patients. This type of hemiparesis is often associated with sensory deficits in the affected side as well. Furthermore, the speech of these patients might also be affected. In less severe cases, the hemiparesis predominantly impacts distal muscles of the limb or the proximal muscles. Another subtype is pure motor hemiparesis. The patients affected by this type of hemiparesis do not experience any somatosensory or visual deficits, and their consciousness is not affected either. In these patients, the lower limb

might not be involved, which means that only the face and arm are affected. Sometimes, the face is not involved either, by which the condition only affects the arm and leg. [Caplan and van Gijn, 2012]

Neurons B

Neurons are the structural elements of the nervous system. A neuron consists of a soma with the nuclei and several processes which form connections to surrounding neurons. [Stanfield, 2013] The main structures are illustrated in figure B.1

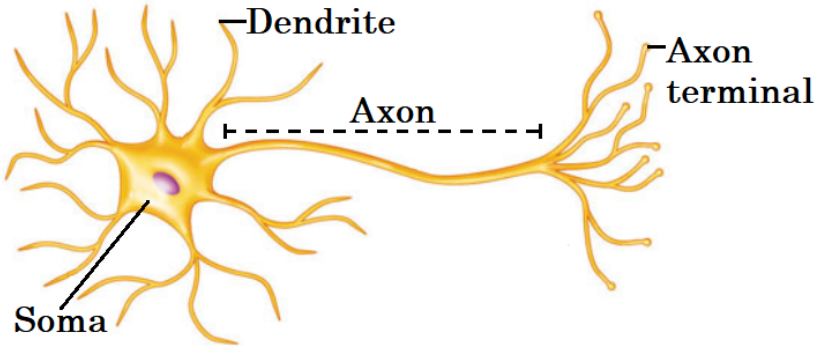


Figure B.1: *Structure of a neuron including dendrites, soma, axon and axon terminals. Modified from Stanfield [2013].*

The soma contains the nuclei, which is involved in continuous protein synthesis to keep the neuron functioning. The dendrites are responsible for receiving signals from other neurons and transmit them to the soma. Each neuron has numerous dendrites, which are structured as trees with a lot of branches. Due to the structure of the dendrites, they create a large receptive area, from which signals can be received. In the part of the soma located in the origin of the axon, a specific type of processes occur. These are responsible for transmitting signals away from the soma. The axon is covered in myelin, which increases the speed of the transmission of a signal from the soma to the axon terminals. As for the dendrites, the axon terminals are structured as trees with a lot of branches to create a large area for transmission of signals from the neuron. Thus, the axon terminals transmit signals to other neurons or to a muscle. Therefore, they are referred to as postsynaptic terminals in a synapse. [Augustine, 2017; Martini et al., 2012]

B.1 Function of Neurons

Based on the function, neurons can either be categorized as sensory, motor and interneurons. The sensory neurons transmit signals from the sensory receptors peripheral in the PNS to the CNS. The human body contains approximately 10 million sensory neurons, which collect and transmit sensory information regarding both internal and external parameters.
Motor neurons transmit signals from the CNS to peripheral structures, by which the processes of the motor neuron are efferent. Approximately 500,000 motor neurons are found in the human body.
Interneurons are involved in the distribution of sensory signals and coordination of motor actions. At least one interneuron is found between a sensory and motor neuron; the number of interneurons is increased dependent on the complexity of the response to stimuli.

Interneurons are furthermore involved in higher-order functions including memorizing. There are approximately 20 billion interneurons in the body, which are mainly localized in the brain and spinal cord. [Martini et al., 2012]
Independent of neuron type, neurons in between or a neuron and a muscle communicate through synapses. The presynaptic terminal is an axon terminal and the postsynaptic terminal are a dendrite or a muscle. When an action potential reaches the presynaptic terminal, it initiates either a chemical synapse or an electrical synapse. For a chemical synapse, the pre- and postsynaptic terminals are separated by a synaptic cleft. When the signal from the axon reaches the presynaptic terminal, it releases neurotransmitters into the synaptic cleft, which bind to receptors on the postsynaptic terminal. The transmitted signal can either be excitatory or inhibitory, which will be elaborated in section B.2. For electrical synapses, the pre- and postsynaptic terminal are separated by a gap junctions, which can conduct electric current. Thereby, a direct change in voltage happens in the postsynaptic terminal. [Martini et al., 2012]

B.2 Membrane Potentials and Action Potentials

When a neuron is at rest, the membrane potential is approximately -70 mV referred to as the resting potential. When the dendrites of a neuron receive a stimuli, the resting potential can be altered depending on the stimuli strength. As mentioned, the signal can either be inhibitory or excitatory. If the transmitted signal from the presynaptic terminal is inhibitory, it causes a hyperpolarization of the membrane potential in the postsynaptic terminal, by which the membrane potential becomes more negatively charged. Thereby, the neuron is less likely to initiate an action potential, which causes an IPSP. On the other hand, if the signal is excitatory, it causes a depolarization of the membrane potential. Thereby, the membrane potential becomes less negatively charged. An excitatory signal creates an EPSP, which might lead to an action potential. In order to create an action potential, multiple EPSP are needed, as one EPSP only depolarize the membrane potential with approximately +0.5 mV. As illustrated in figure B.2, the threshold is around +10 mV from the resting potential. [Martini et al., 2012]

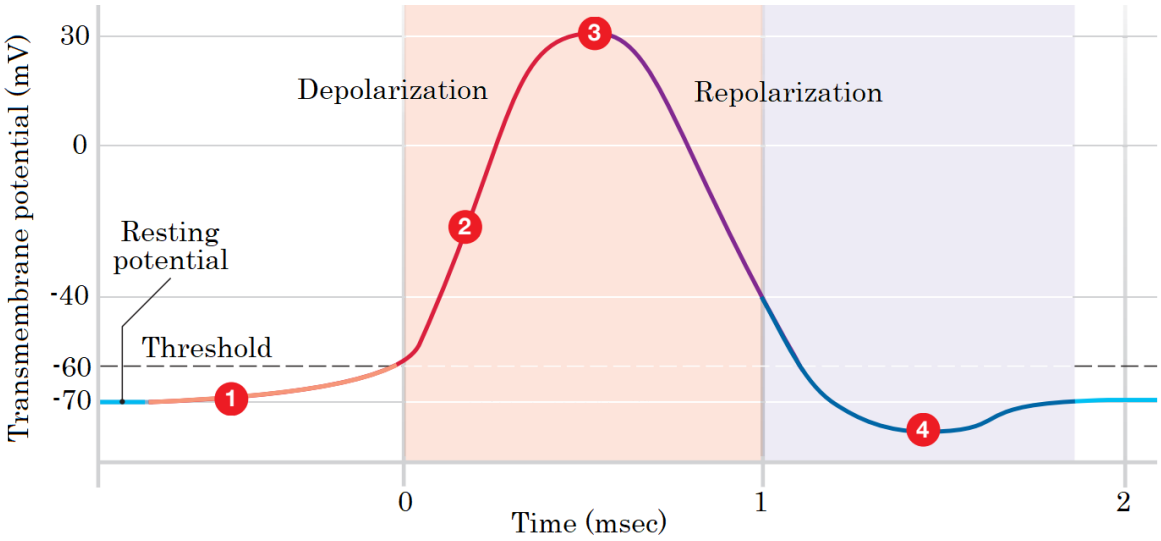


Figure B.2: *Alterations of the membrane potential during an action potential. Modified from Martini et al. [2012].*

The summation of EPSPs and IPSPs can either be temporal or spatial; for a temporal summation, one presynaptic neuron stimulates the postsynaptic neuron multiple times in short intervals. Thereby, the stimuli will sum up and create depolarization above the threshold, as the postsynaptic neuron do not have time to repolarize. For a spatial summation, the postsynaptic neuron receives stimuli simultaneously from multiple different presynaptic neurons, which rapidly depolarize the membrane potential. Phase 1 in figure B.2 illustrates a summation of EPSPs, by which the membrane potential reaches the threshold and an action potential is initiated. This causes a depolarization of the membrane potential until it overshoots and reaches +30 mV, which is illustrated as phase 2 and 3. Then, the membrane potential repolarizes and returns to the resting potential. This process happens rapidly, by which a hyperpolarization occurs, i.e. the membrane potential is more negatively charged than for the resting potential. This is illustrated as phase 4 in figure B.2. After the hyperpolarization, the sodium–potassium pump located in the membrane insures that the potential returns to the resting state. [Martini et al., 2012; Stanfield, 2013]
The strength of the EPSPs, causing the action potential to occur, does not impact the amplitude of the action potential. Either the action potential occurs or not. [Matthews, 2003]

Brain Subdivision and Function C

The largest structure of the brain is the cerebrum, which consists of two cerebral hemispheres. The surface of the cerebrum is covered by a layer of gray matter called the cerebral cortex. This layer is formed by gyri, which help increasing the surface area of the cerebral cortex, sulci and deeper fissures. In general, the cerebrum is involved in complex processes related to e.g. consciousness, handling of memories, sensations and movements. [Martini et al., 2012]

C.1 Cerebral Lobes

The cerebrum can be subdivided into different lobes, each of which handles specific functions [Martini et al., 2012]. This subdivision is illustrated in figure C.1.

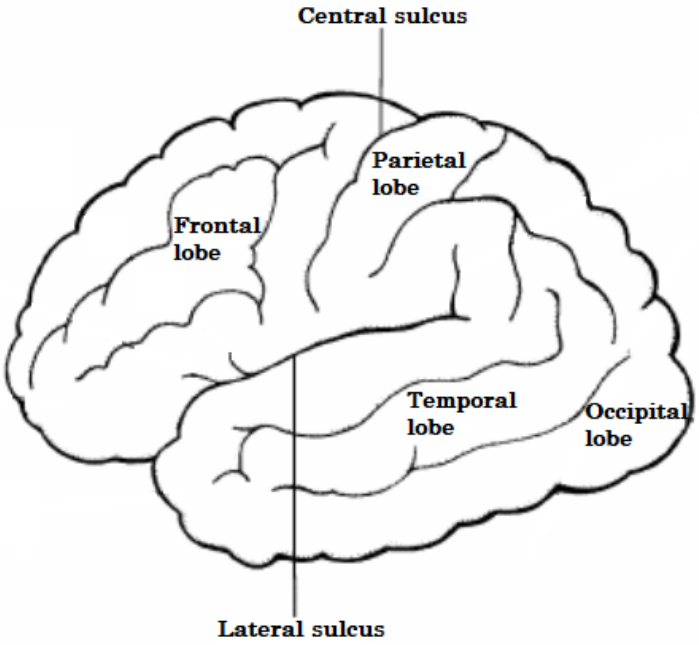


Figure C.1: *The cerebrum from a sagittal view. The subdivision of the cerebrum includes the frontal, parietal, temporal and occipital lobe. Furthermore, the central an lateral sulcus are marked. Modified from Augustine [2017].*

The frontal lobe is involved in the performance of voluntary movements. Furthermore, it is involved in speech and the intelligence and behaviour of a person. The parietal lobe handles processes of recognizing and distinguishing between different types of sensory stimulation. It makes it possible to compare the experienced sensations and is furthermore involved in speech and some eye movements. The occipital lobe handles physiological mechanisms related to vision including color or motion of visual inputs. The temporal lobe handles auditory inputs and certain types of vestibular information. This part of the brain is also involved in

supplementary voluntary movements handled by the frontal lobe. It also assists in producing language and is involved in autonomic actions. [Augustine, 2017]

C.2 Sensory and Motor Cortical Areas

The sensory and motor areas of the cortex are divided by the central sulcus, as illustrated in figure C.1. The primary motor cortex is located anterior to the central sulcus in the outer layer of the precentral gyrus. By controlling somatic motor neurons in both the spinal cord and brain stem, the primary motor cortex is involved in direction of voluntary movements. Thus, when a motor neuron in this cortical area is activated, a specific, corresponding skeletal muscle contracts.
The primary sensory cortex is located posterior to the central sulcus in the outer layer of the postcentral gyrus. This area receives somatosensory signals generated following stimuli as pain or pressure.
The sensory and motor cortical areas are linked to surrounding association areas. The motor association areas handle the interpretation of signals and coordination of motor responses. The sensory association areas are involved in the interpretation of different sensory signals. For example, activity in the primary sensory cortex is registered by the somatic sensory association area. Furthermore, other senses including hearing and sight have other association areas. The ability to coordinate learned movements is due to the somatic motor association area, which transmits information to the primary motor cortex to activate the neurons and thereby initiate voluntary movements. When movements are repeated, a stimulation pattern is saved in the premotor cortex, which creates the ability to smoothly perform the specific movement. Integrative centers receive signals from different types of association areas in order to control motor actions of high complexity and to analyze the result of the different ways of responding to sensory information. [Martini et al., 2012] In the following, a more detailed description of the sensory path is included, to further elaborate how sensory signals are transmitted to the brain.

C.3 Sensory Path

Different types of receptors are located in the different tissues of the body. These receptors are sensitive to different types of sensory stimuli. For example, mechanoreceptors are sensitive to the application of force to an area, thermoreceptors are sensitive to alterations in temperature, while nociceptors are sensitive to painful stimuli. The somatic afferent paths transmit sensory signals from the receptors described above to the CNS. Overall, when a receptor detects sensory stimuli, the signals are transmitted to a first-order neuron. The peripheral process from the neuron is connected to the receptor and the central process transmits the signal to the CNS. They can be located at different places as the dorsal horn or other nuclear groups within the spinal cord and brain stem. The central process of a second-order neuron crosses the median plane within the spine, thus continuing up contralaterally. The soma of third-order neurons is located in the thalamus. Further processing of the sensory signals is performed in different areas of the cerebral cortex. The signals are transmitted from the thalamus to the somatosensory cortex. [Augustine, 2017] A somatic sensory path is illustrated in figure C.2.

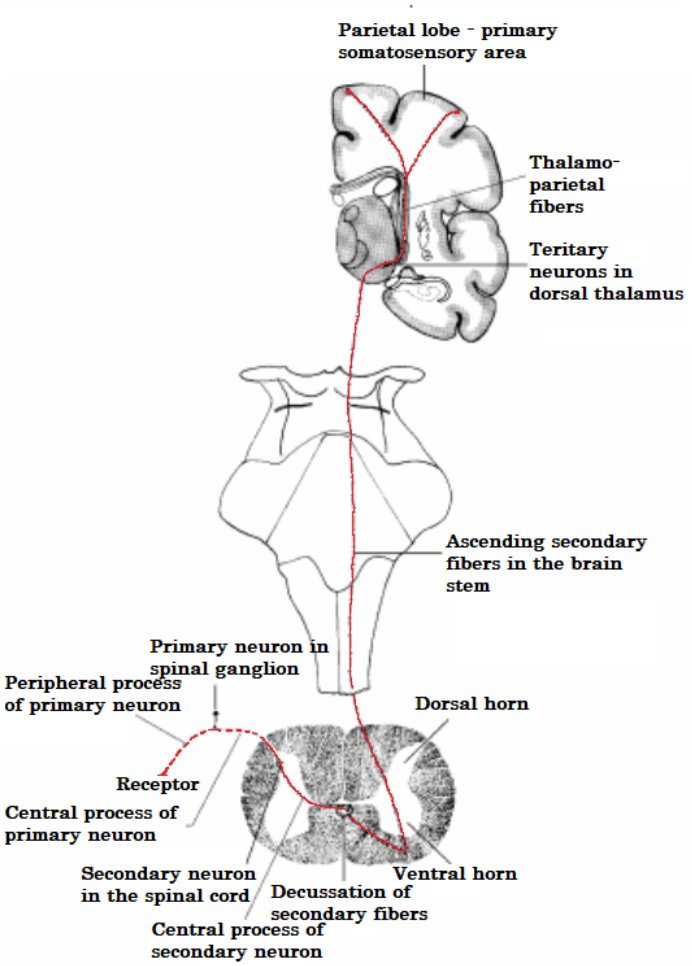


Figure C.2: *An example of a somatic sensory pathway and its different components. Modified from Augustine [2017].*

C.4 Neuroplasticity of the Brain

Neuroplasticity is the ability of the brain to change over time in order to adapt to new conditions or experiences. Examples of alterations caused by neuroplasticity can be an altered proportion of grey matter or a given motor function can be transferred to a different location in the motor cortex. Neuroplasticity can occur on multiple levels from microscopic alterations in a neuron to larger-scale alterations such as cortical remapping as a response to injury. [Wieloch and Nikolich, 2006]

C.4.1 Neuroplasticity after a Stroke

The neuroplasticity is more susceptible to adaptions the first two weeks after a stroke, but is in general strengthened within the first six months after a stroke [Coleman et al., 2017]. Thus, within this time period, the brain can more easily learn and relearn functions [Kleim and Jones, 2008]. Therefore, early rehabilitation is important to improve the condition of the patient. Three phases occur in the brain during recovery from stroke: In the first phase, damaged cells are repaired. This is followed by the second phase, in which plasticity is present in the functional cells, involving alterations of the existing neuronal pathways. Finally, the third phase occurs, involving the generation of new pathways. [Wieloch and Nikolich, 2006] To

cause lasting alterations in the nervous system, several factors might be relevant, including repetitions of the behaviour, intensity of the rehabilitation and the time from the damage occurs until rehabilitation is started. [Kleim and Jones, 2008]

Interview (Danish) D

D.1 Interview Guide

1. Vi har læst os frem til, at I her i centret genoptræner patienter med moderat til svær hjerneskade. Hvilket niveau er apopleksipatienterne så typisk på rent motorisk, når de påbegynder et rehabiliteringsforløb her?
2. Hvor lang tid er apopleksipatienterne typisk indlagt her?
3. Hvor hyppigt træner terapeuterne med patienterne og i hvor lang tid ad gangen?
4. Hvilke behandlingsmetoder bruges der til apopleksipatienter? Og hvilken af disse vurderer du så bruges mest?
5. Bruger du funktionel elektrisk stimulation (FES), når du genoptræner patienter?
   a) Hvis ja: Hvor meget bruger du denne teknik? (dagligt, ugentlig, månedligt?)
   b) Hvis nej: Hvorfor ikke?
6. Ift. de apopleksipatienter med hemiparese, der modtager behandling med FES: Hvornår i genoptræningsforløbet påbegyndes denne behandlingsteknik?
   a) Hvis svaret er bredt: Hvad er afgørende for at behandling med FES kan påbegyndes?
7. Oplever patienter motorisk forbedring ved brug af FES?
   a) Hvis ja:
      i. Oplever du at denne forbedring sker i lige høj grad for alle patienter eller er der stor forskel imellem dem?
      ii. Hvordan oplever du, at patienterne generelt beskriver deres motoriske forbedringer forbundet med behandlingen?
   b) Hvis nej:
      i. Hvad kan årsagen til dette være?
8. Oplever patienter sensorisk forbedring ved brug af FES?
   a) Hvis ja:
      i. Oplever du at denne forbedring sker i lige høj grad for alle patienter eller er der stor forskel imellem dem?
      ii. Hvordan oplever du, at patienterne generelt beskriver deres sensoriske forbedringer forbundet med behandlingen?
   b) Hvis nej:
      i. Hvad kan årsagen til dette være?
9. Ud fra litteratur antager vi, at FES først og fremmest anvendes til at genoptræne motoriske symptomer. Men er genoptræning af sensoriske symptomer noget I fokuserer på under rehabiliteringsforløbet?
10. Hvis FES har forårsaget en ændring (motorisk og/eller sensorisk):
    a) Hvor lang tid skal patienterne have modtaget behandling med FES, før at man begynder at se en ændring - både motorisk og/eller sensorisk?

D.2 Transcription

Udviklingsterapeuten havde på forhånd fået udleveret interview guiden, således vedkommende kunne inkludere sine kolleger i besvarelserne. Disse vil blive henvist til som K1, K2 og K3 i nedenstående transskribering. Personer, der nævnes ved navn, vil blive henvist til med bogstavet X. Inden interviewet gik i gang, introducerede vi os for hinanden, fik lidt kaffe og the og snakkede lidt.
Der blev stillet flere spørgsmål til udviklingsterapeuten omkring rehabiliteringsforløb, både i forhold til frekvens for træningssessioner, motorisk bedring og sensorisk bedring. Herunder ses der et uddrag af interviewet, der har fokus på de sensoriske og motoriske aspekter inden for rehabilitering af apopleksipatienter med FES. Det er netop disse aspekter, som bidrager til dette speciales formål.

Interviewer 1 (I1): Så har vi spørgsmål 7, som også er i forhold til FES. Og det er det her med om patienterne oplever en motorisk forbedring, når man bruger FES?
Udviklingsterapeut (U): Ja. Der siger.. Hvis ja, så skriver K1.. K1 skriver ja, tror jeg. Oplever du at denne forbedring skriver i lige høj grad for alle patienter, eller er stor forskel imellem? Der er stor forskel, skriver K1, ikke også? Nu tager vi lige K1. Hvordan oplever du, at patienterne generelt beskriver deres motoriske forbedringer forbundet med behandlingen? Nu tager jeg lige spørgsmål 7 for K1. Nej, de fleste beskriver ikke, at deres forbedring er forbundet med behandlingen. Og hvad kan årsagen til det være? Så svarer K1: De ser mere en fremgang ud fra alle behandlinger. Nu skal I høre hvad K2 og K3 svarer, de svarer nemlig ikke det samme. De svarer på altså 7.a: Der er store individuelle forskelle. Patienterne beskriver ofte, at de i forbindelse med, eller kort efter FES behandlingen, har bedre øget kontrol svarende til den muskulatur eller ekstremitet der stimuleres med FES, for eksempel øget kontrol af knæet. Det kan være i gang eller under andre aktiviteter. Og så siger de under 7.b, hvis nej, hvad kan årsagen være: Der kan være mange årsager til at de ikke, altså kognitive udfordringer, manglende sensibilitet, nedsat vågenhed, manglende eller nedsat tolerance i forhold til strømintensitet, opmærksomhedsproblemer og neglekt. Og de sidstnævnte skal man simpelthen ikke underkende, at der er opmærksomhedsproblemer. Det var den ene kollega, der svarer sådan. Den anden svarer: Ja. Og så den næste: Nej! Der kan være stor forskel, det er helt afhængig af skadens lokalisation og omfang. Så den er også lidt interessant.
I1: Ja, helt sikkert.
U: Det var de tre fysioterapeuters svar.
Interviewer 2 (I2): Ja. Har du noget, som du vil tilføje så?
U: Altså, det jeg vil tilføje, det er jo at vi.. X og jeg og specielt en af mine kolleger, der stoppede med at arbejde her for et par år siden, vedkommende er en senior terapeut og har arbejdet med elterapi i mange, mange år. Vi lavede projekter sammen, og der sker så det, at vi laver sådan et walking projekt, og så siger min kollega så til X: X, jeg har en hypotese. Jeg tror, at vi godt kan, istedet for at give 20 behandlinger i rap med det her funktionel elektriske terapi under gang, så kunne vi, så tror jeg mere at det er noget der primer systemet, så vi kunne jo, altså jeg opfatter det lidt, når de får det, så får de priming af hele deres system og vi behøver ikke give så mange gange, altså. Så vi prøvede, altså med ganske.. Vi prøvede stimuleringer med meget kortere.. Tid.. Og det er jo ligesom for at sige, jamen er det egentlig en priming af systemet. Vi er inde i det der med det sensoriske eller stimulering, hvad er det egentlig vi gør? Altså behøver vi alt det der, skal vi bare give nerven noget fyring, og så

kommer der noget ud? Og det projekt har vi aldrig fået skrevet. Det skal X og jeg til at sidde og skrive her, så der er noget der, der ikke.. Så resultaterne, de var ikke.. Jeg er ret sikker på, at de ikke var signifikante. Men I kan jo forstå, vedkommende har jo den personlige erfaring, én der bruger rigtig meget elterapi og oplever at de bliver mere opmærksomme på deres ben i det hele taget, altså. De får, det er ligesom baningen, den bliver skærpet. Det er det K1 siger. Og det var jo så det vi efterprøvede, eller hvad kan man sige. Vi efterprøvede det.
I1: Ja, okay.
U: Og det.. Det er også min holdning. At, der sker, om det så er, altså om det er fordi man får noget, der irriterer der ovre, at man så får mere opmærksomhed der ovre, altså. For apopleksi patienter, så er det jo sådan, at de har jo ikke atrofi. De får jo ikke atrofi, fordi banen ligger der jo. Det der er problemet for en apopleksi patient, det er at de kan ikke få adgang til motorprogrammerne, de kan ikke igangsætte det. Hele systemet ligger der, der er jo ikke noget der er skåret over, ledningerne er der. Så hvis vi kan give nogle, lave nogle genveje, lave nogle stimuleringer, der går op og går ned på de rigtige tidspunkter, så er det jo egentlig det vi skal, fordi så kan vi jumpstarte systemet. Og der er elterapi, altså. En brik der er værd at gå efter, er min holdning. Ja.
I1: Ja, fint. Vi bliver lidt i noget af det samme, men så mere med fokus på det sensoriske. Oplever patienterne en forbedring af det sensoriske, når man får FES?
U: Den er spændende. *(...)* Det er jo det sensoriske I spørger om i spørgsmål 8, om de oplever, og der svarer K2 og K3: Nogle gør, andre ikke. Det er nok mere en øget opmærksomhed på den legemsdel eller kropsside, der har været FES på. Siger den ene. Den anden siger: Der er stor forskel imellem patienterne, men generelt er FES god til at øge opmærksomhed på den paretiske ekstremitet. Nej, de siger det samme, ikke også? Og så videre i 8, for der er åbenbart nogle underpunkter, ikke også?
I1: Jo,lige præcis.
U: Så K2 siger til underpunkterne: Patienterne oplever, at de får en bedre fornemmelse af arm og ben, eller den kropsdel der stimuleres via FES. Patienterne får nemmere ved at inddrage ekstremiteten i funktioner bagefter. Der kunne også stå, hvis det var en ergoterapeut, der havde skrevet det, så havde K1 skrevet, eller nej, K3 skrevet: Patienterne får nemmere ved at inddrage ekstremiteten i aktiviteter, og det er vigtigt. Hvis det er sandt, ja..
I1: Ja.. Og i den forbindelse, vi har også et underpunkt der lyder omkring det her med, hvordan patienterne beskriver deres sensoriske forbedringer, det er så det her med, bare lige for at være sikker på at vi forstår det klart, det er så det her med, at de oplever at de nemmere kan inddrage..
U: Ja.. Ja.. Og K1 siger noget andet. K1 siger.. Hvad er det spørgsmål, det er nummer 8 eller hvad?
I1: Ja, det er så. Der er sådan et punkt a og så punkt 1 og 2 derunder.
U: Hvordan oplever du, at patienterne generelt beskriver deres sensoriske.. De beskriver, siger K1, ingen forbedringer i forhold til dette. Hvis nej, hvad kan årsagen til dette være? Det.. De oplever er umiddelbart en meget lille fremgang sensorisk, som ikke nødvendigvis kan tilskrives behandling med FES, og det K1 jo skriver, det er jo, at mange, når de har ret store sensoriske udfald, så er det tit at det er noget af det, der er svært at gendanne. Og jeg ved ikke hvad litteraturen siger på det område, altså, omkring hvor meget, altså, om der har været studier der direkte har været, hvor elterapi har været anvendt i den sammenhæng og hvor man så har kigget og har haft mål for deres sensoriske fremgang, det er jeg faktisk ikke klar over. Er I det?

I2: Ja.. Men det kan vi lige tage senere måske.
U: Ja, for det er jo spændende, for hvis der er det, så skal vi jo måske, altså hvis I kan komme og sige, jamen der er faktisk evidens eller, ja, der er begyndende bevis for at hvis vi gør det, så kan de også på tests vise større, altså, sensorisk, og det.. Hvorfor skulle det ikke være sådan. K1 svarer, at.. At, den er så lille, den K1 oplever, den de oplever, som ikke nødvendigvis kan tilskrives behandlingen med FES. Ja. Det er det K1 skriver.
I1: Ja, okay.
U: Og der kan I jo høre, der kan jeg ikke, der har jeg ikke nogen erfaring eller nogen kontant mening andet end at alt hvad vi kan få ind i det system, så det vågner, det.. Ja..
I2: Ja, fordi når I bruger FES så er det vel hovedsageligt for at fokusere på motorisk genoptræning? Eller hvordan?
U: Ja, det lyder det til, når vi læser K1's svar her. Ja. Det er det. Men som sagt, så ved jeg andre har fokuseret på det sensoriske, og vi ved jo hvordan, at sammenhængen imellem motorisk og sensorik er stor. Så.. Så hvis du får det ene, så er der også stor chance for, at du får det andet, det med at kunne mærke om man holder eller man ikke rører eller hvad, altså, alt det der det er jo ufatteligt vigtigt.
I1: Ja, det er det. Vel også for at nå over i det, som vi snakkede om før, at man når over i den sidste kategori, at kunne fungere i hverdagen?
U: Ja, lige præcis.
I1: Ja.
I2: Men altså, bare baseret på din egen erfaring, oplever du at I fokuserer, altså bare nogle rehabiliteringsmetoder på det sensoriske eller er det oftest..
U: Ja, det gør vi, og det har stigende.. Der kommer også lige lidt om det sensoriske, fordi de går på jer ift. at de vil, der er en af dem der vil have jer til at definere hvad I mener med sensorik, ikke, fordi der er jo mange sanser. Ja, så hvis det er den taktile, kinetiske sansning I taler om, altså der, der er der nogen, der har sådan en stimuleringspakker, hvis I forestiller jer, de laver forskellige former for stimuleringer altså med ru materiale på fingrene, altså de laver stimuleringer, og så ind i en aktivitet og forstærker inputene der hvor de er. Altså, så det har man øje for, det har man. Måske i vejende grad, afhængig af hvor man er uddannet, altså fordi det er ikke sådan, tror jeg, at litteraturen siger til os: Det er den stimuleringspakke, den har vi testet op mod den anden. Sådan er det ikke. Det kan godt være, at I kan fortælle mig noget andet, men det er ihvertfald ikke det jeg oplever, altså så stærkt er det ikke. Så det er jo noget med at.. Lidt og gå sine egne veje men at vi ved, at der er sensomotorik, der hænger sammen, og vi ved at det er en stor gene ikke at kunne mærke sin hånd og lægge den på en kogeplade, altså det.. Og prøve og fange den, og så for øvrigt, give input i forbindelse med opgavespecifik træning, og når I spurgte metode, hvad er det det handler om, ikke? Altså det er jo at træne i dagliglivs opgaver, hvor vi så giver input mens du gør det. Vi laver, vi bryder delopgaver ned og laver en delhandling, det kan for eksempel være functional reach er der jo noget der hedder, at du går hen og når du så er der (red: holder ved koppen), hov den er varm, altså. At du så forstærker de input, som der er i det, ikke?
I1: Ja. Okay.. Ja, det næste spørgsmål, nu har vi allerede været lidt inde på det, det her med at.. Altså, først og fremmest har vi ligesom på baggrund af litteraturen antaget, at det er motoriske symptomer, der bliver fokuseret på, når man bruger FES. Men, om genoptræning af det sensoriske sådan har været i fokus, jeg skal bare lige være sikker på, at vi har været omkring det..
U: Jamen, det kan vi godt lige.. Ja. Det er det med: Ud fra litteratur antager vi, at FES

først og fremmest anvendes til at genoptræne motoriske symptomer ja. Og det har I jo, når I siger det, så er det jo fordi I ud fra litteraturen, sådan er det. Men ja, er genoptræning af sensoriske symptomer noget i fokuserer på under rehabiliteringsforløbet? Ja. Og så skriver K1: Vi fokuserer både på den motoriske og sensoriske opmærksomhed vil jeg sige. Og så er der så en af dem: Hvilke sensoriske symptomer? De fleste patienter har sensoriske symptomer i et eller andet omfang, og det kan være svært at skelne imellem hvorvidt, for eksempel en sensorisk forbedring skyldes FES eller en anden behandling. Og den anden siger: Ja, vi har fokus på det, ja, men måske øger vi bare opmærksomheden. Kan I forestille jer det? Altså, hvad er det vi øger rent faktisk? Er det rent faktisk, hvad hedder det, flow i de neuronale kanaler, eller er det dét, at vi siger: Okay, nu skal du altså virkelig kigge der over, fordi det man ved, når man træner, det er at patienten skal være aktiv medvirkende, når du spørger til metode. Patienten skal for alt i verden være aktiv tænkende, medfølende og.. Ja, alt passiv behandling, det skal vi reducere. Men vi kan ikke undgå nogle gange at mobilisere led, kan I forestille jer det, eller spænde muskler ud, hvor noget af det bliver lidt passivt. Men vi forsøger, at så lidt af det bliver passivt. Patienten skal selv tænke med og kigge der over og sådan noget, og der er elterapi godt og specielt EMG triggered. Det har vi ikke på nuværende tidspunkt.

I1: Nej, okay.

I2: Hverken her eller i jeres anden afdeling i en anden by?

U: Nej. Men vi er lige ved at købe nogle apparater, der kan. Ja.

I1: Ja, det har vi også set, altså det er vi stødt på flere gange i litteraturen, at det der ligesom..

U: Ja, det er det, der er bedst, så vidt jeg kan forstå fra litteraturen i forhold til FES. Og det skal vi altså bare have, så nu er de ved at købe et. Ja.

I1: Det sidste vi spørgsmål vi sådan har skrevet på her, det er i forhold til, hvis nu at FES motorisk og eller sensorisk har forårsaget en ændring, hvor lang tid skal patienten så have modtaget den her behandling før man begynder at se ændringen?

U: Ja, og det er et rigtig godt spørgsmål. Og der svarer K2 og K3: Det er meget forskelligt. Nogen de reagerer med det samme, andre nogen dage efter, og nogen patienter reagerer først efter uger af behandlingen. Igen afhænger det af skadens omfang og lokalisering, jo mindre skade, siger de (red: Kollegaerne), jo hurtigere effekt. Det er også helt afgørende at FES er placeret på den rigtige måde. Husk, at FES bare et et supplement. Og den anden siger på samme måde: Der kan være øjeblikkelig respons efter en behandling, og der kan gå op til dage eller uger. Ofte står FES ikke alene men som et supplement til mange andre behandlingsformer, så det kan være svært at vurdere om fremgang skyldes spontan remission, fordi vi jo også har den akutte, styrketræning, konditionstræning, gangtræning, FES, NMES. Hvad det er vi gør. Og K1 svarer: Det er individuelt ud fra patientens skade. Så de svarer det samme.

Experimental Protocol for the Pilot Trial

E

E.1 Aims

The aims of the pilot trial were to:

- Identify the most optimal immobilization procedure for the experiment.
- Identify the most optimal order of the measurements during the experimental sessions.
- Identify a suitable cutoff frequency for the lowpass filter in the preprocessing of SEPs.
- Identify the minimum required number of SEPs.
- Verify the motor impact of short-term upper limb immobilization as previously described [Huber et al., 2006; Moisello et al., 2008; Bassolino et al., 2012].
- Explore the sensory impact of short-term upper limb immobilization.
- Examine if similar baselines were obtained from the two parts of the experiment.
- Identify the most optimal electrode sites from which the single SEP components of interest should be analyzed.

E.2 Material List

- For SEP recording
  - g.GAMMAcap
  - Measuring tape
  - Alcohol swaps
  - Abrasive paper
  - Marker
  - Syringe with unsharp tip
  - Conductive gel
  - g.HIamp EEG amplifier
  - Ambu Neuroline 700 single patient surface electrodes for electrical stimulation
  - NI USB-6221 24 DIO USB Multifunction I/O Device
  - Computer with the program g.recorder
  - Computer with the program Mr.Kick III
  - Comfortable chair
- For JPS test
  - Stopwatch
  - Comfortable horizontal surface for the subject to lie on as e.g. a bed
  - Camera that can be placed in the exact same position between sessions based on a construction of the following items:
    - Spirit level
    - String or tape
    - Four pencils
- For QST
  - Marker
  - Von Frey hairs (Somedic Senselab Aesthesiometer)
  - Ambu Neuroline 700 single patient surface electrodes
  - NI USB-6221 24 DIO USB Multifunction I/O Device
  - Computer with the program Mr.Kick III

  - Comfortable chair
- For modified JTHF test
  - Stopwatch
  - Horizontal board at table height with markings indicating the placements of included items
  - Items from the Jebsen Test of Hand Function test kit from Patterson Medical Ltd:
    * Five cards
    * One spoon
    * Five kidney beans
    * One bowl
    * Four checkers
- For immobilization of the upper limb
  - Cotton glove
  - Gauze
  - Element to stabilize the wrist and arm (wooden spoon)
  - Arm sling

E.2.1 Subjects

Two healthy subjects were included in the pilot trial with mean age 25.5 years with SD=±0.7.

E.3 Measurements and Tests

E.3.1 Setup for Somatosensory Evoked Potentials Recordings

The recording of SEPs was based on the IFCN recommended standards [Nuwer et al., 1994].

- The subject was seated in the chair and initial information about the subject was obtained.
- The programs g.recorder and Mr.Kick III were opened on the computers and specific configurations were loaded.
  - In the program g.recorder, the configurations included choosing a virtual ground, selection of the trigger, activating 32 electrodes of the 64 available and setting the sample frequency to 9600 Hz.
  - In the program Mr.Kick III, the configurations included setting the number of stimulations to 500, which was repeated four times in order to obtain 2000 epochs, with a frequency of 2.85-3.33 Hz.
- The EEG cap was placed on the head of the subject by identifying the location of the central point Cz. The electrode placement followed the 10-20 system, as described in subsection 3.2. The cap was then connected to the EEG amplifier.
- The skin on each earlobe was prepared with abrasive paper and alcohol wipes.
- A reference electrode was placed on each earlobe.
- The EEG electrodes were filled with gel and the scalp area under each included electrode was rubbed with the tip of the syringe until the impedance of the electrodes was below 30 kΩ [Emmerling, 2017]. This was checked in the g.recorder program.
- The skin on the wrist of the non-dominant hand was prepared with abrasive paper and alcohol wipes.
- Two stimulating electrodes were placed on the wrist: The cathode was placed approximately 2 cm proximal to the wrist crease and the anode was placed on the wrist crease.
- The stimulating electrodes from the wrist were linked to the stimulator.

After the initial setup of the equipment and preparation, the motor threshold for visible thumb activity was identified.

- The electrical stimulator was set to send a 200 μsec square pulse with an amplitude of 1.0 mA.
- Single impulses were transmitted to the median nerve. The intensity was gradually increased with approximately 1.0 mA until visible thumb twitches of 1-2 cm end movement were obtained.
- When the specific threshold was reached, the intensity of the impulses was increased with 1.0 mA. This intensity was used for electrical stimulation throughout the SEP recording.

After the preparation and setup was finished, the actual recording was performed.

E.3.2 Experimental Procedure for Somatosensory Evoked Potentials Recording

- The channels were viewed in g.recorder and the impedances of the electrodes were controlled again.
- The subject was asked to relax, keep the upper limbs relaxed and placed in a supinated position, and to focus on a central fixing point in front of them.
- The recording was started.
- After 500 stimulations were given to the subject, the examiner checked that the subject was still doing fine. Then another 500 stimulations were given. This was repeated until 2000 SEPs were obtained.
- The data was recorded by g.recorder and stored as .HDF5-files on the computer.
- After obtaining the 2000 SEPs, the EEG-recording was ended and the cap was removed from the subject. The electrodes for electrical stimulation were not removed, as they were used later for the QST.

E.3.3 Setup for Joint Position Sense Test

- The four pencils were hanged from the ceiling in a horizontal construction, checked with a spirit level, 95 cm above the surface of the bed. This was done in order to have a standardized point from which the picture of the subject's upper limbs could be taken. This setup is illustrated in figure E.1
- The subject was instructed to lie supine on a bed, with the head placed at a specific place and bending the knees similarly to the JPS test in the case report by Bustamante et al. [2016].

(a) *(b)*

Figure E.1: *The four pencils were taped together to make a straight surface and hanged horizontal from the ceiling. This was checked with a spirit level (a). They were placed 95 cm above the bed in a place which was approximately above the head of the subject (b).*

E.3.4 Experimental procedure for Joint Position Sense Test

- The subject was instructed to close the eyes.
- The examiner moved the non-dominant upper limb and placed it so that the shoulder, elbow and wrist were in specific angles.
- The examiner asked the subject to place the dominant upper limb in the same position as the non-dominant upper limb with the eyes closed. The stopwatch was started.
- When the subject was convinced that the dominant upper limb was in the same position, the stopwatch was stopped and a picture was taken of the subject.

E.3.5 Setup for Quantitative Sensory Testing

- The QST with Von Frey hairs for identifying the tactile detection threshold was performed followed by a test of the ability to sense electrical stimulation.
- For the QST subtest involving electrical stimulation:
  - The electrodes used for providing electrical stimulation for SEPs were once again connected to the electrical stimulator NI USB-6221.
  - The computer with the program Mr.Kick III was prepared by setting the stimulation up as single squared pulses of 200 μsec and with an amplitude of 1.0 mA.

E.3.6 Experimental procedure for Quantitative Sensory Testing

- A mark indicating the center of the back of the non-dominant hand was drawn on the subject.

- The subject was instructed to close the eyes and relax and place the non-dominant hand on the arm rest in a prone position.
- Starting from the hair with the smallest length and diameter, each hair was pressed against the back of the hand of the subject at a specific marked point until they bended in an s-shape. The contact time with the skin should be about 2 seconds.
- The subject gave notice when a specific hair provided a detectable tactile sensation (upper threshold). The number of the hair was noted.
- By using the staircase method, hairs with a decreasing length and diameter were again pressed against the back of the hand until the subject could not feel the hair anymore (lower threshold).
- When five upper and lower thresholds were obtained, the subtest with Von Frey hairs was ended.
- After the setup for detection of electrical stimulation, the subject kept sitting with the eyes closed in the chair and was given one impulse with an amplitude of 1.0 mA.
- Until the subjects gave notice that the electrical stimulation provided a detectable tactile sensation, the amplitude of the impulse was increased gradually by 0.1 mA.
- When the subject gave notice, the amplitude was noted (upper threshold).
- By using the staircase method, the amplitude of the electrical stimulation was decreased with 0.1 mA again until the subject could not feel it anymore (lower threshold).
- When three upper and lower thresholds for detection of electrical stimulation were obtained, the test with detection of electrical stimulation was ended.

E.3.7 Setup for the Modified Jebsen-Taylor Hand Function Test

- Five cards, a spoon, five kidney beans, a bowl and four checkers were placed on a horizontal board at table height in a standardized manner.
- The stopwatch was prepared for measurement.

E.3.8 Experimental procedure for the Modified Jebsen-Taylor Hand Function Test

The order of the modified JTHF test followed the order used in a study by Simonsen et al. [2017].

- The subject was instructed in how to perform the test:
  - With one hand, flip over five cards from left to right, spoon five kidney beans into a bowl, one at a time, and stack four checkers on top of each other.
- The subject was allowed to begin and the examiner started the stopwatch.
- When the subject had completed the three tasks, the stopwatch was stopped and the time was noted.
- The cards, beans, spoon and checkers were placed at a standardized manner again.
- The subject conducted the test three times for each hand, starting with the dominant hand.

E.3.9 Immobilization of Non-Dominant Upper Limb

Two different procedures for the immobilization were used for the pilot trial in order to evaluate which one that was more comfortable for the subject. Each subject included in the pilot trial underwent both immobilization procedures.

Procedure 1

- A cotton glove was placed on the non-dominant hand.
- One roll of gauze was wrapped in between the fingers and around the hand.
- The wooden spoon used for stabilizing the wrist and arm was placed with the head of the spoon at the tip of the fingers and the handle in the palm and along the wrist.
- Another roll of gauze was wrapped around the spoon, hand and arm.
- The limb was placed in an arm sling which was tightened around the shoulder and torso.
- The subject was instructed to keep the immobilized upper limb in this position and use it as little as possible within the next 24 hours. However, the subject was allowed to sleep without the sling tightened around the shoulder and torso, if it became too uncomfortable.

Procedure 2

- Identical to procedure 1, except that the wooden spoon was not included in this procedure.

E.4 Experimental Pipeline

The pipeline for the pilot trial for each subject was as follows:

- The measurements described above were performed for baseline.
- The non-dominant upper limb of the subject was immobilized for 24 hours with immobilization procedure 1.
- After the immobilization, the measurements were performed again.
- Three days later, the baseline measurements were performed again.
- The non-dominant upper limb of the subject was immobilized for 24 hours with immobilization procedure 2.
- After the immobilization, the measurements were performed again.

Experimental Protocol for the Experiment F

F.1 Aim

The aim of the experiment was to examine how a single session of functional electrical stimulation alters proprioception and tactile sensation in a hemiparetic stroke model based on upper limb immobilization. Given that alterations occurred, the aim was furthermore to examine which associations that could be found between the neurophysiological and clinical measurements.

F.2 Material List

- For QST
  - Marker
  - Von Frey hairs (Somedic Senselab Aesthesiometer)
  - Ambu Neuroline 700 single patient surface electrodes
  - NI USB-6221 24 DIO USB Multifunction I/O Device
  - Computer with the program Mr.Kick III
  - Comfortable chair
- For SEP recording
  - g.GAMMAcap
  - Measuring tape
  - Alcohol swaps
  - Abrasive paper
  - Marker
  - Syringe with unsharp tip
  - Conductive gel
  - g.HIamp EEG amplifier
  - Ambu Neuroline 700 single patient surface electrodes for electrical stimulation
  - NI USB-6221 24 DIO USB Multifunction I/O Device
  - Computer with the program g.recorder
  - Computer with the program Mr.Kick III
  - Comfortable chair
- For JPS test
  - Stopwatch
  - Comfortable horizontal surface for the subject to lie on as e.g. a bed
  - Camera that can be placed in the exact same position between sessions based on a construction of the following items:
    - Spirit level
    - String or tape
    - Four pencils
- For modified JTHF test
  - Stopwatch
  - Horizontal board at table height with markings indicating the placements of included items
  - Items from the Jebsen Test of Hand Function test kit from Patterson Medical Ltd:
    - Five cards

    - One spoon
    - Five kidney beans
    - One bowl
    - Four checkers
- For immobilization of the upper limb
  - Cotton glove
  - Gauze
  - Arm sling
- For the TOT session
  - Bottle filled with cold water
  - Items for the FES-assisted TOT:
    - Dura stick premium 50x90 mm electrodes
    - Dura stick premium 32 mm round electrodes
    - Programmable FES stimulator (specially produced for AAU research purposes)

F.3 Measurements and Tests

F.3.1 Setup for Quantitative Sensory Testing

As the electrodes used for obtaining SEPs were also used for the QST test, the preparation and placement of these electrodes were performed prior to the QST test. Additionally, the motor threshold for visible thumb twitches was identified prior to the QST.

- The subject was seated in the chair and initial information about the subject was obtained.
- The skin on the wrist of the non-dominant hand was prepared with abrasive paper and alcohol wipes.
- Two stimulating electrodes were placed on the wrist: The cathode was placed approximately 2 cm proximal to the wrist crease and the anode was placed on the wrist crease.
- The stimulating electrodes from the wrist were linked to the stimulator.
- The computer with the program Mr.Kick III was prepared by setting the stimulation up as single squared pulses of 200 μsec and with an amplitude of 1.0 mA.
- Single impulses were transmitted to the median nerve. The intensity was gradually increased with approximately 1.0 mA until visible thumb twitches of 1-2 cm end movement were obtained.
- When the specific threshold was reached, the intensity of the impulses was increased with 1.0 mA. This intensity was used for electrical stimulation throughout the SEP recording.

The following setup was applied for the QST:

- The QST with Von Frey hairs for identifying the tactile detection threshold was performed followed by a test of the ability to sense electrical stimulation.
- For the QST subtest involving electrical stimulation:
  - The computer with the program Mr.Kick III was prepared by setting the stimulation up as single squared pulses of 200 μsec and with an amplitude of 1.0 mA.

F.3.2 Experimental procedure for Quantitative Sensory Testing

- The subject was instructed to close the eyes and relax and place the non-dominant hand on the arm rest in a prone position.

- Until the subjects gave notice that the electrical stimulation provided a detectable tactile sensation, the amplitude of the impulse was increased gradually by 0.1 mA.
- When the subject gave notice, the amplitude was noted (upper threshold).
- By using the staircase method, the amplitude of the electrical stimulation was decreased with 0.1 mA again until the subject could not feel it anymore (lower threshold).
- When three upper and lower thresholds for detection of electrical stimulation were obtained, the test with detection of electrical stimulation was ended.
- After the test involving electrical stimulation, the subject kept sitting in the chair.
- A mark indicating the center of the back of the non-dominant hand was drawn on the subject.
- The subject was again instructed to close the eyes and relax and place the non-dominant hand on the arm rest in a prone position.
- Starting from the hair with the smallest length and diameter, each hair was pressed against the back of the hand of the subject at a specific marked point until they bended in an s-shape. The contact time with the skin should be about 2 seconds.
- The subject gave notice when a specific hair provided a detectable tactile sensation (upper threshold). The number of the hair was noted.
- By using the staircase method, hairs with a decreasing length and diameter were again pressed against the back of the hand until the subject could not feel the hair anymore (lower threshold).
- When five upper and lower thresholds were obtained, the subtest with Von Frey hairs was ended.

F.3.3 Setup for Somatosensory Evoked Potentials Recordings

The recording of SEPs was based on the IFCN recommended standards [Nuwer et al., 1994].

- The programs g.recorder and Mr.Kick III were opened on the computers and specific configurations were loaded.
  - In the program g.recorder, the configurations included choosing a virtual ground, selection of the trigger, activating 32 electrodes of the 64 available and setting the sample frequency to 9600 Hz.
  - In the program Mr.Kick III, the configurations included setting the number of stimulations to 500, which was repeated four times in order to obtain 1500 epochs, with a frequency of 2.85-3.33 Hz.
- The EEG cap was placed on the head of the subject by identifying the location of the central point Cz. The electrode placement followed the 10-20 system, as described in subsection 3.2. The cap was then connected to the EEG amplifier.
- The skin on each earlobe was prepared with abrasive paper and alcohol wipes.
- A reference electrode was placed on each earlobe.
- The EEG electrodes were filled with gel and the scalp area under each included electrode was rubbed with the tip of the syringe until the impedance of the electrodes was below 30 kΩ [Emmerling, 2017]. This was checked in the g.recorder program.
- The stimulating electrodes from the wrist were linked to the stimulator.

After the preparation and setup was finished, the actual recording was performed.

F.3.4 Experimental Procedure for Somatosensory Evoked Potentials Recording

- The channels were viewed in g.recorder and the impedances of the electrodes were controlled again.
- The subject was asked to relax, keep the upper limbs relaxed and placed in a supinated position, and to focus on a central fixing point in front of them.
- The recording was started.
- After 500 stimulations were given to the subject, the examiner checked that the subject was still doing fine. Then another 500 stimulations were given. This was repeated until 1500 SEPs were obtained.
- The data was recorded by g.recorder and stored as .HDF5-files on the computer.
- After obtaining the 1500 SEPs, the EEG-recording was ended.

F.3.5 Setup for Joint Position Sense Test

- The four pencils were hanged from the ceiling in a horizontal construction, checked with a spirit level, 95 cm above the surface of the bed. This was done in order to have a standardized point from which the picture of the subject's upper limbs could be taken. This setup is illustrated in figure E.1
- The subject was instructed to lie supine on a bed, with the head placed at a specific place and bending the knees similarly to the JPS test in the case report by Bustamante et al. [2016].

(a)

(b)

Figure F.1: *The four pencils were taped together to make a straight surface and hanged horizontal from the ceiling. This was checked with a spirit level (a). They were placed 95 cm above the bed in a place which was approximately above the head of the subject (b).*

F.3.6 Experimental procedure for Joint Position Sense Test

- The subject was instructed to close the eyes.
- The examiner moved the non-dominant upper limb and placed it so that the shoulder, elbow and wrist were in specific angles.
- The examiner asked the subject to place the dominant upper limb in the same position as the non-dominant upper limb with the eyes closed. The stopwatch was started.
- When the subject was convinced that the dominant upper limb was in the same position, the stopwatch was stopped and a picture was taken of the subject.

F.3.7 Setup for the Modified Jebsen-Taylor Hand Function Test

- Five cards, a spoon, five kidney beans, a bowl and four checkers were placed on a horizontal board at table height in a standardized manner.
- The stopwatch was prepared for measurement.

F.3.8 Experimental procedure for the Modified Jebsen-Taylor Hand Function Test

The order of the modified JTHF test followed the order used in a study by Simonsen et al. [2017].

- The subject was instructed in how to perform the test:
  - With one hand, flip over five cards from left to right, spoon five kidney beans into a bowl, one at a time, and stack four checkers on top of each other.
- The subject was allowed to begin and the examiner started the stopwatch.
- When the subject had completed the three tasks, the stopwatch was stopped and the time was noted.
- The cards, beans, spoon and checkers were placed at a standardized manner again.
- The subject conducted the test three times for each hand, starting with the dominant hand.

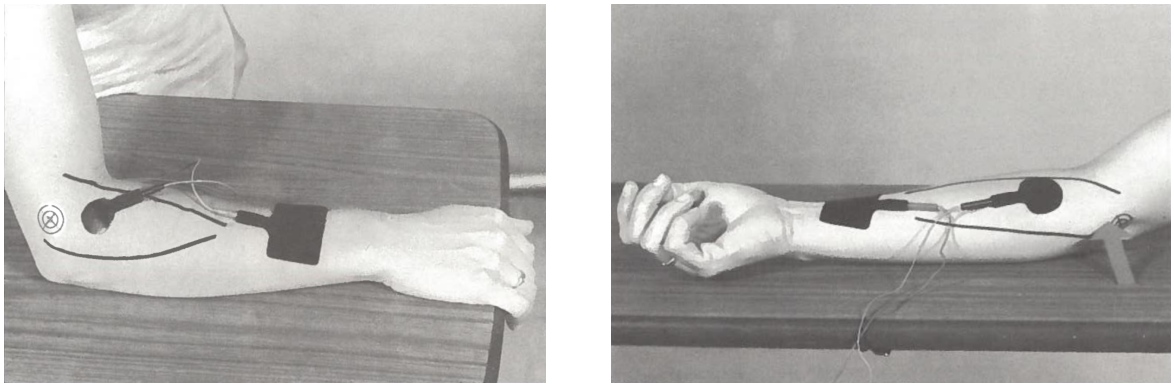
F.3.9 Immobilization of Non-Dominant Upper Limb

- A cotton glove was placed on the non-dominant hand.
- One roll of gauze was wrapped in between the fingers and around the hand.
- Another roll of gauze was wrapped around hand and arm.
- The limb was placed in an arm sling which was tightened around the shoulder and torso.
- The subject was instructed to keep the immobilized upper limb in this position and use it as little as possible within the next 24 hours. However, the subject was allowed to sleep without the sling tightened around the shoulder and torso, if it became too uncomfortable.

F.3.10 Setup for the Task-Oriented Therapy with Functional Electrical Stimulation Assistance

- The bottle with cold water was placed on a horizontal surface at table height.
- The FES electrode placement followed the principles illustrated in figure F.2 [Baker et al., 2000].
  - One of the dura stick premium 50x90 mm electrodes was used as a positive electrode and placed dorsally on the forearm, just proximal to the wrist joint.

  - One of the dura stick premium 32 mm round electrodes was used as a negative electrode and placed proximally on the forearm near the lateral condyle of the humerus.
  - One of the dura stick premium 50x90 mm electrodes was used as a positive electrode and placed distally on the forearm near the wrist.
  - One of the dura stick premium 32 mm round electrodes was used as a negative electrode and placed on the proximal third of the volar forearm.
- The FES stimulator was set to start channel 1 (wrist extension) after 1500 msec and last 1500 msec.
- The FES stimulator was set to start channel 2 (wrist flexion) after 3000 msec and last 1500 msec.
- Both channels were set to stimulate with a frequency of 50 Hz and the pulse width was 300 μsec.
- The intensity of the stimulations was defined individually for each subject by increasing the amount of mA until clear wrist extension and flexion were seen.



(a) *(b)*

Figure F.2: *Electrode placement for wrist extension (a) and wrist flexion (b) [Baker et al., 2000].*

F.3.11 Experimental procedure for the Task-Oriented Therapy with Functional Electrical Stimulation Assistance

- The subject was instructed in how to perform the task:
  - Lift the non-dominant upper limb and move it towards the bottle.
  - When the wrist extensors activate, help opening the hand.
  - When the wrist flexors activate, help closing the hand around the bottle and lift the bottle.
  - When the wrist flexion deactivates, put the bottle back down on the table.
  - When the wrist extensors activate again, help opening the hand and release the bottle.
  - When the wrist flexors activate again, pull the upper limb back down by the torso while you help closing the hand.
- The subject was allowed to begin when the examiner said so.
- The examiner started to count the number of repetitions.
- Another examiner activated the FES stimulator every time the subject started over with the task or had lifted the bottle up.
- When the subject had repeated the task 30 times, the session was completed.

F.3.12 Setup for the Task-Oriented Therapy without Functional Electrical Stimulation Assistance

- The bottle with cold water was placed on a horizontal surface at table height.

F.3.13 Experimental procedure for the Task-Oriented Therapy without Functional Electrical Stimulation Assistance

- The subject was instructed in how to perform the task:
  - Lift the non-dominant upper limb and move it towards the bottle.
  - Open the hand.
  - Close the hand around the bottle and lift the bottle.
  - Put the bottle back down on the table.
  - Release the bottle.
  - Pull the upper limb back down by the torso while closing the hand.
- The subject was allowed to begin when the examiner said so.
- The examiner started to count the number of repetitions.
- When the subject had repeated the task 30 times, the session was completed.

F.4 Experimental Pipeline

The pipeline for the experiment for each subject was as follows:

- The measurements described above were initially performed as a baseline measurement.
- The non-dominant upper limb of the subject was immobilized for 24 hours.
- After the immobilization, the measurements were performed again.
- Immediately after, the subject performed TOT either with or without FES assistance.
- The measurements were performed once more.
- Between three and seven days later, the baseline measurements were performed again.
- The non-dominant upper limb of the subject was immobilized for 24 hours.
- After the immobilization, the measurements were performed again.
- Immediately after, the subject performed TOT either with or without FES assistance. If the first TOT session was with FES assistance, this one would be without assistance and vice versa.
- The measurements were performed again.

Notice for Recruitment of Subjects G

The notice for recruitment of subjects is found on the next page.

Notice for recruitment of subjects

AALBORG UNIVERSITET

Project title: Exploring Sensory and Neuroplastic Changes Induced by Functional Electrical Stimulation in the Rehabilitation of Upper Limb Hemiparesis

We are two students writing our thesis at the master's program Biomedical Engineering and Informatics, who want to examine the sensory impact of functional electrical stimulation (FES) on healthy subjects after 24 hours immobilization of an arm. The immobilization can induce a short lasting, but harmless, condition, which is similar to the motor weakness in the upper limb experienced by some stroke patients. For the experiment, we want to include subjects without heart- and lung-diseases or any neurological disorders, notwithstanding gender, age, height, size, ethnicity etc. Pregnant women and persons with metal implants, e.g. pacemakers, cannot participate in the experiment.

As a participant you will have your non-dominant arm immobilized over two sessions lasting 24 hours each (with three to seven days between each session) by covering it with gauze and placing it in a sling. After each immobilization, we will ask you to perform a simple grasping task, performed with and without assistance from FES respectively. Whether FES assistance will be included after the first or second immobilization is random. Before and after both immobilizations and after performing the grasping exercise in each experimental session, we are going to do some measurements of your brain activity with a cap placed on your head, while you receive mild electrical stimulation of your wrist. The stimulation will not be painful. After the measurements of your brain activity, we are going to perform some different simple tests of your sense of touch and motor function, including your ability to replicate a position, which your arm has been placed in, to detect touch and mild electrical stimulation of applied on the hand, and how fast you can solve three tasks in which you have to move and place some objects. The sessions will last approximately 2-2½ hours each.

The experiment will be performed in the laboratory Fredrik Bajers Vej 7, D3-109, 9220 Aalborg Ø from week 17 to 20. If a day in a weekend is better for you, we can arrange that as well.

You will not receive any economical compensation for your participation in the experiment, but you will help generating new knowledge, which can be used for further research and potentially contribute to a better understanding of the rehabilitation of stroke patients. Furthermore, there will be sweets after each session.

If you are interested in the experiment or have any questions, please contact us:

Cecilie Topp
Mobile: XX XX XX XX
E-mail: X

Nikoline Kristensen
Mobile: XX XX XX XX
E-mail: X

Participant Information Sheet and Consent Form

H

The participant information sheet and consent form is found on the next page.

Participant Information Sheet

AALBORG UNIVERSITY
DENMARK

Study title:	**Exploring Sensory and Neuroplastic Changes Induced by Functional Electrical Stimulation in the Rehabilitation of Upper Limb Hemiparesis**
Locality:	**Fredrik Bajers Vej 7, D3-109** **9220 Aalborg E**
Main supervisor:	**Erika G. Spaich**
Co-supervisor:	**Federico Arguissain**

You are invited to take part in a study examining how functional electrical stimulation (FES) impacts your sensation and brain activity. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide, if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the possible benefits and risks might be and what will happen after the study ends. We will go through this information with you and answer any questions you may have.
If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document.

This document is 5 pages long, including the Consent Form. Please make sure you have read and understood all the pages.

What is the purpose of the study?

In this study, we will explore the sensory changes which occur during rehabilitation of stroke patients with FES. This will be done in healthy subjects, in which a short lasting condition similar to the weakness that can be experienced in one side of the body by stroke patients, is induced by upper limb immobilization.

What will my participation in the study involve?

You have been asked to participate in this study, because we are seeking participants without any neurological disorders or heart or lung diseases. Furthermore, participants must not have any metal implants as e.g. pacemakers or be pregnant. If you participate in this study, you will have your non-dominant arm immobilized for 24 hours by placing a glove on your hand, wrapping your hand and arm in gauze and placing the arm in a sling. This is done to ensure that you move your arm as little as possible, and to decrease the sensory input to the lower part of your arm. Before the immobilization of your arm, we will perform an assessment consisting of some measurements and tests. In this

document, these will be referred to as the first assessment. A similar assessment will also be done immediately after the immobilization and after a training session, referred to as the second and third assessment respectively.

The measurements and tests included in an assessment are described in the following:

- We will initially run a test to examine your ability to sense electrical stimulation applied to the wrist, by slowly changing the stimulation, while you sit with your eyes closed and verbally indicate when you can feel the stimulation.
- The next test examines your ability to sense touch on your hand. This is done by applying a light pressure with hairs of different thickness to the back of your hand while you sit with your eyes closed and inform the examiner, when you are able to feel the touch from a hair.
- Then we will examine how your brain responds to mild electrical stimulation applied to your wrist, which is going to be sufficiently strong to cause a small twitch in your thumb. The stimulation will not be painful. Your brain activity will be recorded from a cap placed on your head, in which gel is applied through holes in the cap over your scalp. The test involves you sitting passively while focusing your gaze on a fixing point in front of you while electrical stimulation is applied to your wrist. The stimulation can feel like you are being 'flicked' by a finger.
- Your ability to feel the position of your upper limbs will then be assessed. This is done while you are lying on a mattress with your eyes closed. The examiner will then place your non-dominant arm in a certain position over your head. Then you will be instructed to place your dominant arm in a similar position.
- The final test evaluates your fine motor skills of the dominant and non-dominant hand respectively by having you perform three different tasks where you have to flip cards, put beans into a jar with a spoon and finally stack checkers.

Before each measurement or test, we will once again explain what you are going to do, and you are welcome to ask questions again.

In the second assessment after the immobilization, we will initially run all the tests described above again. Then you will be asked to perform a simple grasping exercise, involving you sitting in front of a table, using your non-dominant hand to grasp and lift a bottle placed in front of you, then place it on the table again and finally withdraw your hand. You must perform the grasping exercise 30 times. The exercise can be assisted by FES, which means that a mild electrical stimulation will be applied to the muscles of the wrist to activate the muscles which you use to grasp and release the bottle. Otherwise, you will have to perform the exercise without any assistance. After performing the grasping exercise, the third assessment takes place with all the described measurements and tests performed once again.

3-7 days after your first immobilization period, you will undergo the second part of the experiment which is similar to the first part described above. The only difference is that if you performed the exercise with FES assistance in the first part, you will perform it without FES assistance in this part and vice versa.
You are free to withdraw from the study at any time if you find any of the procedures too uncomfortable or for any other reason.

We will record health information from you before starting the experiment to ensure that you are qualified for participation.

What are the possible benefits and risks of this study?

The risks associated with participation in this study are considered to be very low. The immobilization method is similar to the method used in case of an arm fracture and lasts considerably shorter. Furthermore, a study has shown that the effect of the short-time immobilization gradually fades shortly after the immobilization is stopped.
The electrical stimulation applied during the recording of brain activity and the FES assisted exercise is mild and not painful. The skin might be a little red and irritated after the stimulation, but this should only last a few hours.
The benefits associated with participation in the study is that you contribute to the understanding of how FES can help restoring sensation in stroke patients with sensory impairments.

Who pays for the study?

You will not incur any costs for being involved in this study and you will not receive any payment for being involved in the study.

What are my rights?

Participation in this study is voluntary, so you are free to decline to participate, or to withdraw from the research at any practicable time, without experiencing any disadvantage. You have the right to access information about you that is collected as part of the study, and you will be told of any new information about adverse or beneficial effects related to the study that becomes available during the study that may have an impact on your health.

What happens after the study or if I change my mind?

Your confidentiality will be respected at all times during this study. No material which could personally identify you will be used in any reports on this study. Your record will be securely stored by the examiners and your records will be kept for up to 1 year after the study and then destroyed. They will not be used for any other purposes that are not related to this study.

WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Cecilie Sophie Rosenkrantz Topp
e-mail: X
mobile: XX XX XX XX

Or

Nikoline Suhr Kristensen
e-mail: X
mobile: XX XX XX XX

Consent Form

Please tick to indicate you consent to the following

I have read, or have had read to me, the Participant Information Sheet, and I understand it.	Yes ☐	No ☐
I have been given sufficient time to consider whether or not to participate in this study.	Yes ☐	No ☐
I am satisfied with the answers I have been given regarding the study and I have been offered a copy of this consent form and information sheet.	Yes ☐	No ☐
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time.	Yes ☐	No ☐
I consent to the examiners collecting and processing my information, including information about my health.	Yes ☐	No ☐
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes ☐	No ☐
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.	Yes ☐	No ☐
I know who to contact if I have any questions about the study in general.	Yes ☐	No ☐
I understand my responsibilities as a study participant.	Yes ☐	No ☐

Declaration by participant:
I hereby consent to take part in this study.

Participant's name: _____

Signature: _____ Date: _____

Declaration by examiner:
I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Examiner's name: _____

Signature: _____ Date: _____

Results from the Experiment I

I.1 Somatosensory Evoked Potential Component Alterations

Threshold for Thumb Twitch [mA] for TOT Session without FES							
	S1	S2	S3	S4	S5	S6	S7
Baseline	3.5	4.0	4.0	4.8	3.6	3.5	4.0
Pre-TOT	5.0	5.3	5.0	4.6	5.6	4.5	3.4
Post-TOT	3.2	4.9	4.8	3.8	6.5	3.5	3.0

Table I.1: *The amplitude for the square pulse which induced a detectable thumb twitch for each subject at baseline, before (Pre-TOT) and after (Post-TOT) TOT without functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the threshold had increased compared to the previous measurement. When a cell is red, it indicates that the threshold had decreased compared to the previous measurement.*

Threshold for Thumb Twitch [mA] for TOT Session with FES							
	S1	S2	S3	S4	S5	S6	S7
Baseline	3.3	5.9	3.8	4.2	3.9	3.7	3.9
Pre-TOT	8.0	7.6	5.3	5.6	4.2	4.0	4.3
Post-TOT	4.9	7.2	3.9	4.2	4.0	3.2	3.8

Table I.2: *The amplitude for the square pulse which induced a detectable thumb twitch for each subject at baseline, before (Pre-TOT) and after (Post-TOT) TOT with functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the threshold had increased compared to the previous measurement. When a cell is red, it indicates that the threshold had decreased compared to the previous measurement.*

Somatosensory Evoked Potential - Component Amplitude [μV] for TOT Session without FES								
		S1	S2	S3	S4	S5	S6	S7
Baseline	N20	-0.17	-1.31	-1.02	-1.19	0.26	-1.56	-1.51
	N30	-0.37	-2.86	-1.92	-1.96	-0.41	-3.35	-2.57
	P45	2.02	3.49	0.51	0.10	1.61	0.62	3.10
	N90	-2.07	-2.28	-0.79	-0.14	-0.75	-3.60	1.08
Pre-TOT	N20	-1.08	-1.65	-0.86	-1.25	-0.18	-1.67	-0.73
	N30	-0.11	-3.63	-3.98	-1.53	-0.41	-3.87	-1.79
	P45	2.38	5.30	-0.09	0.10	1.10	3.78	3.35
	N90	-1.25	-2.50	0.54	0.06	-1.83	-2.00	0.44
Post-TOT	N20	-0.81	0.18	-0.25	-1.66	-0.81	-2.45	-0.26
	N30	-0.18	-1.47	-3.56	-1.69	-1.16	-1.72	-1.57
	P45	1.89	5.15	0.30	-0.64	1.71	1.70	2.26
	N90	-1.27	-2.07	0.94	0.45	-2.58	-0.66	-0.94

Table I.3: *The amplitude of the SEP components of interest for baseline, before (Pre-TOT) and after (Post-TOT) TOT respectively for each subject without functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the amplitude of the component had increased compared to the previous measurement. When a cell is red, it indicates that the amplitude of the component had decreased compared to the previous measurement. When a cell is white, it indicates that the amplitude remained unchanged compared to the previous measurement.*

Somatosensory Evoked Potential - Component Amplitude [μV] for TOT Session with FES								
		S1	S2	S3	S4	S5	S6	S7
Baseline	N20	-0.51	-0.92	-0.39	-1.15	-0.16	-3.02	-2.35
	N30	0.01	-2.52	-2.73	-1.99	-3.56	-2.09	-3.36
	P45	1.68	4.97	0.06	0.85	1.04	3.16	3.37
	N90	-0.60	-3.48	-0.78	1.08	-1.30	-4.76	-2.51
Pre-TOT	N20	-0.43	-0.93	-1.18	-1.56	0.38	-3.61	-0.27
	N30	-0.52	-2.73	-3.54	-3.07	-4.70	-1.98	-1.52
	P45	2.67	5.31	0.76	1.33	1.19	2.32	0.64
	N90	-2.09	-1.35	-1.09	0.09	-2.05	-2.85	-1.10
Post-TOT	N20	-0.32	-0.50	-1.03	-0.31	0.38	-3.47	-0.54
	N30	-0.75	-2.72	-2.70	-2.24	-4.79	-1.88	-1.13
	P45	1.98	5.41	0.51	1.22	0.98	1.84	0.04
	N90	-1.13	-1.90	-0.82	0.53	0.14	-1.79	-1.21

Table I.4: *The amplitude of the SEP components of interest for baseline, before (Pre-TOT) and after (Post-TOT) TOT respectively for each subject with functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the amplitude of the component had increased compared to the previous measurement. When a cell is red, it indicates that the amplitude of the component had decreased compared to the previous measurement. When a cell is white, it indicates that the amplitude remained unchanged compared to the previous measurement.*

Somatosensory Evoked Potential - Component Latency [msec] for TOT Session without FES								
		S1	S2	S3	S4	S5	S6	S7
Baseline	N20	17.19	13.23	14.90	14.90	16.56	21.56	15.42
	N30	30.31	34.06	32.92	30.83	25.81	31.98	32.08
	P45	53.96	55.83	64.17	43.65	57.81	42.19	60.10
	N90	85.10	96.35	85.73	83.54	96.15	86.04	86.98
Pre-TOT	N20	16.88	13.65	14.69	14.06	16.77	20.31	15.31
	N30	37.08	33.02	33.75	30.73	26.88	32.08	32.81
	P45	55.94	57.29	66.56	43.65	60.73	42.40	66.67
	N90	84.48	95.10	83.23	83.23	92.29	88.75	94.69
Post-TOT	N20	17.19	12.92	14.38	13.96	16.88	20.42	15.31
	N30	33.33	36.15	32.71	32.90	33.75	33.13	32.60
	P45	55.52	58.75	64.90	43.75	59.38	43.44	66.04
	N90	89.58	92.08	91.56	85.94	83.85	90.21	93.96

Table I.5: *The latency of the SEP components of interest for baseline, before (Pre-TOT) and after (Post-TOT) TOT respectively for each subject without functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the latency of the component had increased compared to the previous measurement. When a cell is red, it indicates that the latency of the component had decreased compared to the previous measurement. When a cell is white, it indicates that the latency remained unchanged compared to the previous measurement.*

Somatosensory Evoked Potential - Component Latency [msec] for TOT Session with FES								
		S1	S2	S3	S4	S5	S6	S7
Baseline	N20	17.50	13.85	15.00	14.17	16.67	21.67	13.85
	N30	30.63	33.85	31.35	30.00	32.71	30.21	31.88
	P45	55.10	56.35	62.50	44.79	57.08	42.50	55.00
	N90	89.38	95.00	85.00	83.33	86.67	89.27	84.06
Pre-TOT	N20	17.08	13.75	15.00	25.10	16.98	21.77	15.21
	N30	33.44	31.67	34.17	31.88	34.17	32.92	32.81
	P45	57.50	58.23	66.67	48.23	58.23	42.29	51.46
	N90	88.23	94.27	96.04	85.52	84.17	88.54	84.38
Post-TOT	N20	17.50	15.31	14.79	13.96	16.67	22.08	15.52
	N30	35.31	33.13	31.98	32.40	33.96	32.81	31.92
	P45	56.77	55.94	65.73	43.13	58.23	42.08	50.21
	N90	92.08	96.25	93.23	84.38	87.29	86.15	85.63

Table I.6: *The latency of the SEP components of interest for baseline, before (Pre-TOT) and after (Post-TOT) TOT respectively for each subject with functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the latency of the component had increased compared to the previous measurement. When a cell is red, it indicates that the latency of the component had decreased compared to the previous measurement. When a cell is white, it indicates that the latency remained unchanged compared to the previous measurement.*

I.2 Joint Position Sense Alterations

Joint Position Sense Test - Difference between Left and Right Upper Limb [°] for TOT Session without FES								
		S1	S2	S3	S4	S5	S6	S7
Baseline	Shoulder	11	3	13	8	8	-8	-2
	Elbow	14	3	3	17	9	2	2
	Wrist	-8	6	6	6	-13	0	8
Pre-TOT	Shoulder	13	6	24	6	14	-2	-6
	Elbow	12	-8	3	19	8	0	-3
	Wrist	-8	19	-13	17	-9	-6	8
Post-TOT	Shoulder	22	16	3	7	10	-4	4
	Elbow	26	15	-2	8	7	8	2
	Wrist	3	6	11	7	-16	2	10

Table I.7: *The angular difference between the dominant and non-dominant upper limb for the JPS test for each subject at baseline, before (Pre-TOT) and after (Post-TOT) TOT without functional electrical stimulation (FES) assistance. It was calculated by subtracting the angle of the joints from each upper limb. A negative value indicates that the angle of the dominant upper limb was larger than the angle of the non-dominant, immobilized upper limb and vice versa. When a cell is green, it indicates that the angular difference relative to 0 had increased compared to the previous measurement. When a cell is red, it indicates that the angular difference relative to 0 had decreased compared to the previous measurement. When a cell is white, it indicates that the angular difference remained unchanged compared to the previous measurement.*

Joint Position Sense Test - Difference between Left and Right Upper Limb [°] for TOT Session with FES								
		S1	S2	S3	S4	S5	S6	S7
Baseline	Shoulder	18	5	1	3	-3	-1	-1
	Elbow	11	6	-1	2	9	-7	2
	Wrist	-10	3	-3	-2	3	-9	6
Pre-TOT	Shoulder	21	6	-3	7	3	10	1
	Elbow	15	10	6	4	5	-5	6
	Wrist	-7	-8	9	8	-4	0	8
Post-TOT	Shoulder	13	9	2	-6	13	6	4
	Elbow	6	7	6	1	1	5	-2
	Wrist	-4	-3	7	3	-14	-8	12

Table I.8: *The angular difference between the dominant and non-dominant upper limb for the JPS test for each subject at baseline, before (Pre-TOT) and after (Post-TOT) TOT with functional electrical stimulation (FES) assistance. It was calculated by subtracting the angle of the joints from each upper limb. A negative value indicates that the angle of the dominant upper limb was larger than the angle of the non-dominant, immobilized upper limb and vice versa. When a cell is green, it indicates that the angular difference relative to 0 had increased compared to the previous measurement. When a cell is red, it indicates that the angular difference relative to 0 had decreased compared to the previous measurement. When a cell is white, it indicates that the angular difference remained unchanged compared to the previous measurement.*

Joint Position Sense Test - Time [sec] for TOT Session without FES							
	S1	S2	S3	S4	S5	S6	S7
Baseline	7.47	6.45	13.93	8.39	16.75	9.90	2.17
Pre-TOT	7.07	4.85	9.95	8.12	11.25	15.86	3.27
Post-TOT	8.97	4.97	9.49	8.17	15.70	14.51	2.36

Table I.9: *The time it took for each subject to place the dominant upper limb during the JPS test at baseline, before (Pre-TOT) and after (Post-TOT) TOT without FES assistance. When a cell is green, it indicates that the time period had increased compared to the previous measurement. When a cell is red, it indicates that the time period had decreased compared to the previous measurement.*

Joint Position Sense Test - Time [sec] for TOT Session with FES							
	S1	S2	S3	S4	S5	S6	S7
Baseline	8.77	4.98	10.21	9.64	8.12	10.55	4.99
Pre-TOT	6.67	4.69	11.26	11.33	13.48	14.33	3.41
Post-TOT	7.05	4.45	7.54	9.60	11.20	12.17	3.01

Table I.10: *The time it took for each subject to place the dominant upper limb during the JPS test at baseline, before (Pre-TOT) and after (Post-TOT) TOT with functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the time period had increased compared to the previous measurement. When a cell is red, it indicates that the time period had decreased compared to the previous measurement.*

I.3 Quantitative Sensory Testing Alterations

Detection Threshold for Von Frey Hairs [gf] for TOT Session without FES							
	S1	S2	S3	S4	S5	S6	S7
Baseline	3.27	4.52	6.12	3.76	4.72	3.00	7.27
Pre-TOT	4.37	7.38	6.25	3.61	5.30	7.48	4.96
Post-TOT	5.71	6.09	5.83	3.25	4.52	6.35	4.04

Table I.11: *The detection threshold for the Von Frey hairs for each subject calculated as the geometric mean of the upper and lower thresholds at baseline, before (Pre-TOT) and after (Post-TOT) TOT without functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the detection threshold had increased compared to the previous measurement. When a cell is red, it indicates that the detection threshold had decreased compared to the previous measurement.*

Detection Threshold for Von Frey Hairs [gf] for TOT Session with FES							
	S1	S2	S3	S4	S5	S6	S7
Baseline	4.61	7.17	6.46	5.64	3.09	7.36	5.66
Pre-TOT	4.45	7.24	5.40	4.28	3.34	6.01	5.63
Post-TOT	4.83	5.70	5.65	4.79	4.44	6.06	5.27

Table I.12: *The detection threshold for the Von Frey hairs for each subject calculated as the geometric mean of the upper and lower thresholds at baseline, before (Pre-TOT) and after (Post-TOT) TOT with functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the detection threshold had increased compared to the previous measurement. When a cell is red, it indicates that the detection threshold had decreased compared to the previous measurement.*

Detection Threshold for Electrical Stimulation [μA] for TOT Session without FES							
	S1	S2	S3	S4	S5	S6	S7
Baseline	674.40	493.24	447.21	662.66	482.39	447.21	447.21
Pre-TOT	748.33	470.15	447.21	470.15	478.47	447.21	447.21
Post-TOT	626.52	582.84	447.21	464.16	754.38	447.21	447.21

Table I.13: *The detection threshold for electrical stimulation for each subject calculated as the geometric mean of the upper and lower thresholds at baseline, before (Pre-TOT) and after (Post-TOT) TOT without functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the detection threshold had increased compared to the previous measurement. When a cell is red, it indicates that the detection threshold had decreased compared to the previous measurement. When a cell is white, it indicates that the detection threshold remained unchanged compared to the previous measurement.*

Detection Threshold for Electrical Stimulation [μA] for TOT Session with FES							
	S1	S2	S3	S4	S5	S6	S7
Baseline	545.16	418.85	447.21	527.73	547.72	447.21	447.21
Pre-TOT	490.93	464.15	426.28	531.33	748.33	447.21	447.21
Post-TOT	464.16	447.21	447.21	447.21	731.86	447.21	447.21

Table I.14: *The detection threshold for electrical stimulation for each subject calculated as the geometric mean of the upper and lower thresholds at baseline, before (Pre-TOT) and after (Post-TOT) TOT with functional electrical stimulation (FES) assistance. When a cell is green, it indicates that the detection threshold had increased compared to the previous measurement. When a cell is red, it indicates that the detection threshold had decreased compared to the previous measurement. When a cell is white, it indicates that the detection threshold remained unchanged compared to the previous measurement.*

I.4 Modified Jebsen-Taylor Hand Function Test Alterations

Modified JTHF Test Time Ratios (unaffected[sec]/affected[sec]) for TOT Session without FES							
	S1	S2	S3	S4	S5	S6	S7
Baseline	0.98	0.94	1.01	1.01	0.95	0.80	0.94
Pre-TOT	0.86	0.78	0.93	0.72	0.92	0.70	0.76
Post-TOT	0.85	0.79	0.95	0.86	0.94	0.79	0.91

Table I.15: *The time for performing of the modified Jebsen-Taylor Hand Function (JTHF) test expressed as the ratio between the mean time for the unaffected (dominant) and affected (non-dominant) hand respectively, i.e. the mean time for the unaffected hand divided by the mean time for the affected hand. The more similar the performance of the hands was, the closer the value is to 1. A value below 1 indicates that the performance for the unaffected hand was faster than the affected. A value above 1 indicates that the performance for the affected hand was faster that the unaffected hand. This data for each subject at baseline, before (Pre-TOT) and after (Post-TOT) TOT respectively is for the TOT session without functional electrical stimulation (FES). When a cell is green, it indicates that the range relative to 1.00 had increased compared to the previous measurement. When a cell is red, it indicates that the range relative to 1.00 had decreased compared to the previous measurement.*

Modified JTHF Test Time Ratios (unaffected[sec]/affected[sec]) for TOT Session with FES							
	S1	S2	S3	S4	S5	S6	S7
Baseline	1.00	0.83	1.00	0.84	1.04	0.83	0.80
Pre-TOT	0.81	0.76	0.93	0.73	0.88	0.77	0.78
Post-TOT	0.91	0.82	0.97	0.83	1.11	0.82	0.84

Table I.16: *The time for performing of the modified Jebsen-Taylor Hand Function (JTHF) test expressed as the ratio between the mean time for the unaffected (dominant) and affected (non-dominant) hand respectively, i.e. the mean time for the unaffected hand divided by the mean time for the affected hand. The more similar the performance of the hands was, the closer the value is to 1. A value below 1 indicates that the performance for the unaffected hand was faster than the affected. A value above 1 indicates that the performance for the affected hand was faster that the unaffected hand. This data for each subject at baseline, before (Pre-TOT) and after (Post-TOT) TOT respectively is for the TOT session with functional electrical stimulation (FES). When a cell is green, it indicates that the range relative to 1.00 had increased compared to the previous measurement. When a cell is red, it indicates that the range relative to 1.00 had decreased compared to the previous measurement.*

I.5 Test for Normal Distribution

I.5.1 Baseline versus Data before TOT

Table I.17 depicts the results for the datasets from the session without FES assistance and table I.18 depicts the results for the datasets from the session with FES assistance.

Results from the Shapiro-Wilk Test for the Data prior to the Session **without** FES Assistance			
	P-value		
Data	Baseline	Pre-TOT	Test
Threshold for thumb twitch [mA]	0.080	0.415	Paired sample t-test
N20 amplitude [μV]	0.234	0.715	Paired sample t-test
N20 latency [msec]	0.219	0.295	Paired sample t-test
N30 amplitude [μV]	0.338	0.191	Paired sample t-test
N30 latency [msec]	0.219	0.321	Paired sample t-test
P45 amplitude [μV]	0.485	0.686	Paired sample t-test
P45 latency [msec]	0.412	0.233	Paired sample t-test
N90 amplitude [μV]	0.954	0.234	Paired sample t-test
N90 latency [msec]	0.020	0.057	Wilcoxon signed-rank test
JPS - angular difference for shoulder [°]	0.519	0.892	Paired sample t-test
JPS - angular difference for elbow [°]	0.057	0.981	Paired sample t-test
JPS - angular difference for wrist [°]	0.068	0.144	Paired sample t-test
JPS - time [sec]	0.925	0.927	Paired sample t-test
QST - Electrical stimulation [μA]	0.008	0.000	Wilcoxon signed-rank test
QST - Von Frey hairs [gf]	0.517	0.625	Paired sample t-test
Modified JTHF test (ratio)	0.050	0.354	Paired sample t-test

Table I.17: *The results from the Shapiro-Wilk test of each individual dataset, concluding whether data is normally distributed (P>0.05) or not (P<0.05). The data is for the TOT session without functional electrical stimulation (FES) assistance. The P-values indicating that data is not normally distributed are written in red for clarity.*

Results from the Shapiro-Wilk Test for the Data prior to the Session **with** FES Assistance			
	P-value		
Data	Baseline	Pre-TOT	Test
Threshold for thumb twitch [mA]	0.015	0.138	Wilcoxon signed-rank test
N20 amplitude [μV]	0.407	0.338	Paired sample t-test
N20 latency [msec]	0.078	0.189	Paired sample t-test
N30 amplitude [μV]	0.251	1.000	Paired sample t-test
N30 latency [msec]	0.635	0.421	Paired sample t-test
P45 amplitude [μV]	0.689	0.084	Paired sample t-test
P45 latency [msec]	0.245	0.833	Paired sample t-test
N90 amplitude [μV]	0.954	0.684	Paired sample t-test
N90 latency [msec]	0.384	0.322	Paired sample t-test
JPS - angular difference for shoulder [°]	0.037	0.602	Wilcoxon signed-rank test
JPS - angular difference for elbow [°]	0.875	0.579	Paired sample t-test
JPS - angular difference for wrist [°]	0.457	0.126	Paired sample t-test
JPS - time [sec]	0.123	0.347	Paired sample t-test
QST - Electrical stimulation [μA]	0.061	0.007	Wilcoxon signed-rank test
QST - Von Frey hairs [gf]	0.592	0.972	Paired sample t-test
Modified JTHF test (ratio)	0.048	0.361	Wilcoxon signed-rank test

Table I.18: *The results from the Shapiro-Wilk test of each individual dataset, concluding whether data is normally distributed (P>0.05) or not (P<0.05). The data is for the TOT session with functional electrical stimulation (FES) assistance. The P-values indicating that data is not normally distributed are written in red for clarity.*

I.5.2 Before and after TOT Data

Table I.19 depicts the results for the difference between the obtained results before and after TOT, with versus without FES assistance respectively.

Results from the Shapiro-Wilk Test for the Difference Pre- and Post-TOT either with or without FES			
	P-value		
Data	Excl. FES	Incl. FES	Test
Threshold for thumb twitch [mA]	0.808	0.108	Unpaired sample t-test
N20 amplitude [μV]	0.455	0.087	Unpaired sample t-test
N20 latency [msec]	0.451	0.000	Mann Whitney U test
N30 amplitude [μV]	0.120	0.254	Unpaired sample t-test
N30 latency [msec]	0.765	0.910	Unpaired sample t-test
P45 amplitude [μV]	0.865	0.846	Unpaired sample t-test
P45 latency [msec]	0.745	0.059	Unpaired sample t-test
N90 amplitude [μV]	0.653	0.630	Unpaired sample t-test
N90 latency [msec]	0.953	0.089	Unpaired sample t-test
JPS - angular difference for shoulder [°]	0.123	0.745	Unpaired sample t-test
JPS - angular difference for elbow [°]	0.990	0.196	Unpaired sample t-test
JPS - angular difference for wrist [°]	0.696	0.395	Unpaired sample t-test
JPS - time [sec]	0.132	0.717	Unpaired sample t-test
QST - Electrical stimulation [μA]	0.142	0.194	Unpaired sample t-test
QST - Von Frey hairs [gf]	0.046	0.463	Mann Whitney U test
Modified JTHF test (ratio)	0.132	0.014	Mann Whitney U test

Table I.19: *The results from the Shapiro-Wilk test of the difference for each individual dataset before (Pre-TOT) and after (Post-TOT) TOT either with or without functional electrical stimulation (FES) assistance, which concludes if the data is normally distributed (P>0.05) or not (P<0.05). The P-values indicating that data is not normally distributed are written in red for clarity.*