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by

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Armita received her Bachelor's and Master's degree in Biomedical Engineering from Amirkabir University, Tehran, Iran, in 2013 and 2017, respectively. She was also a part-time lecturer at Ruzbahan University and the National Organization for Development of Exceptional Talents, Mazandaran, Iran, between 2014 and 2018. Following her graduation, she received a Marie-curie Ph.D. fellow scholarship and started her Ph.D. at the Center for Neuroplasticity and Pain in the Neural Engineering and Neurophysiology research group at Aalborg University under the supervision of Professor Winnie Jensen. Her Ph.D. project was co-supervised by Andrew James Thomas Stevenson and Eugen Romulus Lontis, Aalborg University, Denmark. Additionally, external collaboration with S. Farokh Atashzar at New York University, and Herta Flor at Central Institute of Mental Health (CIMH), Mannheim, Germany, developed her knowledge and brought the opportunity to establish an international network with highly experienced researchers. Armita has had the chance to speak and present his work at several international conferences. Her main research interests include but are not limited to neurorehabilitation, pain, neuroscience, and biological signal processing.

ENGLISH SUMMARY

Following amputation, almost two-thirds of amputees experience painful sensations localized in or around the area of the amputated limb, termed phantom limb pain (PLP). Literature demonstrated anatomical and physiological changes at the peripheral, spinal, and central levels as possible mechanisms generating PLP. While the underlying mechanisms of PLP are not fully understood, several therapeutic interventions have been suggested with the goal of PLP alleviation. Transcutaneous electrical nerve stimulation (TENS) is one of these approaches as a non-invasive, safe, and inexpensive pain treatment. To improve rehabilitation efficacy, the literature has recently focus on alternative temporal patterns like burst and pulse width modulated (PWM) rather than classical TENS. However, the mechanism of modulated TENS on the cortical and corticospinal level, which might lead to improvement of the PLP alleviation, has not explored yet. The objective of this Ph.D. project was, therefore, to investigate possible cortical plasticity following modulated TENS.

The Ph.D. thesis consists of four studies. Studies I-III were carried out in healthy subjects, while a pilot study included one PLP patient. Study I was conducted to compare the effect of modulated TENS patterns with the classical TENS on the corticospinal (CS) activity. We assessed motor evoked potentials (MEP) elicited by transcranial magnetic stimulation (TMS). The results revealed significant facilitation of CS excitability and enhancement in the volume of the corticomotor map of the stimulated muscle following the PWM intervention, which were suggested as the possible desired effects to reduce PLP in the future. In Study II, we utilized somatosensory evoked potentials (SEPs) to investigate the possible alteration in cortical activity at the somatosensory cortex and the perceived sensation by PWM TENS intervention and compared with induced changes by classical TENS. Our findings showed that suppression of selected SEP features (N1 and P2 amplitude) following PWM TENS was associated with a greater perceived sensation reduction in average (not significantly). In Study III, the functional brain network from eight brain areas involved in pain processing was examined. The results demonstrated several significant changes in local and global brain network indices following the application of PWM TENS compared to classical TENS. Finally, in Study IV, it was evaluated if induced cortical plasticity by PWM TENS as was found in healthy subjects could also be found in a PLP patient. The findings indicated the same trend of changes in cortical response (suppression of SEP activity and oscillations) with the reduction in PLP.

In conclusion, this work provides further evidence for the potential of PWM TENS as the alternative pattern in PLP treatment.

DANSK RESUME

Efter amputation oplever næsten to treidedele smertefulde følelser lokaliseret omkring området af det amputerede lem, også kaldet fantomsmerter. I litteraturen er det tidligere vist at der sker både anatomiske og fysiologiske forandringer perifert, spinalt og kortikalt, der kan være mulige årsager til at fantomsmerter opstår. Selvom man i dag ikke forstår de underliggende mekanismer, så er der udviklet mange terapeutiske interventioner med det mål at lindre fantomsmerter. For eksempel er transcutan elektrisk nerve stimulation (TENS) en mulig intervention, da det er en ikke-invasiv, sikker og billig teknik. For at forbedre teknikken, har der i litteraturen været fokus på at teste alternative temporale stimulations mønstre, så som 'burst' og pulsbredde moduleret (PBM) stimulations mønstre fremfor klassisk TENS. Effekten af moduleret TENS på kortikal eller kortiospinalt niveau og hvordan det kan lede til lindring af fantomsmerter har endnu ikke været undersøgt. Formålet med dette Ph.D. projekt var derfor at undersøge effekten af moduleret TENS på den kortikale plasticitet.

Ph.D. afhandlingen består af fire studier. Studierne I-III blev gennemført med raske forsøgspersoner, og i Studie IV blev et pilotforsøg gennemført med en fantomsmerte patient. Studie I blev udført for at sammenligne effekten af moduleret TENS med klassisk TENS på kortio-spinal aktivitet (KS). Vi målte motor evokerede potentialer forårsaget af transcranial magnetisk stimulation. Resultaterne viste en signfikant facilitering af KS aktiviteten og en forøgelse af kortio-motor volume efter PBM interventionen. Det blev foreslået, at dette var en ønsket effekt ift. at reducere fantomsmerter i fremtiden. I Studie II målte vi somato-sensoriske evokerede potentialer (SEP) for at undersøge den mulige ændring af kortikal aktivitet i den somato-sensoriske kortex og den følelse som PBM TENS inducerede og sammenlignede med klassisk TENS. Vores resultater viste en suppression af SEP features (N1og P2) der korrelerede med en reduktion af den opfattede sensoriske følelse. I Studie III blev hjernens funktionelle netværk fra otte udvalgte områder relateret til smerte processering undersøgt. Resultaterne viste flere signifikante ændringer i lokale og globale netværk efter PBM TENS. I Studie IV blev det til sidst undersøgt om den inducerede kortikale plasticitet efter PBM TENS som blev fundet i raske forsøgspersoner kunne tilsvarende induceres i en patient med fantomsmerter. Resultaterne viste samme trend ift. kortikale ændringer (dvs. supression af SEP aktivitet) og en reduktion i fantomsmerter.

Som en konklusion, så har dette arbejde leveret mere evidens for PBM TENS som en mulig alternativ metode til fantomsmerte lindring.

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CHAPTER 1. INTRODUCTION

Phantom limb pain (PLP) was first described by Ambroise Pare, a French surgeon who was in close contact with injured amputated soldiers in 1552 (Finger et al., 2003). After several years of assigning this pain to the category of psychological disease, the International Association for the Study of Pain defined PLP as 'pain perceived as arising in the missing limb' (Bogduk & Merskey, 1994). Painful sensations perceived in or around the area of the removed limb may be characterized by, e.g., 'throbbing', 'squeezing', 'pressing', 'pricking', or 'burning' (Herta Flor, 2002b; T. S. Jensen et al., 1983). While the underlying mechanism of PLP remains to be fully clarified, it has been shown that changes at the peripheral, spinal, and central nervous systems levels are associated with PLP onset (Collins et al., 2018).

It is estimated that one out of 190 American citizens (approximately 1.9 million) are currently living with an amputated limb, and that occurrence is predicted to double by 2050 (Ziegler-Graham et al., 2008). Although the PLP prevalence has been reported to vary (50% to 80%), PLP influences the quality of amputees' life and emotional well-being negatively, and it can partially or entirely disturb the daily living activities of amputees (Collins et al., 2018; Urits et al., 2019). However, there is still no fully effective treatment, and finding novel effective treatments is therefore important. Several non-invasive interventions have been tested, including transcutaneous electrical nerve stimulation (TENS). The neurobiological effect of TENS depends on the electrical current characteristics, e.g., frequency and intensity (Chesterton et al., 2003; L. S. Chipchase et al., 2011; Peng et al., 2019). Due to the growing use of conventional (high-frequency, low-intensity) TENS in clinical trials (Gibson et al., 2019; Hu et al., 2014), recent studies focused on the underlying mechanism of this treatment. However, the importance of the temporal patterns of TENS are gaining more attention as a new dimension (Grill, 2018). The effectiveness of burst and pulse width modulated (PWM) patterns in pain relief has been determined in clinical studies (Bouafif & Ellouze, 2018; D. Tan et al., 2016). Even so, the impact of these interventions on the nervous system, which may cause pain alleviation, specially PLP relief, is still not known.

The focus of the present thesis was to provide new insights in the use of modulated TENS for PLP relief. Therefore, the induced changes by the application of PWM TENS are assessed in cortical, corticospinal pathways, and brain functional connectivity network to extract possible biomarkers supporting the effectiveness of modulated TENS in PLP relief.

CHAPTER 2. STATE-OF-THE-ART

2.1. PHANTOM LIMB PAIN

Phantom limb sensation (PLS) often appears following a limb loss due to traumatic injuries, e.g., car accidents, war wounds, or chronic vascular diseases like diabetes. PLS has also been linked to amputations caused by cancer, infection, and congenital disabilities (Sinha et al., 2011). Regardless of the event causing limb removal, most amputees report experiencing various sensations in the limb that was once present, namely touch, itching, warmth, and cold (Ketz, 2008; Kooijman et al., 2000). In addition to somatosensory experience, kinesthetic sensations, including size, position, and shape of the lost limb, are reported following amputation. Moreover, voluntary (e.g., making a fist) (Weinstein, 1998) and involuntary movement (e.g., occupying a posture) of the removed limb are also categorized in PLS.

Besides the non-painful phantom sensation, the majority of amputees experience painful sensations perceived in the stump or around the area of the lost limb, known as residual limb pain and phantom limb pain (PLP), respectively (Ahmed et al., 2017). PLP is frequently described by amputees as shooting, pricking, burning, tingling, or any combination of these sensations in the phantom limb (Herta Flor, 2002b). PLP onset may depend on anesthetic technique, amputation site, time since amputation, and pre-amputation pain. Moreover, depression, stress, and other emotional experience can be plays a triggering role in PLP exacerbation (Larbig et al., 2019; Richard A Sherman et al., 1989). PLP influences the quality of amputees' life and emotional well-being negatively. It can disturb the daily living activities of amputees partially or entirely, and the majority of patients have reported impaired personal hygiene, loss of appetite, loss of focus, and depression and anxiety symptoms (Petersen et al., 2019).

PLP is reported to begin in about half of all amputees within the first 24 hours after amputation, and for another 25%, PLP might occur one week after amputation (T. S. Jensen et al., 1983). In part of patients, phantom limb pain decreases and eventually disappears, but in some cases, it can persist for a lifelong with no change in severity and frequency. Even after 25 years, 70% of amputees still perceived painful sensations (Richard A. Sherman et al., 1984). In addition, it has been noted that PLP is more prevalent in females than males and more common among adult amputees or patients with upper limb amputation than in children or lower limb amputees (Bosmans et al., 2010).

Prevalence of PLP among amputees is reported in a varying range of 50% to 80% in the literature. Ephraim et al. showed that in the patient population of 914 amputees, 79.9% had PLP experience at least once, while 38.4% stated severe pain scored higher than 7 on the visual analog scale (VAS, range 0-10) (Ephraim et al., 2005). In a

prospective study performed on lower limb amputees, the mean pain intensity was scored 22 (3-82 range) on a VAS (ranging from 0 to 100) after six months of amputation (Nikolajsen et al., 1997). Pain onset frequencies are most commonly reported on a daily basis or at daily/weekly intervals. For instance, in a survey of 141 upper-limb amputees, the reported pain duration was seconds or a few minutes in 43%, several minutes to hours in 20%, and longer in the remaining amputees (Desmond & MacLachlan, 2010).

2.2. UNDERLYING MECHANISMS OF PHANTOM LIMB PAIN

Several different mechanisms have been proposed to explain PLP. However, PLP's neurological mechanism is not yet fully understood. Although the initial theories of PLP were rooted in psychological points, recent studies evidenced the underlying mechanism of PLP as a neurological disorder (Herta Flor et al., 2006; Makin & Flor, 2020; Raffin et al., 2016).

Amputation is known to result in a variety of chemical, physiological, and morphological changes in the central nervous system (CNS), spinal mechanism, and peripheral nervous system (PNS). Due to the heterogeneity of the phenomenon, the underlying mechanism of phantom limb pain is inherently difficult to explain, which has led to a wide range of explanations and theories (Collins et al., 2018; Herta Flor, 2002b). The PNS, spinal cord, and supraspinal mechanism have been reported as potential origins of PLP, which are summarized in the following sections.

PERIPHERAL MECHANISMS

Several clinical observations have demonstrated that phantom limb pain is caused by peripheral mechanisms (stump or central parts of the sectioned afferents). The theories about the role of PNS in PLP include, but are not limited to, the following reports.

Phantom pain is linked to muscle activity and the muscle temperature at the stump (Nikolajsen et al., 2000; R. A. Sherman & Bruno, 1987). There is an inverse association between pressure and phantom pain thresholds at the stump early after amputation. The fact that stump temperature is linked to phantom pain suggests a role of the sympathetic nervous system (R. A. Sherman & Bruno, 1987). In a study with 28 amputees, including 11 patients with phantom pain, nine with PLS, and eight with no phantom phenomena, Kats et al. found that in the amputees with phantom phenomena, the temperature at the stump was dramatically lower compared with their contralateral limb, but not in the amputees without phantom phenomena (Katz, 1992).

Phantom pain is linked to stump pathology and increased stump sensibility. A possible explanation of pain following severe nerve damage is the sprouting of nerves in the periphery fibers. A transection of a peripheral nerve will form a tangled mass at the nerve end, called a neuroma (Lago & Navarro, 2007). The formation of neuromas

typically leads to abnormal activity of sodium channels, such as hyperactivity of the injured nerves (Devor et al., 1989). Neuromas may lead to neuropathic pain by inducing abnormal evoked activity after peripheral nerve injury, such as PLP after amputation (Matzner & Devor, 1994).

The clinical observation of regional anesthesia and the role of peripheral afferent input on phantom pain reduction is still a topic of debate. Birbaumer et al. investigated the effects of regional anesthesia on phantom pain and cortical reorganization in 6 upper-limb amputees. They discovered that a blockade of the brachial plexus eliminated pain and altered the cortical reorganization in three phantom pain amputees. However, cortical reorganization remained unchanged in the three amputees whose pain was not relieved by brachial plexus blockade (Birbaumer et al., 1997). This suggests that peripheral afferent input plays a role in phantom pain. On the other hand, studies have reported the persistency of PLP following a neuroma nerve block. Their results demonstrated that although the touch-induced ectopic discharge and the touch-induced PLP were eliminated by anesthetic blocking the neuroma (using lidocaine), the spontaneous discharge and spontaneous PLP remained unchanged (Wu et al., 2002). As a result, spinal and supraspinal function factors have become more prominent in the last few decades as a possible explanation for PLP.

SPINAL MECHANISMS

Understanding mechanisms of PLP also includes the spinal level. Several studies have reported the contribution of spinal factors to PLP by assessing the PLP severity following spinal cord anesthesia (Schmidt et al., 2005).

The dorsal root is the root that enters the spinal cord and handles afferent sensory information. At the same time, the ventral root exits the spinal cord and carries the efferent motor information. Sapunar et al. reported that an enhancement of afferent sensory input to the dorsal root ganglion (DRG) would lead to PLP in amputees (Sapunar et al., 2012). Vaso et al. found a significant PLP relief in PLP patients following intraforamina nerve block, indicating the essential role of DRG in generating phantom limb pain (Vaso et al., 2014).

Moreover, although some dorsal horn projection neurons are excitatory, several neurons act as an inhibitory gate, preventing the brain from misinterpreting a sensation as painful when the body is not in danger (Melzack, 1996; Melzack & Wall, 1965). A severed or damaged limb leads to the loss of inhibitory signals sent to the spinal cord and, consequently, ectopic discharge in DRG, which increases pain sensation (Wall & Devor, 1983). Because amputation causes immediate disinhibition, this theory may be a better fit for the rapid onset of PLP than the neuroma theory (Herta Flor, 2002b). However, PLP has been seen in people who have no nerve injury, e.g., paraplegia, and in people who have had their spinal cord transection (Melzack et al., 1997). Moreover, PLP has been shown to last despite nerve/plexus blockade and anesthetic blocking of

the neuroma (Birbaumer et al., 1997; Ramachandran & Hirstein, 1998). As a result, recent PLP research has focused on the CNS as the source of PLP.

SUPRASPINAL MECHANISMS

While spinal plasticity has been interpreted as a process involved in PLP, supraspinal mechanisms have gained much attention as a rational explanation for PLP. The complex and vivid sensations associated with phantom phenomena (such as telescoping and spontaneous phantom movements) suggest cortical mechanisms are involved (Ehde et al., 2000; Montoya et al., 1997; Pezzin et al., 2000). In addition, other factors such as stress have been reported to increase phantom limb pain, whereas distraction and attention can help reduce phantom pain (Katz & Melzack, 1990; Kooijman et al., 2000; Pezzin et al., 2000).

Supraspinal changes are thought to be involved in several amputation observations, and here we discuss the various supraspinal factors such as cortical reorganization in greater depth. Cortical reorganization is identified as the cause in PLP theories involving the CNS. Several brain areas have been reported to be affected in phantom limb pain, including but not limited to the following areas. The primary somatosensory cortex (S1) (located within the post-central gyrus) is responsible for the somatotopic representation of the body and sensory discrimination of the peripheral nociceptive stimulation (Herta Flor & Andoh, 2017; Herta Flor et al., 2006). Also, the intensity of nociceptive input is believed to be recognized at the secondary somatosensory cortex (SII) (which lies adjacent to the SI) (Makin & Flor, 2020). Additionally, the primary motor cortex (MI) also plays an essential role in PLP (Herta Flor et al., 2006; Karl et al., 2001). M1 is located within the precentral gyrus, anterior to SI, and is responsible for controlling and executing limb movements. At the same time, the premotor cortex controls movement planning and handles movement's spatial and sensory guidance (Makin, Scholz, et al., 2015).

Significant cortical reorganization in SI has been reported in patients with hand amputation compared with healthy subjects (Makin, Scholz, et al., 2015). The lack of sensory input has been proposed as the mechanism for starting the reorganization. Several studies have reported the reorganization of S1, S2, premotor cortex, and M1 after limb amputation, demonstrating that the reorganization extent and PLP severity are positively correlated in amputees (H. Flor et al., 1995; Karl et al., 2001; Makin & Flor, 2020; Raffin et al., 2016). For instance, reorganization of the SI following limb amputation has been investigated, and results demonstrated the functional invasion of the somatosensory regions of the lips into a cortical representation of the amputated hand, suggesting that cortical shifting extent and PLP severity are strongly correlated (Makin & Flor, 2020).

Moreover, although phantom sensation has been shown to elicit by ipsilateral stimulation of the limb, contralateral stimulation could also evoke a phantom

sensation, which indicates the involvement of the interhemispheric structures in PLP (Giuffrida et al., 2010; Tilak et al., 2016).

Besides the reorganization and shift of cortical activity between the representative area of the removed limb and the neighboring area in the sensorimotor cortex, it has been hypothesized that PLP onset is associated with disturbances in somatosensory cortex activity (Herta Flor et al., 1997; Zhao et al., 2016). Facilitation in the activity of the SI is suggested as a cortical biomarker in the PLP mechanism. Flor et al. reported the enhancement of brain response to both noxious and non-painful stimuli in the somatosensory cortex of patients with chronic pain (Herta Flor et al., 1997).

2.3. TREATMENT APPROACHES FOR PHANTOM LIMB PAIN

Several interventions have been examined with the eventual goal of PLP alleviation, which are classified into pharmacological, invasive, and noninvasive treatments (Collins et al., 2018; Knotkova et al., 2012). Each category is described briefly in the following sections.

PHARMACOLOGICAL

There are clinical trials with reports positive results following pharmacological treatment (e.g., opioids and tramadol) in PLP management (Attal et al., 2010; Knotkova et al., 2012). For instance, morphine has been indicated to be beneficial for relieving the symptoms of PLP. Wu et al. also noted PLP alleviation in 50% of the PLP patients in their study following using morphine (Wu et al., 2002). Moreover, several articles have investigated the impact of antidepressants, anticonvulsants, neuroleptics, and muscle relaxants in PLP alleviation (Alviar et al., 2016; Wolff et al., 2011). However, the lack of supportive evidence regarding the effectiveness of pharmacological treatment in large population besides the temporary impact and frequently reported side effects such as dizziness, tiredness, nausea, shortness of respiration, and sedation reveal the importance of non-pharmacological conservative therapies.

INVASIVE, NON-PHARMACOLOGICAL TREATMENTS

Deep brain stimulation (DBS) and stump revision are examples of invasive treatments for PLP alleviation. Stump revision therapy is generally performed to help with prosthesis fitting and/or neuroma treatment (Tintle et al., 2012). Skin scarring, bone shape, or chronic ulcers prevent the prosthesis from fitting properly, and stump revision is known as a possible solution. The stump revision success rate is extremely high in case the pain has a known cause (generally stump pain) (Tintle et al., 2012). Stump revision procedures are time-consuming, accompanied by extensive surgeries, resulting in a slew of complications such as infection risks.

Furthermore, DBS intervention consists of deep brain electrode placement delivering an electrical stimulus to specific brain areas, either regulating abnormal activity (much like a pacemaker) or triggering the neurotransmitters (J. P. Nguyen et al., 2011). DBS has been shown as a beneficial treatment for chronic pain, Parkinson's disease, and depression when other treatments (e.g., medications) have failed to help (Daneshzand et al., 2018; Farrell et al., 2018). For instance, DBS of the sensory thalamus and periventricular grey (PVG) has been indicated to decrease PLP intensity up to 60% (Bittar et al., 2005). However, evidence suggests that the effectiveness of DBS is controversial, and future studies with a larger population are needed to verify the efficiency of DBS.

NON-INVASIVE, NON-PHARMACOLOGICAL TREATMENTS

Repetitive transcranial magnetic stimulation (rTMS)(Malavera et al., 2016; Nardone et al., 2019; Scibilia et al., 2018), peripheral electrical stimulation (PES)(Johnson et al., 2015; Mulvey et al., 2013; Tilak et al., 2016), and visual feedback (mirror-box therapy)(Diers et al., 2010; Foell et al., 2014; Tilak et al., 2016) are example approaches of non-invasive PLP treatment. Transcutaneous electrical nerve stimulation (TENS) as a subset of PES is discussed in detail later in the next section.

TMS requires delivering electrical stimuli induced by a magnetic field through the magnetic coil positioned over the scalp (Rossi et al., 2009; Rossini et al., 1994, 2015). Generally, it can be delivered as a single pulse (including paired-pulse stimulation) to study brain functionality or as a set of pulses (rTMS) to induce plasticity and with a therapeutic aim. Literature has shown that following several consecutive sessions of high (Di Rollo & Pallanti, 2011; Malavera et al., 2016) and low-frequency (Di Rollo & Pallanti, 2011; J. H. Lee et al., 2015; Töpper et al., 2003) rTMS, patients experienced transient pain relief, and the effect was prolonged in some cases (Di Rollo & Pallanti, 2011; Malavera et al., 2016; Nardone et al., 2019) . While rTMS is considered a successful chronic pain treatment, the efficiency of this intervention in PLP relief was just investigated in a series of case studies but not sham-control trials. In addition, the temporal durability of the induced effect by rTMS is a major limitation for the therapeutic use of this intervention.

Moreover, mirror therapy (MT) was suggested as an approach to compensate for the theorized motor/sensory input mismatch and eventual PLP alleviation goal (Diers et al., 2010; Ramachandran & Rodgers-Ramachandran, 1996; Tilak et al., 2016). MT entails placing a mirror in the sagittal (lateral) plane and moving the intact limb while looking in the mirror at its reflection, giving the impression that the missing limb is present. Mirror neurons are discussed as one of the main underlying mechanisms of MT (Foell et al., 2014).

Furthermore, acupuncture has been shown to help people with PLP in some cases (Hu et al., 2014). Besides that, case reports and studies with small sample sizes have

introduced many other treatments for PLP, e.g., electroconvulsive therapy, far-infrared rays, mental imagery, massage, ultrasound, and vibration (Bókkon et al., 2011; Huang et al., 2009; Lundeberg, 1985; MacIver et al., 2008; Rasmussen & Rummans, 2000).

Transcutaneous electrical stimulation

Recently, TENS has become widely popular as a non-invasive, safe, and inexpensive treatment for several neurological conditions. The potential of TENS intervention in pain relief has been examined in the animal and human-based literature (Báez-Suárez et al., 2018; DeSantana et al., 2008; Johnson et al., 2015; Khadilkar et al., 2008; Mulvey et al., 2013; Peng et al., 2019). Over therapeutic sessions with TENS, an electric current is delivered through electrodes placed on the skin surface.

Depending on TENS characteristics (including intensity, frequency, duration, and application site), the underlying mechanism and respective therapeutic effect of the treatment may differ (L. S. Chipchase et al., 2011; Lucy S. Chipchase et al., 2011; Faghani Jadidi et al., 2022; Jadidi et al., 2020; Mang et al., 2010; Peng et al., 2019). For pain alleviation, TENS parameters generally are divided into the following categories. 1) Conventional TENS with high frequency (50-120 Hz) and low intensity (above sensory threshold)(Leonard et al., 2010; Peng et al., 2019). It has been theorized that conventional TENS works by activating large-diameter fibers (Aβ), resulting in the prevention of nociceptive signal transmission (through Aδ and C fibers) by blocking the dorsal horn gate at the spinal level (Melzack & Wall, 1965). 2) Acupuncture TENS with a low-frequency rate (4-10 Hz) and high intensity (Han, 2003). Studies have shown that the action mechanism of this type of TENS resulting in pain analgesia is caused by endogenous release in the rostral ventral medulla (RVM) and periaqueductal gray (PAG) by noxious stimulus based on a phenomenon known as the diffuse noxious inhibitory control (Le Bars et al., 1992). In terms of cortical activity, studies have presented the decrease in sensory evoked potentials (SEPs) following both conventional and acupuncture TENS interventions associated with pain and sensory suppression (Peng et al., 2019; A. A. Zarei et al., 2021). However, a recent study revealed that conventional TENS leads to greater experimental pain reductions and cortical activity suppression than acupuncture TENS (Peng et al., 2019). Furthermore, alteration in cortical oscillation in different frequency bands (from theta to high-gamma) was suggested as a TENS-induced cortical biomarker accompanying pain relief (Ebrahimian et al., 2018; Peng et al., 2019; A. A. Zarei et al., 2022).

A series of clinical case studies determined the positive contribution of TENS sessions to PLP alleviation. Significant reduction in both stump and phantom limb pain were stated by patients following TENS sessions (Hu et al., 2014; Mulvey et al., 2014; Tilak et al., 2016). Within the EU project EPIONE at Aalborg University, non-painful sensations were evoked in the PL by 1-s bursts or by continuous surface electrical

stimulation of referred sensation areas (RSAs). In most subjects (upper limb amputees, lower limb amputees as well as brachial nerve damage patients), a significant temporary change in the perception of the PL was observed, and that also was associated with a reduction of PLP (W. Jensen, 2017). Longitudinal studies with repetition of TENS sessions (from month to a year) have also been revealed the effectiveness of TENS intervention (Hu et al., 2014). Interestingly, the meaningful effect of TENS treatment was reported in clinical studies, even when the stimulation was applied to the contralateral limb (Tilak et al., 2016). However, the lack of studies with placebo control groups and the small number of investigated patients are two key points that should be taken into consideration.

Modulated transcutaneous electrical stimulation

Over the last decades, researchers and clinicians have focused on new dimensions of stimulation to enhance the effectiveness of intervention sessions. The temporal pattern of TENS stimulation is a new approach to therapeutic innovation (Brocker et al., 2017; Chen & Johnson, 2009; Grill, 2018; D. Tan et al., 2016). In this regard, modulated TENS patterns are delivered to the skin instead of continuous non-modulated ones with the final aim of increasing rehabilitation efficiency. While the underlying mechanism of modulated TENS is not fully understood, minimizing the habituation effect (Cruccu et al., 2008) and dynamic neuron recruitment (D. W. Tan et al., 2014) might be possible explanations for different induced effects. For instance, it has been recently shown that intermittent TENS application can induce greater facilitation in corticospinal (CS) excitability compared to the continuous pattern (Faghani Jadidi et al., 2022). In addition, frequency modulated TENS varied in a range of 1-250 Hz (leading to various endogenous opioids release) has been examined on patients after abdominal surgery. Notable pain reduction has been reported following this TENS pattern compared to placebo and control groups (Tokuda et al., 2014).

Moreover, burst intervention is the popular temporal pattern in rehabilitation sessions (Buchmuller et al., 2012; De Ridder et al., 2013; Macedo et al., 2015). This pattern is delivered with high-intensity and low-frequency (2 - 5 Hz) modulated in high-frequency carrier waves (40-100 Hz)(DeSantana et al., 2008; Macedo et al., 2015; Sherry et al., 2001). Both gate control theory and opioid receptors activation contribute to pain alleviation mechanism by burst pattern. The effectiveness of burst pattern in pain study has been tested on patients with chronic back pain, and the lowest pain score was stated following this intervention compared to non-modulated intervention and placebo groups(De Ridder et al., 2013). In addition, burst stimulation was shown to induce PLP suppression when the stimulation was applied on the intact (contralateral) limb of lower limb amputees (Tilak et al., 2016).

Finally, pulse width modulated (PWM) is a new temporal pattern suggested and examined in chronic back pain patients (D. Tan et al., 2016). Patients reported the same level of pain alleviation following both PWM and conventional stimulation. The

result supports this pattern's efficiency with a more comfortable and natural perceived sensation due to dynamic and sequential axonal activation. This reveals the potential of this type of modulation to help PLP patients.

2.4. ASSESSMENT OF CORTICAL CHANGES FOLLOWING ELECTRICAL STIMULATION THERAPY

Neuroimaging approaches such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), TMS, and electroencephalography (EEG) have evidenced cortical alteration and reorganization following sensory and painful experiences (Dos Santos Pinheiro et al., 2016; Lenoir et al., 2020; Peng et al., 2019; Strelnikov et al., 2015), including PLS and PLP (Kew et al., 1994; Makin & Flor, 2020; Makin, Scholz, et al., 2015) and even intervention effectiveness for pain alleviation.

fMRI as a hemodynamic noninvasive imaging modality, detects the blood flow changes in the brain. Blood oxygen level-dependent (BOLD) fMRI is widely utilized to evaluate cortical reorganization due to the capability of relating activation to specific cortical structures (Buxton, 2013; Gore, 2003). Comparative analyses of the cortical activity of healthy subjects and amputees regarding possible reorganization following amputation have been conducted in several studies (Gunduz et al., 2020; Makin & Flor, 2020; Makin et al., 2013; Makin, Scholz, et al., 2015). Herta et al. demonstrated the shifted lip representation in the deprived somatosensory cortex into the hand area among upper limb amputees with phantom limb pain (PLP) using event-related BOLD fMRI. In the same study, it has been shown there is a correlation between the extents of reorganization in lip representation with PLP severity (Makin & Flor, 2020).

There are some drawbacks when BOLD fMRI is used for studying cortical differences, and the length of the period needed for measurement is the main one. In addition to the time dynamics of the biological system, signal-to-noise ratio (SNR) issues have a probability in prolonged experimentation paradigms. For alleviating the poor SNR, signal averaging is utilized by fMRI paradigms. For analyzing fMRI results, detection power (capability for detecting activation) and estimation efficiency (capability for estimating the hemodynamic response function) should be understood as described by Frank and Liu.

TMS AND MOTOR EVOKED POTENTIAL (MEP)

fMRI and PET both cause difficulties in discriminating neuronal firing rates (exciting vs. inhibiting effects) and distinguishing the temporal sequence of a phenomenon (poor temporal resolution). However, TMS is a non-invasive approach to probing focal neural excitability and provides data regarding the density and localization of cortical motor neurons with higher temporal resolution compared to the

aforementioned modalities (Rossi et al., 2009; Rossini et al., 2015). As recently explained in section 2.3, TMS stimulation is directly administered to the scalp through an intense and brief magnetic field in single and paired-pulse mode, allowing for investigation of the excitatory/inhibitory functionality and mapping the cortical representation areas placed under the coil by recording the evoked responses.

Motor evoked potentials (MEP) are induced by applying TMS stimulation at the hotspot, defined as a location eliciting maximal evoked response with the constant stimulation intensity. MEP amplitude is a non-invasive, highly variable measure of cortical and spinal excitability(Rossi et al., 2009; Rossini et al., 2015).

Recently, researchers have been focusing on alteration in the excitability of the CS tract following chronic pain onset (Chowdhury et al., 2022; Nardone et al., 2019; Rohel et al., 2021). For instance, Seminowicz et al. showed that those subjects who experienced corticomotor depression following injection of nerve growth factor stated higher pain levels than subjects with facilitation in CS activity. In a separate study, they examined the hypothesis that motor cortex excitability may be suppressed during muscle pain (Seminowicz et al., 2019). This study using paired-pulse TMS as an evaluation technique showed that experimental pain caused suppression in intracortical inhibition (ICI) and enhancement of intracortical facilitation (ICF)(Nardone et al., 2019; Seminowicz et al., 2019).

Moreover, TMS has been widely used to study the cortical reorganization in patients with chronic pain, including phantom limb pain, and provide evidence to optimize the rehabilitation of individuals with PLP in clinical trials. For motor cortex mapping, single TMS pulses are delivered at different sites on the scalp neighboring the hotspot (Grab et al., 2018; S. M. Schabrun et al., 2016). A recent systematic review of TMS motor mapping studies among amputees represented a shift in the center of gravity (CoG) of neighboring limb regions toward the deafferented limb regions as a cortical biomarker induced following amputations (Gunduz et al., 2020). In terms of treatment effectiveness, literature using transcranial direct current stimulation (tDCS) and PES to alleviate pain investigated the possible changes in the corticomotor map before and following interventions. The results revealed enhancement in the volume of the motor map of the target muscle after the combination of tDCS and PES was associated with significant pain relief (Siobhan M. Schabrun et al., 2014).

The present understanding of motor neuroplasticity has been considerably advanced by presenting TMS as a research method. The main strengths of the TMS modality are safety, rapid assessment, decent temporal (higher than PET and fMRI), and spatial resolution (higher than EEG). However, as a drawback of this approach, the induced electric current can spread away from the stimulation's focal point and result in lower spatial resolution compared to PET and fMRI. In addition, the investigation of cortical functionality by the TMS technique is limited to the motor cortex and the CS tract and not somatosensory cortex playing primary role in pain/sensory processing.

ELECTROENCEPHALOGRAPHY (EEG)

EEG is a noninvasive recording technique for neurophysiological assessment of the brain's electrical activities, which is able to accurately reflect the brain dynamics on a millisecond basis (highest temporal resolution compared to aforementioned modalities). EEG signals are collected by multiple electrodes positioned on the scalp (Niedermeyer, Ernst et al., 2005) . The cortex's pyramidal neurons with similar spatial orientation are considered the primary source of the EEG signal (Crosson et al., 2010). Recorded electrical signals on the scalp are the combination of electrical activity generated by different brain sources passing through several brain layers, including cerebrospinal fluid, skull, and scalp, and subsequently causing a phenomenon known as volume conduction (Nunez & Srinivasan, 2009).

The power spectrum of the resting EEG in individuals with chronic neurogenic pain was indicated with higher spectral power over the frequency range of 2~25 Hz compared to healthy individuals (control group). In addition, the theta EEG power experienced a reduction in the cases of pain following a thalamic surgery, and it approached normal values a year after the operation, which suggests a possible association between EEG power and the degree of neurogenic pain and the reduction of EEG power due to the pain relief (Sarnthein et al., 2006).

Although the low spatial resolution and volume conduction impose some limitations, EEG has some crucial advantages in comparison with other neuroimaging techniques. While PET and fMRI have poor time resolutions between minutes and seconds, the EEG modality is more advantageous than the available neuroimaging techniques due to combining high temporal resolution (millisecond), safety, and low-cost hardware requirements.

Another approach to assess the cortical activity is to segment the induced brain activity following external stimulations.

SOMATOSENSORY EVOKED POTENTIALS (SEP)

Evoked potentials are the brain event-related activity induced by an external stimulus (electrical, visual, thermal, etc.). Evoked potentials have been reported as a powerful method for investigating the somatosensory system's function, such as the nociceptive pathways (Herta Flor, 2002a; Mouraux & Iannetti, 2018). SEPs are electrical potentials produced in sensory pathways at spinal, cortical, or peripheral levels in reaction to peripheral stimulation. SEP could provide information about the cortical time and dynamic activity. Although SEP responses depend on the stimulus type and characteristics, the SEP components are generally described based on their polarities (positive (P), negative (N)), amplitudes, and latencies. This method has been used in studies with PLP patients to investigate somatosensory and cortical functionality (Herta Flor, 2002a, 2003; Liu et al., 2020). Alteration in somatosensory cortex activity

has been reported in PLP patients by assessing the short (<50 ms after stimulus) (Granata et al., 2018) and long term (>50 ms after stimulus) (D'Anna et al., 2017; Zhao et al., 2016) SEP components. Also, SEP has been used to evaluate the conventional TENS effect on cortical processing (Peng et al., 2019; A. A. Zarei et al., 2020, 2021).

Another approach to investigate the cortical alteration could be analyzing the SEP's dynamic activity. Oscillatory dynamics of signals are generally studied in different frequency bands ranging from delta (0.2-3 Hz), theta (4-7.5 Hz), alpha/mu (8-13 Hz), beta (14-30 Hz), and gamma (30-90) Hz. Previous studies have indicated that pain experience is associated with changes in spectral power in theta (Bjørk et al., 2009; Sarnthein et al., 2006; Vuckovic et al., 2014), beta (Stern et al., 2006), and delta (Sarnthein et al., 2006) bands. For example, Vuckovic et al. presented a significant enhancement of event-related desynchronization within 16-24 Hz frequency range among patients with central neuropathic pain compared to the able-bodied group (Vuckovic et al., 2014).

BRAIN FUNCTIONAL CONNECTIVITY

Brain functional connectivity can be defined as the correlations between spatially remote neurophysiological events extracted from neuroimaging methods (EEG and fMRI) (Friston, 1994). The information about the correlation between two time series of electrophysiological measurements can be assessed by computing the connectivity. In this regard, nodes are defined as the individual EEG scalp electrodes and brain area (region of interest, ROI) in the channel and source domain, respectively.

Methods to measure functional connectivity can be categorized as time and frequency-based algorithms (for review (Bastos & Schoffelen, 2016)). However, there is no ideal solution for estimating the functional connectivity as some limitations warrant caution with respect to the interpretation of the estimated connectivity (David et al., 2011). Volume conduction has been reported to play an important role in analyzing functional connectivity, especially when the measurements are established on non-invasive recordings (Schoffelen & Gross, 2009; Srinivasan et al., 2007). Volume conductance issues would lead to a wrong estimation (strong correlation) between EEG scalp electrodes, particularly with neighboring electrodes (Cornelis J. Stam et al., 2007). However, while the source localization methods have considered the volume conductance issue, analyzing the functional connectivity in the source domain has been suggested as a better solution for brain functional connectivity analysis (Dos Santos Pinheiro et al., 2016). Moreover, analyzing the functional connectivity across EEG frequency bands of theta (4-8Hz), delta (0-4Hz), alpha (8-13Hz), beta (13-32Hz), and gamma (32-60Hz) has been commonly presented in previous research, including pain field.

Correlation

Correlation is a time-domain functional connectivity analysis method that measures the linear relationship between two nodes and has been used in previous studies (Pizzagalli et al., 2003; Savva et al., 2019). The extent of a signal variance (activity of a node) which can be described by another node, is represented by the squared correlation coefficient (R^2). The temporal structure in the data is not considered by correlation, and the time activity of the nodes is treated as random variables.

Coherence

The phase synchrony is generally computed from the frequency representation of two-time series (epoched data) based on the amplitude and the phase of the oscillations (Leblanc et al., 2014; Miskovic & Keil, 2015; Sun et al., 2004). The coherence coefficient is a well-known frequency-based functional connectivity method to assess the phase synchrony of the pair-wise connection of two signals. This method is mathematically equivalent to the cross-correlation in the time-based functional analysis methods. Assume y and x as two signals at different nodes, and to obtain the coherence between y and x at frequency f, the ratio between the cross-spectrum of the two signals and their auto-spectra is used:

$$\boldsymbol{Coh_{xy}} = \frac{\left|\boldsymbol{S_{xy}(f)}\right|^2}{\boldsymbol{S_{xx}(f)S_{yy}(f)}}$$

Where $\boldsymbol{S_{xy}}$ is the cross-spectral density between two nodes (brain areas) activity, and $\boldsymbol{S_{xx}}$ and $\boldsymbol{S_{yy}}$ are the auto-spectral densities at each node.

In the EEG scalp electrode domain, coherence is highly affected for adjacent electrodes due to the instantaneous state of volume conduction to all electrodes (Bastos & Schoffelen, 2016). However, while this issue can be handled by a proper source localization method, considering the coherence as a functional connectivity method in the source domain has been suggested previously (Gysels & Celka, 2004).

Phase Synchrony

The phase-locking values (PLV) are computed from the frequency representation of two-time series (epoched data) based on the amplitude and the phase of the oscillations. The PLV is a well-known frequency-based functional connectivity method to assess if the pair-wise connection of two signals is phase-locked (Gysels & Celka, 2004; Lachaux et al., 1999). The Hilbert transform needs to apply to the signal to determine the signal's phase synchrony. Then, PLV is obtained as the mean value over all trials of the phase differences (Lachaux et al., 1999).

$$\boldsymbol{PLV_t = \frac{1}{N}\sum_{1}^{N}(\exp\left(j\Delta\varphi(t,n)\right))}$$

Consistent synchronization of two nodes across trials would lead to a PLV value of 1, while a PLV of 0 represents no pair-wise synchronization.

NETWORK ANALYSIS (GRAPH THEORY)

Recent studies in analyzing brain functional connectivity move beyond pair-wise functional connectivity, conceptualizing the brain as a network of interconnected regions (Colombo, 2013; Sporns, 2018). In this framework, complex behaviors can be assessed by considering the activities among all the brain regions. Graph theory has been shown as a powerful mathematical tool to understand the brain network by presenting a summarized view of a complicated system as the brain (for review (Farahani et al., 2019)).

Brian functional connectivity is represented by a collection of nodes (brain regions) and edges between node pairs as the pair-wise functional connectivity (Bassett & Sporns, 2017). The network can be considered a binary network by imposing a threshold and discretization of edges. While edges of a binary network represent the absence or presence of a connection, a binary network has also been used to simplify a complicated network (Lord et al., 2017; Newman & Girvan, 2004). Despite the application of binary networks in several studies in the literature (Achard & Bullmore, 2007; C. J. Stam et al., 2007), higher interest is paid to weighted (non-binary) network analysis (Haartsen et al., 2020; A. A. Zarei et al., 2022).

Several studies have investigated the pain-related brain regions and identified a complicated brain network involved in pain processing (the pain matrix) (Mouraux et al., 2011). The insular cortex and anterior cingulate cortex (ACC) have been shown to play an important role in the pain-processing brain network (Bolaños et al., 2013). Results from a study on chronic pain patients reported functional and structural abnormalities between and within these brain areas (Nickel et al., 2020; Stern et al., 2006; Ta Dinh et al., 2019).

Researchers have suggested a small-world architecture for human brain connectivity, which is described by two critical characteristics, including integration and segregation (Achard & Bullmore, 2007; Farahani et al., 2019; Lenoir et al., 2021). For quantifying the segregated processing potential, analyzing the local characteristics of the network (between nodes) has been suggested. However, global measures such as the average path length can assess the network's integration. Strength, efficiency, and clustering coefficient are the network indexes features which are explained below.

Strength is one of the basic structural features of a non-binary network and represents the extent of the interconnectivity of the nodes in a network (Guo et al., 2019; Yuan et al., 2013). The local strength of node ‘i’ is given as the sum of the edges’ values connected to the node. Additionally, network efficiency investigates the extent of functional integration and the efficiency of information flow in a network (Latora & Marchiori, 2001; Petruo et al., 2018; Watts & Strogatz, 1998). Nodal efficiency evaluates the capability of efficient information flow of a node. It is obtained as the average inverse of the length of the shortest path, capturing the shortest covered distance from one node to another. This index illustrates the node's ability to effectively share information with neighboring nodes (Harrington et al., 2015). From the global perspective, efficiency is considered as the average of inverse shortest path lengths between all pairs of ROIs.

The clustering coefficient is a network characteristic representing the degree to which nodes tend to cluster together (local cluster coefficient) (Milo et al., 2002). This characteristic assesses the extent of the network’s functional segregation by measuring the fraction of the node’s neighbors that are also connected around (Guo et al., 2019; J. M. Lee et al., 2020; Newman, 2004; Rubinov & Sporns, 2010). A high clustering coefficient indicates the availability of local cliques that form specialized functional units. The clustering coefficient can also be measured as a global metric for the whole graph by averaging the local values. Having node ‘i’ in the center of a fully interconnected cluster would lead to the clustering coefficient of 1. In contrast, the clustering coefficient of 0 depicts that node ‘i’ is not connected to neighbors.

CHAPTER 3. OUTLINE OF PH.D. WORK

3.1. THESIS OBJECTIVE

Although many PLP therapies have been tried or are currently being employed, the majority appear to only have a temporary impact or have been presented with limited evidence regarding their effectiveness. As a result, the importance of developing effective PLP treatments with longer-lasting effect keeps growing. TENS intervention is one of the approaches that revealed promising results. Recent work investigated the effect of conventional TENS on cortical activity and network. While the application of modulated TENS over clinical studies on chronic pain patients indicated noticeable results, the effect of these interventions on the CS and cortical level is still debated.

Therefore, the objective of the thesis was to investigate the neurobiological effects and possibly alteration in sensory/pain response by modulated TENS intervention.

3.2. SPECIFIC RESEARCH QUESTIONS AND SOLUTION STRATEGY

To address the thesis objective, the following specific research questions were formulated.

STUDY I. How do CS pathway activity and motor cortical map alter following modulated and non-modulated TENS?

As it is mentioned in chapter 2, reorganization in the MI cortex is believed as a significant contributor to chronic pain, specially PLP. The alteration in corticomotor excitability has also been reported following pain onset. While recent articles illustrated chronic pain relief associated with facilitation in the CS excitability and corticomotor map (reversing effect), this study aimed to provide evidence regarding possible alteration in CS tract and cortical motor map by modulated TENS.

Two modulated TENS patterns in pain therapy (PWM and burst) were selected based on the literature. Conventional TENS patterns were also included to compare the effect of frequency besides the modulation dimension on the activity of the CS tract and corticomotor map. Therefore, TMS was chosen as evaluation modalities, and the MEP signals were recorded before and after each intervention phase. Study I was conducted on healthy subjects.

Findings are reported in paper I: “Effect of Modulated TENS on Corticospinal Excitability in Healthy Subjects”, Armita Faghani Jadidi, Andrew James Thomas Stevenson, Ali Asghar Zarei, Winnie Jensen, and Romulus Lontis, Neuroscience, Volume 485, March 2022.

STUDY II. How does the modulated TENS intervention induce changes in somatosensory cortex activity and perceived sensation compared to alteration induced by non-modulated TENS?

The literature revealed a positive correlation between the effectiveness of conventional TENS in pain/sensation suppression and reduction of cortical activity. Therefore, the aim of the second study was to investigate the effect of modulated TENS on the cortical response of the SI cortex (SEPs) and perceived sensation.

A modulated pattern with the desired effects to potentially cause pain relief was selected based on the result of Study I. We recorded EEG signals and sensory information from healthy subjects and performed temporal and time-frequency analyses on SEP waves as a valid evaluation method for assessing plasticity in the somatosensory cortex.

Findings are reported in the paper II: "Alteration in Cortical Activity and Perceived Sensation following Modulated TENS", Armita Faghani Jadidi, Winnie Jensen, Ali Asghar Zarei, and Romulus Lontis. Submitted in Neuromodulation: Technology at the Neural Interface.

STUDY III. How does modulated TENS affect the functional cortical network among brain areas involved in sensory and pain perceptions? How distinguishable are the changes with the results following conventional TENS?

Literature has shown network reorganization in chronic pain patients, including patients with PLP. Moreover**,** previous works suggested the enhancement of functional connectivity between pain-related brain areas, including SI, SII, ACC, the medial prefrontal cortex (mPFC), and the facilitation of the network characteristic in these regions as a possible biomarker for analgesic effect of TENS. Therefore, the third study focused analyzing on cortical network alteration as an effect of applying modulated TENS.

The data collected in study II were utilized with different processing perspectives to address this question. The alteration in functional connectivity and cortical network following modulated and conventional TENS patterns were compared up to an hour after the intervention phase.

Findings are reported in paper III: "From Pulse Width Modulated TENS to Cortical Modulation: Based on EEG Functional Connectivity Analysis", Armita Faghani Jadidi, Winnie Jensen, Ali Asghar Zarei, Romulus Lontis, and Farokh Atashzar. (In preparation).

STUDY IV: How does modulated TENS intervention affect cortical response and pain profile in an upper limb amputee?

As a last step, we tried to verify our findings in a clinical trial with an upper limb amputated patient with PLP. Experimental sessions were conducted with an amputee, and EEG signals were recorded before and after modulated TENS sessions. The changes in SEP signal recorded from affected hemisphere and perceived pain level were evaluated and compared with findings obtained from Study II.

Findings are reported in paper IV: "Altered Cortical Activity by Modulated TENS in Upper Limb Amputee, A Case Study," Armita Faghani Jadidi, Winnie Jensen, Ali Asghar Zarei, Romulus Lontis (In preparation).

CHAPTER 4. METHODOLOGICAL APPROACHES

4.1. PROCEDURES AND STUDY DESIGN

The design of each study is explained briefly in the following sections (visual overview in Fig.1). The experimental procedures were approved by North Denmark Region Committee on Health Research Ethics (N-20190016). All participants signed the consent form after written and verbal instruction. The design of the first study was separately and conducted on different populations compared to Study II and III based on the defined goals. Meanwhile, the procedure of Study IV had been planned similarly to Study II with minor modifications to optimize the experimental design for patient's situation.

STUDY I

Experimental setup

Forty-four right-handed healthy subjects were tested in this study. They were divided into four groups which eleven subjects in each group received a specific TENS pattern on the right median nerve for 30 min. Measurements, including the activity of the CS pathway of the predefined muscles and cortical motor map of the target muscle (by TENS), were performed before (Pre), immediately (Post), and 30 min (Post30) after the intervention phase.

TENS

Four selected TENS patterns were randomly assigned between different groups. The characteristics of TENS interventions (intensity, frequency, and pulse width) were adjusted based on literature showing significant pain reduction following TENS therapy (Hu et al., 2014; Johnson et al., 2015; Mulvey et al., 2014, 2013). Two conventional TENS patterns were delivered with frequencies 100Hz (NMHF) and 40 Hz (NMLF) with 500 µs pulse width to consider the frequency effect on induced changes. Moreover, PWM TENS, as the third pattern, was generated with a 100 Hz frequency rate and pulse width varying between 0 to 500 µs (modulated in 1 Hz sinusoidal wave)(D. Tan et al., 2016). Burst modulated was programmed as the last pattern while groups of five pulses with 100 Hz were delivered every 250 ms (4 Hz)(Macedo et al., 2015). Subjects received the intervention through pair of oval electrodes (Dura Stick, 4 x 6 cm) for 30 min on their right median nerve with 20 s on 10 s off repetition (Lagerquist et al., 2012; Mang et al., 2010). The intensity of each intervention was individually adjusted at 80 % of the discomfort threshold (measured by staircase procedure) to induce a strong but comfortable sensation(Faghani Jadidi et al., 2022; Manresa et al., 2018; A. A. Zarei et al., 2021).

Data recording

Ten single TMS pulses were delivered by a figure-of-eight shaped coil (MagVenture, MC-B70) connected to a Magstim 200 stimulator at each hotspot of right and left abductor pollicis brevis (APB) muscles to investigate the alteration in excitability of the CS tract. Induced MEP waves followed by stimulation of left motor cortex hemisphere were recorded by two pairs of the surface electrode (Ambu Neuroline 720) placed on the right APB and right abductor digiti minimi (ADM) as median nerve innervated and non-target muscles. MEP signals from left APB (TMS stimulation at the contralateral hemisphere) were also collected as control muscle. Furthermore, the corticomotor map of the target muscle (right APB) was extracted before and after each TENS intervention by applying single TMS pulses in pseudo-random order at sites (over 9x9 cm grid) neighboring hotspot with 1x1 spatial resolution oriented towards the cranial vertex as point (0,0)(S. M. Schabrun et al., 2016; Seminowicz et al., 2019). The intensity of TMS stimulation was set at 120% resting motor threshold (rMT) (Cavaleri et al., 2019; D. T. A. Nguyen et al., 2019; Rossini et al., 2015) measured at the beginning of the experimental session, defined as minimum intensity induced 5 out of 10 MEP with an amplitude higher than 50 mV(Rossini et al., 2015).

STUDY II AND STUDY III

Experimental setup

The experimental procedure of Study II and Study III were similar and conducted on the same group of subjects. Fourteen healthy subjects (right-handed) were recruited to participate in two experimental sessions with two different TENS patterns. Outcome measures, including the EEG signal and perceived sensation to electrical sensory pulses, were collected at the following time phases, Pre (baseline), Post (immediately after TENS), and Post60 (60 min after intervention phase).

TENS

Based on Study I findings, the modulated pattern with the potential effects on pain reduction (PWM TENS) was selected and further investigated. Non-modulated pattern with the same frequency but constant pulse width (NMHF) was also included in these studies to perform a comparative analysis between induced changes by two patterns. The characteristic of delivered TENS interventions was adjusted at the same level as explained in Study I.

Data recording

Continues EEG was recorded from a 64-channel EEG set up connected to MR plus amplifiers (brain product) with a 5 kHz sampling rate. The EEG signals were collected while the subject was seated in a relaxed position. In total, 100 single sensory pulses (with 500 μs pulse width) were delivered to the median nerve of two hands alternately through the surface electrodes. The intensity of stimulation was set 2.5 times the sensory threshold measured at the beginning of the experiment using the stare case procedure (Faghani Jadidi et al., 2022; A. A. Zarei et al., 2021). Besides the cortical

activity, the subjects were asked to rate the magnitude and area of the received sensory pulses.

STUDY IV

Experimental setup

The fourth study's experimental procedure was performed close to Study II and III with minor changes. We evaluated the effect of selected modulated TENS (PWM) on an upper limb amputee's cortical activity (EEG recording) and pain profile. Fifty years old women with amputation below the elbow participated in two experimental sessions. Patient had a frequent PLP, and phantom stump pain rated around eight (0 no pain to 10 worst possible) on a VAS scale up to 12 months ago. Following a neuroma removal (surgery) her pain level dropped to four (varying in rang 4-6) both at the stump and lost limb (PLP). The first session was allocated to extract the referred sensation area (RSAs) and proper location and characteristics for the TENS application. Over the second session, EEG signals and sensory information were collected before and following PWM TENS intervention.

TENS

Following extraction of the RSAs on a day I (Eugen Lontis et al., 2018), several combinations of electrodes position were examined, and eventually, the surface electrodes were positioned in the proper location, which the patient reported comfortable feeling on the phantom limb in response to electrical stimulation. Several series of pulses with frequency and pulse width were delivered, and the patient was asked to describe the type of sensation in the stump and phantom limb. Eventually, TENS characteristic, including frequency (100 Hz) and pulse width (varied from 0 to 500 μs), was adjusted based on the patient statement. Over the experimental session on Day 2, the PWM TENS was delivered at an intensity to elicit a strong sensation in the phantom hand around seven on the VAS scale (0 no sensation to 10 uncomfortable feelings). The stimulation was delivered for 30 min with 15 s on-time and 15 s off-time (Lagerquist et al., 2012; Mang et al., 2010).

Data recording

Due to a technical issue with one of the MR amplifiers (brain product, GmbH) the EEG data were only collected from a 32-channel EEG system. To study the SEP alteration of affected hemispheres following TENS intervention, 25 single sensory pulses were delivered to the ulnar nerve at the cubital tunnel of amputated hand. Besides the stimulation of the ulnar nerve, 50 sensory pulses (two sets of 25 pulses with 5 min rest in between) were delivered to RSA. The intensity of stimulations was set at 3 times of sensory threshold of the phantom limb, which induced clear sensation in the removed limb, rated around 4 on the VAS scale (0 no sensation to 10 uncomfortable feelings) by patients.

4.2. DATA ANALYSIS

STUDY I

The stored data by ‘‘Mr. Kick” (custom-made software, Knud Larsen, Aalborg University) was already pre-amplified (×1K) and filtered (50-2k Hz). Ten recorded MEPs from each muscle were inspected, and waves contaminated by background activity were excluded (Cavaleri et al., 2019; D. T. A. Nguyen et al., 2019). Next, the peak-to-peak amplitude of remaining MEP within 20-50 ms after TMS stimulation was extracted and averaged across subjects at Pre, Post, and Post60 representing the excitability of the CS tract.

In terms of the cortical map of the target muscle, the root means square (RMS) value was chosen to quantify MEP amplitude due to the shape of the MEP wave in some trials (Tsao et al., 2010). The RMS value was calculated for each wave in 20-50 ms window following TMS stimulation, and background activity in 55 to 5 ms preceding to TMS pulse was subtracted (Siobhan M. Schabrun et al., 2014; Tsao et al., 2010). The average RMS value of net MEP at each site generated the motor map of the right APB muscle in the left hemisphere for each subject. Three highly suggested features in pain literature, including map volume, the number of active sites, and coordinate of the center of gravity (CoG) were extracted at Pre ad Post. More detail are explained in (Faghani Jadidi et al., 2022).

Study II and IV

EEG data were pre-processed using brain vision analyzer software (Version 2.2.2), and further analysis was performed by EEGLAB (v14.1.2) (Delorme & Makeig, 2004) and a custom-made Matlab script. After pre-processing steps (explained in detail in paper 2), segmented data with 2s length (500 ms before to 1.5 s after sensory pulse onset) were assigned to the right and left-hand groups, and epochs were averaged across each hand group. Following that, the changes in the amplitude of N1 and P2 component as the first negative and positive peaks of the SEP from the Cz channel within 150-250 ms were studied over three time phases (Cruccu et al., 2008; A. A. Zarei et al., 2021).

Moreover, the alteration in SEP oscillation was also evaluated as changes in the activity of different frequency bands have been reported following chronic pain onset (examples in chapter 2). The time-frequency analysis was performed by assessing the event-related spectral perturbation (ERSP) using EEGLAB. The time-frequency map was extracted at Pre, Post, Post60, and significant changes induced by TENS intervention in all time-frequency regions were detected by a non-parametric, cluster-based permutation test (A. A. Zarei et al., 2021). In addition, alterations in the alpha-band activity of SEP components (N1 and P2) were investigated in all channels, and those experiencing significant changes in this frequency band were detected by a false discovery rate (FDR) correction ($p < 0.05$).

Study III

To analyze the cortical network, EEG data were pre-processed and segmented into 1500 ms length epochs (500 ms before to 1000 ms after sensory pulse onset) using a Brain vision analyzer (Version 2.2.2). Brain cortical source activity of each epoch has been estimated using the build-in LORETA toolbox of the Brain-vision analyzer.

The source-level electrophysiological activity was estimated from eight different brain areas involved in sensory-pain processing, including the SI, SII, and anterior insula (on both hemispheres) beside the mPFC and the ACC. Epochs with the source activity of these ROIs were then band-passed filtered into seven predefined frequency bands (delta: 0.5-4 Hz, theta: 4-8 Hz, alpha: 8-13 Hz, beta: 14-40 Hz, gamma: 40-49 Hz, high gamma: 51-90 Hz and 0.5-90 Hz)(Tottrup et al., 2020; A. A. Zarei et al., 2022). The functional connectivity for each pairwise ROIs (28 pairs in total) at each frequency band (7 frequency bands), time phase (Pre, Post, Post60), and TENS pattern (PWM TENS and NMHF TENS) were measured using Pearson coefficient and PLV. The constructed functional connectivity network from all 28 pairwise connections was then considered for network analysis. Then after, the well-known local and global indexes, namely strength, efficiency, and cluster coefficient, were calculated for phase and amplitude-based networks for further analysis regarding alteration in the quality and quantity of information transmission in both networks by each TENS intervention.

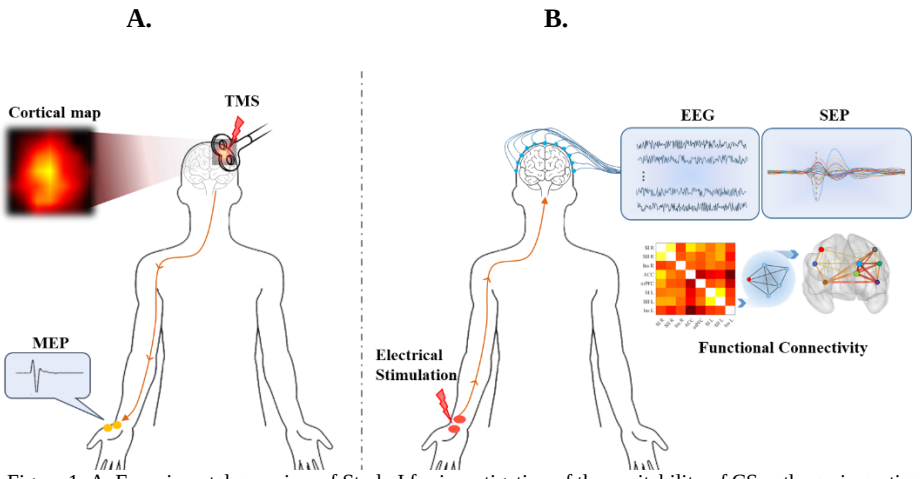

Figure 1. A. Experimental overview of Study I for investigation of the excitability of CS pathway in cortico motor map following TENS intervention. B. EEG recording for assessment of the effect of TENS intervention on SEP (Study II, and IV) and functional connectivity (Study III).

CHAPTER 5. SUMMARY OF MAIN FINDINGS

This chapter summarizes the main findings of four studies and how these results address each of the research questions introduced in chapter 3. The summary of main findings from Study I-IV is presented in Table 1.

5.1. SUMMARY OF STUDY I RESULT

Study I was designed to compare the effect of modulated TENS and conventional TENS on the excitability of the CS tract and the corticomotor map.

The findings revealed that CS activity showed an increasing trend immediately after PWM, NMHF, and NMLF. However, the result of the statistical tests (repeated measure (RM) ANOVA or Friedman, depending on data distribution) only showed a significant effect of time for the PWM pattern when evaluating the mean amplitude of MEP from the right APB muscle. The result of the post hoc test indicated a significant facilitation at the "Post" time phase compared to the baseline value. The statistical analysis on the features extracted from the non-target and control muscles showed no significant changes after the intervention phases, and the amplitude of MEP remained almost at the same level over time phases.

In terms of alteration in the corticomotor map of the target muscle (right APB muscle), three features showed an expansion trend of the representative area in the motor cortex (map volume and number of active sites). Statistical analysis (paired t-test/Wilcoxon test) revealed that PWM and NMLF induced a significant enhancement in the volume of the APB muscle map.

5.2. SUMMARY OF STUDY II RESULT

Study II aimed to investigate and compare the effect of the selected modulated pattern (PWM) and conventional TENS on the cortical response to sensory input and alteration of perceived sensation. Moreover, the SEP components from the right hemisphere were also analyzed to understand the effect of TENS interventions on the ipsilateral hemisphere.

The statistical analysis (RM ANOVA) showed no significant difference between induced changes by the two patterns. Both interventions lead to immediate suppression of N1 components (statistically significant) of the contralateral (left) hemisphere. Moreover, following 30 min of PWM application, the P2 amplitude was significantly reduced, while the non-modulated pattern induced cortical suppression

after 60 min. Importantly, despite the decreasing trend in SEP components recorded from the ipsilateral hemisphere, only conventional TENS suppressed the N1 amplitude after one hour (statistically significant). Besides that, the time-frequency analysis revealed a significant reduction of theta to low beta bands oscillation in the Cz channel, which was associated with a notable suppression in perceived sensation reported by subjects. Interestingly, while the level of reduction in sensation was not statistically different between the two patterns, the average perceived sensation following PWM TENS was lower than conventional TENS (14% less).

5.3. SUMMARY OF STUDY III RESULT

The aim of the third study was defined to investigate the possible alterations in cortical networks following modulated TENS intervention. The statistical results (Wilcoxon/paired t-tests) revealed no significant difference between induced alterations in global indexes right after both interventions. However, 60 min after the PWM TENS, enhancement in all three features (strength, efficiency, and cluster coefficient) of both networks in the high gamma-band was significantly different compared to induced changes by conventional TENS.

Moreover, the local features of selected brain regions involved in pain/sensory procedures showed that the efficiency and strength of ipsilateral and contralateral somatosensory cortices (left and right SI, SII) increased one hour following PWM TENS and was significantly greater than the conventional pattern. In terms of phase-based network segregation, besides the sensory cortices, the cluster coefficient value for mPFC and ACC increased 60 minutes after the PWM phase, which was statistically different compared with the conventional TENS phase. In line with this, the statistical analysis of the correlation-based network indicated significant differences in strength, efficiency, and cluster coefficient alteration an hour following PWM and conventional TENS in left SI, mPFC, and ACC.

5.4. SUMMARY OF STUDY IV RESULT

The last study was designed to eventually evaluate the effectiveness of PWM TENS on brain response to sensory input and pain level of amputee with PLP.

The subject reported pleasant and clear sensation (scored seven on the VAS ranging from 0 = no sensation to 10 = painful stimuli) in phantom hand (digit 3, 4, 5) and the entire palm and wrist by receiving PWM TENS at RSA. Moreover, patient reported tactile/vibration sensation spread in a larger area during TENS intervention (with varying pulse width) than by single pulses stimulation with constant parameters. At the end of the TENS session, the subject stated a reduction in phantom pain to 3.5 (in the range of 3 to 3.5).

Regarding cortical activity, both extracted SEPs (at Cz channel) induced by stimulation of RSA and ulnar nerve at the elbow (RSA_SEP and Ulnar_SEP, respectively) were suppressed following TENS intervention. The amplitude of N1 and P2 components in RSA_SEP decreased from -3 μV and 3.4 μV to -2.2 μV and 2.89 μV, respectively. In line with this, the magnitude of both N1 and P2 peaks extracted from Ulnar_SEP dropped by 28% (N1: -3.4 to -2.48 and P2: 3.96 to 2.89). Moreover, the alteration in N1 and P2 peaks distribution in scalp topographies has been investigated, and a reduction was shown in the amplitude of both peaks in central and frontal electrodes at Post compared to baseline. In terms of dynamic activity, the time-frequency maps calculated from RSA_SEP of Cz channel at Pre and Post time phases illustrated the reduction in the activity of theta to the beta frequency band in the time window of 0 - 400 ms.

Tabel 1. Summary of the main significant findings in Study I-IV.

Studies	Pre	TENS Intervention	Post	Post 30	Post 60	Summery of significant outcome
	-30	0	30		60	
Study I (N = 44)	TMS	NM_LF (n=11) NM_HF (n=11) PWM (n=11) BM (n=11) **Applied on right median nerve at wrist**	TMS	TMS	-	❑ Increase in CS activity of right ABP by PWM TENS ❑ Corticomotor enhancement of right ABP by PWM and NMHF TENS
Study II (N = 28)	SEP	PWM (n=14) NM_HF (n=14) **Applied on right median nerve at wrist**	SEP	-	SEP	❑ Suppression of N1 and P2 amplitude by PWM and NMHF TENS lasted 60 min ❑ Suppression in perceived intensity by PWM and NMHF TENS lasted 60 min
Study III (N = 24)	FC	PWM (n=12) NM_HF (n=12) **Applied on right median nerve at wrist**	FC	-	FC	**Comparison between PWM and NMHF TENSs' effects in high-gamma band and Post 60 – Pre time phase** ❑ Greater enhancement in local strength and efficiency indexes by PWM TENS in SI_L, SII_L, SI_R and SII_R (plus ACC and mPFC for cluster coefficient) ❑ Greater enhancement in global strength, efficiency and cluster coefficient indexes
Study IV (N = 1) PLP patient	SEP	PWM **Applied on RSA**	SEP	-	-	❑ Suppression of N1 and P2 amplitude by PWM TENS ❑ PLP reduction

TMS: Transcranial magnetic stimulation, **SEP**: Somatosensory evoked potential, **FC**: Functional connectivity
NM_LF: Frequency = 40 Hz, Pulse width = 500 µs
NM_HF: Frequency = 100 Hz, Pulse width = 500 µs
PWM: Frequency = 100 Hz, Pulse width modulated from 0 to 500 µs
BM: Carrier Frequency = 100 Hz, Modulated Frequency = 4 Hz, Pulse width = 500 µs

SI: Primary somatosensory cortex
SII: Secondary somatosensory cortex
ACC: Anterior cingulate cortex
mPFC: Medial prefrontal cortex

CHAPTER 6. DISCUSSION

6.1. STUDY I. HOW DO THE CS PATHWAY ACTIVITY AND MOTOR CORTICAL MAP ALTER FOLLOWING MODULATED AND NON-MODULATED TENS?

Study I mainly focused on providing evidence regarding the effects of modulated TENS (burst and PWM pattern) on the CS tract and corticomotor map.

The result of Study I showed that while NMHF, NMLF, and PWM patterns could induce enhancement in the MEP amplitude of the target muscle (right APB) immediately after the intervention phase, only alteration by PWM was statistically significant. The averaged MEP magnitude of right APB at Post30 was still higher than Pre vale, but the statistical difference was not detected anymore. In contrast, the burst pattern could not cause an alteration in the activity of the CS pathway, which may be due to the applied intensity as literature reported the effect of this pattern at high intensity eliciting painful (tolerable) sensation with even visual movement (Buchmuller et al., 2012; Macedo et al., 2015). The induced effect by PWM TENS on the CS activity may suggest its effectiveness in pain analgesic as facilitation in the CS tract by rTMS and tDCS interventions has been shown to be associated with chronic pain (PLP) alleviation (André-Obadia et al., 2006; Malavera et al., 2016; Roux et al., 2001). The possible explanation was supported by the hypothesis that an increase in excitability causes the indirect suppression effect on the thalamus and subsequently alteration of ascending pathway for nociceptive signals (Bolognini et al., 2013; Garcia-Larrea & Peyron, 2007). Moreover, our result revealed that all four patterns indued focal and specified effect only on median nerve innervated muscle (targeted by TENS) and not non-target (right ADM) and control (left APB) muscles (Kaelin-Lang et al., 2002; Peng et al., 2019; D. Tan et al., 2016).

In addition, the result of the present study revealed a significant enhancement in the volume of motor maps of target muscle following PWM and NMLF intervention compared to baseline maps. In this regard, Seminowicz et al. showed that individuals experiencing corticomotor depression in experimental pain reported higher pain levels (Seminowicz et al., 2019). Chowdhury et al. also suggested suppression of corticomotor excitability as a cortical biomarker in chronic pain cases (Chowdhury et al., 2022). In addition, experimental investigation of interventions leading to enhancement in the motor cortex indicated improvement in chronic pain alleviation. For example, the combination of tDCS with PES treatment reduced the pain severity among chronic back patients (CBP), while the activity of the cortical motor map (volume) was increased (Siobhan M. Schabrun et al., 2014).

6.2. STUDY II. HOW DOES THE MODULATED TENS INTERVENTION INDUCE CHANGES IN SOMATOSENSORY CORTEX ACTIVITY AND PERCEIVED SENSATION COMPARED TO ALTERATION INDUCED BY NON-MODULATED TENS?

Based on the results of Study I, further investigation on PWM TENS has been conducted in Study II by analyzing the possible alteration in the somatosensory cortex following the application of PWM TENS and its correlation with changes in sensation profile.

Results from Study II revealed a significant suppression in somatosensory activity following the application of both NMHF and PWM TENS with a significant reduction in perceived sensation in the TENS stimulated hand. These findings supported previous work indicating SEP suppression following conventional TENS associated with sensation reduction compared with the sham group (A. A. Zarei et al., 2021; A. Zarei et al., 2019).

As mentioned in chapter 2 and alongside cortical reorganization, changes in cortical excitability have been reported as a cortical biomarker of PLP prevalence. Previous studies have reported enhancement in cortical excitability in chronic pain patients (Duarte et al., 2020; Herta Flor et al., 1997; Zhao et al., 2016). For instance, Flor et al. showed an increase in brain response to sensory and painful stimulations in the SI cortex of patients with chronic pain (Herta Flor et al., 1997). Jacobs et al. also detected significantly larger P2 potentials for subjects with CBP compared to healthy subjects (Jacobs et al., 2016). Additionally, the artificial restoring of sensory congruence has been suggested to reverse this issue (i.e., decrease the somatosensory cortex excitability) with possible pain reduction (Daenen et al., 2012; Thieme et al., 2016). Peng et al. reported the correlation between SI activity reduction following conventional and acupuncture TENS and notable pain reduction (Peng et al., 2019).

Study II showed suppression of the N1 component following PWM and NMHF TENS. N1 has been reported to represent the early sensory processing with the magnitude positively correlated with the memory of pain, and the suppression of this component might be the possible biomarker for causing the analgesic/sensory suppression effect of PWM TENS. P2 has been represented as another SEP component correlated with pain and responsible for perceived stimulus translation (M. C. Lee et al., 2009). While the recent study revealed a positive correlation between P2 suppression and pain relief (Peng et al., 2019), a significant reduction in P2 amplitude in this study at Post and Psot60 also suggested PWM TENS potential as a therapeutic intervention in pain rehabilitation.

Moreover, several changes in cortical dynamic activity, such as enhancement of theta, alpha, and the beta band, especially in SI and medial prefrontal cortex, have been linked to chronic pain onset (Ploner et al., 2017). The findings from Study II regarding

the dynamic activity revealed a significant reduction in the alpha oscillation of N1 and P2 at the Cz and central/frontal channels. This result may suggest the underlying mechanism of the PWM TENS analgesic effect correlating with the alpha band's cortical dynamic alteration.

6.3. STUDY III. HOW DOES MODULATED TENS AFFECT THE FUNCTIONAL CORTICAL NETWORK AMONG BRAIN AREAS INVOLVED IN SENSORY AND PAIN PERCEPTIONS? HOW DISTINGUISHABLE ARE THE CHANGES WITH THE RESULTS FOLLOWING CONVENTIONAL TENS?

As it was mentioned in Chapter II, the brain functional connectivity network has also been identified to alter in chronic pain patients. Studies have suggested several brain areas involved in pain processing (e.g., SI, SII, ACC, mPFC) and investigated the changes in the characteristics of the network constructed by these areas (pain matrix) in chronic patients (Green et al., 2009; Kandić et al., 2021; Makin & Flor, 2020; Zheng et al., 2020). Considering the role of the functional connectivity network in pain processing, Study III examined the possible information integration and segregation changes in the network constructed by eight sensory/pain-related ROIs. Statistically significant differences in topographical changes induced by NMHF TENS and PWM were found in the high-gamma band one hour following the intervention phases.

PLP has been reported to correlate with the suppression of sensory cortices' inter-hemisphere FC and intra-hemisphere FC between SI in the affected hemisphere and subcortical nuclei (Zhang et al., 2018). Moreover, a recent study reported PLP reduction linked with an increase in sensory network functional connectivity and unaffected hemisphere (Scibilia et al., 2018). In this regard, Study III demonstrated that delivering PWM TENS to both intact and amputated hands of amputees might improve segregation and integration of information from SI and SII more significantly compared to conventional TENS, which may lead to PLP alleviation.

The cortical functional connectivity beyond the sensory cortex has also been reported to represent the chronic pain-evoked reorganization. Considering the ACC and mPFC to be responsible for the affective aspect of pain and regulating endogenous pain, influencing their activity could be assumed to affect the pain processing (Kucyi & Davis, 2015; Singh et al., 2020; Wager et al., 2016; Xiao et al., 2019). Additionally, the enhancement of local efficiency in these brain regions following TENS has been reported as a possible mechanism for TENS' analgesic effect (A. A. Zarei et al., 2022). In line with this, Study III illustrated a significant improvement in information transmission efficiency of ACC and mPFC areas 60 min following PWM TENS compared to the conventional TENS suggesting PWM TENS as a long-lasting therapeutic intervention.

Regarding the global investigation of brain FC network characteristics, several studies have shown the role of this cortical factor in chronic pain (Lenoir et al., 2021; Ta Dinh et al., 2019; Zheng et al., 2020). A recent study assessed brain activity using fMRI and reported that global metrics of brain networks of upper limb amputees are significantly different compared to healthy subjects (Makin, Filippini, et al., 2015). Additionally, studies have shown that these brain network characteristics could be more informative by analyzing them in various frequency bands (DeSouza et al., 2020; Ta Dinh et al., 2019). For example, Ta Dinh et al. reported a significant decrease in high gamma-band global efficiency in chronic pain patients (Ta Dinh et al., 2019). In accordance with this, Study III revealed a significant enhancement in global indexes of functional connectivity networks following PWM TENS and highlighted the advantages of PWM TENS compared to conventional TENS as a possible solution in pain therapy.

6.4. STUDY IV. HOW DOES MODULATED TENS INTERVENTION AFFECT CORTICAL RESPONSE AND PAIN PROFILE IN AN UPPER LIMB AMPUTEE?

Over the last three studies, some evidence was obtained regarding the neurobiological effects of PWM TENS on healthy subjects. However, while the eventual goal was to benefit from this pattern in PLP alleviation, study IV aimed to investigate the effectiveness of this pattern on an upper limb amputee with PLP.

Besides the alteration in pain and perceived sensation level, the changes at the cortical level were also evaluated in Study IV. The result revealed suppression in the activity of the SI cortex after delivering 30 min PWM TENS at RSA. The reduction in the magnitude of SEPs components (N1 and P2) was accompanied by a decrease in the PLP level, which was in line with studies covering cortical suppression with pain/sensory reduction (Peng et al., 2019; A. A. Zarei et al., 2021; A. Zarei et al., 2019). It has been hypothesized that pain prevalence, e.g., PLP, is associated with disturbances in SI activity (Duarte et al., 2020; Herta Flor et al., 1997; Jacobs et al., 2016), and interventions that could cause the reverse effect lead to positive contributions to pain alleviation (Daenen et al., 2012; Mccabe et al., 2007; Peng et al., 2019; Perry et al., 2014). In addition to pain reduction, the subject stated the 'pleasant' sensation (termed in vibration and tactile) in a larger area of a phantom limb by PWM TENS compared to pulses with constant parameters.

Regarding dynamic activity, it has also been shown that pain onset alters abnormal cortical oscillations in a wide range of frequency bands (Ploner et al., 2017). In this regard, the depressive effects of PWM TENS have also been observed in the extracted time-frequency maps from SEP elicited by RSA stimulation in the present study, and the intervention suppressed the activity of theta to the beta frequency bands. These findings may support the positive correlation between reduction in cortical oscillations and pain level.

CHAPTER 7. CONCLUSION

This Ph.D. thesis included four studies that aimed to investigate the CS and cortical changes in sensory/pain perception following modulated TENS. Significant facilitation in the activity of the CS pathway and enhancement in the corticomotor map were detected following PWM TENS but not in conventional TENS as shown by analyzing MEP waves induced by TMS pulses. In addition, cortical changes in the SI cortex were examined by collecting EEG data in response to sensory stimulation. The results revealed significant suppression in cortical activity and oscillations associated with a reduction in the perceived level of sensory stimulation. The exploration of cortical networks between pain-involved areas also indicated a late significant increase in PLV-based and correlation-based network indexes following PWM TENS as compared to conventional TENS. Moreover, the effectiveness of the PWM pattern was tested in a case study with an upper limb amputee and the result showed suppression in cortical activity and PLP alleviation. Altogether, these findings provide novel evidence regarding the neurobiological effect of PWM and the potential of this pattern as an alternative intervention for PLP therapy. Nevertheless, further investigation with a large number of PLP patients could substantiate the present knowledge.

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