Modulation of Sensory Perceptions and Cortical Responses Following TENS

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by

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AALBORG UNIVERSITY
DENMARK

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CV

Ali received his Bachelor's degree in Electronic Engineering from Sadjad University, Mashhad, Iran, in 2013, and his Master's degree in Biomedical Engineering from Amirkabir University, Tehran, Iran, in 2017. Following a short period working in the industry as a software developer, he received the Marie Curie Ph.D. scholarships. Ali enrolled as a Ph.D. fellow working 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. Co-supervision is by Romulus Lontis, Aalborg University, Aalborg, Denmark. Ali has had the opportunity to speak and present his work at several international conferences. His main research interests include pain, neuroscience, biological signal processing, and neurorehabilitation.

ENGLISH SUMMARY

Nearly two million people live with limb loss in the US caused by vascular diseases, trauma, and cancer. Recent increasing awareness of diminishing quality of life and societal impact of phantom limb pain (PLP) pose an increasing burden on rehabilitation within the health care system. Amputation deprives the nervous system of sensory input leading to anatomical and physiological changes at the peripheral and central level, contributing to the mechanisms generating PLP. Today the underlying mechanism of PLP is not well known, however modulation of cortical plasticity has shown to be correlated with onset and relief of PLP. Several treatments have been suggested for PLP relief. Transcutaneous electrical nerve stimulation (TENS) has been suggested as a possible noninvasive, drug-free pain treatment for chronic and neuropathic pain (e.g., PLP and back pain). However, the underlying mechanism of TENS's analgesic effect on the central nervous system in amputees to induce phantom limb pain relief is not yet understood. In line with these, the objective of this Ph.D. project is to investigate possible altered cortical responses following TENS in amputees and healthy subjects. The Ph.D. thesis was based on a series of three studies. The first study was conducted on forty healthy subjects to investigate the cortical change following TENS intervention using somatosensory evoked potnd a significant suppression in N100 and P200 components at least for an hour following TENS intervention compared to the sham group. The SEP component changes were associated with a reduction in theta and alpha oscillation and perceived intensity. The second study assessed the effect of TENS on brain functional connectivity FC and pain network. The pairwise functional connectivity between different brain regions (i.e., Brain areas corresponding to pain and sensation) across five frequency bands was compared for the TENS and sham groups. The extracted functional connectivity networks were analyzed using graph theory methods. The results of this study demonstrated the effect of TENS on gamma-band functional connectivity between the primary somatosensory cortex and anterior cingulate cortex. Results from network analysis showed significant changes in both local and global network indices. The third study was conducted on two upper limb amputees with PLP. The capability of TENS applied as surface electrical stimulation of Referred Sensation Areas (RSAs) in amputees to induce phantom limb pain relief was investigated. SEP and functional connectivity characteristics of this study were compared to the results from the first two studies. The finding of this study reported the same changes in SEP pattern and FC features. At the same time, a PLP reduction following TENS was found. In conclusion, the results denote the underlying mechanism of TENS intervention on the CNS, which was associated with alternation in sensation and possible PLP relief.

DANSK RESUME

Næsten to millioner mennesker i USA lever med en amputation som følge af vaskulære sygdomme, traumer og kræft. Den øgede bevidsthed om forringet livskvalitet og samfundsmæssig belastning af sundhedssystemet der følger på grund af fantomsmerter (PLP phantom limb pain) har sat fokus på behov for rehabilitering væsentligt. En amputation fratager nervesystemet sensorisk input, hvorved der sker anatomiske og fysiologiske ændringer på det perifere og centrale nervesystemer, hvilket man mener bidrager til de mekanismer, der genererer PLP. I dag forstår man ikke de underliggende mekanismer der forårsager PLP, men modulering af kortikal plasticitet har vist sig at hænge sammen med både opståen og lindring af PLP. Der er i dag flere behandlingsmuligheder til lindring af PLP, bl.a Transkutan Elektrisk NerveStimulering (TENS), som er en ikke-invasiv, ikke-farmakologisk smertebehandling. Imidlertid er der endnu utilstrækkelig forståelse for hvordan TENS virker smertelindrende, dvs. hvordan påvirker behandlingen central nerve systemet og de underliggende mekanismer. Formålet med dette ph.d. projekt var derfor at undersøge hvordan den kortikale respons ændrer sig efter TENS hos raske forsøgspersoner og amputerede. Ph.D. afhandlingen er baseret på resultater fra tre studier. Formålet med det første forsøg var at undersøge den kortikale ændring efter TENS-intervention hos 40 raske forsøgspersoner. Analyse af SEPs (somatosensory evoked potentials) viste et statistisk signifikant fald i N100- og P200-komponenterne i op til én time efter TENS-intervention sammenlignet med sham-gruppen (placebo). Ændringerne i SEP var forbundet med en reduktion i hjernens theta- og alfa bølge aktiviteten og personens opfattelse af intensiteten af TENS. I det andet studie blev effekten af TENS på hjernens funktionelle forbindelser (functional connectivity, FC) og smertenetværk undersøgt. FC mellem udvalgte hjerneområder (dvs. hjerneområderne for smerte og sensoriske følelse) blev analyseret og sammenlignet for TENS- og sham-grupperne ved hjælp af grafteoretiske metoder. Resultaterne demonstrerede en effekt af TENS på hjernens gamma bølge aktivitet mellem den primære SI (somatosensoriske cortex) og ACC (anterior cingulate cortex). Resultater fra netværksanalysen viste signifikant ændringer i både lokale og globale netværksindekser. Det tredje studie inkluderede to arm amputerede der oplevede PLP. Det blev undersøgt om TENS leveret til de amputeredes RSAs (referred sensory areas) kunne lindre deres fantomsmerter. Analyse af SEP og FC blev sammenlignet med resultaterne fra de to første forsøg. Resultaterne viste de samme ændringer i SEP-mønster og FC-funktioner. På samme tid blev der målt en reduktion af PLP efter TENS. Samlet set, idet at resultaterne viste en modulering af sensoriske følelser og mulig PLP reduktion, så peger resultaterne på at en mulig forklaring på den underliggende mekanisme af TENS.

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CHAPTER 1. INTRODUCTION

Losing a limb by amputation is a traumatizing experience and is known to profoundly impact both the physical and mental health of the amputee. The reasons for amputation include but are not limited to amputation by accident, peripheral vascular disease, neurological injury, wartime conflicts, terrorist attacks, and landmine explosions. The impact and prevalence of amputation have been estimated in different studies, which has been linked with a decrease in the quality of life (Sinha et al. 2011). In the United States, approximately 185,000 amputations occur each year (Pokras et al. 1997; Ziegler-Graham et al. 2008). In 2005, around 1.6 million Americans had experienced the loss of a limb, and 65% of these individuals had lower extremity amputations (Ziegler-Graham et al. 2008). The diagnosis of dysvascular disease was linked to 54% of the amputation cases (Ziegler-Graham et al. 2008). In addition to the high prevalence rate, post-amputation pain makes it evident that phantom pain demands to be addressed. Phantom limb pain (PLP) is the painful phantom sensation in the amputated limb which most amputees experienced it following amputation. Several invasive and non-invasive treatment has been mentioned for PLP reduction.

Transcutaneous electrical nerve stimulation (TENS) is a treatment for chronic pain reduction and rehabilitation (Black et al. 2009; Cornwall 2007; Lai et al. 2016; Peng et al. 2019). It has been shown that electrical current pulses delivered by TENS leads to different results by changing the TENS parameters such as frequency and pulse width (Schabrun et al. 2012). The underlying mechanism of TENS include both peripheral and central nervous system (Gozani 2019; Peng et al. 2019). However, the effect of TENS on pain and sensation with and associated cortical alternation following TENS is not fully understood.

Several neuroimaging techniques have been suggested to examine the induced changes at the central nervous system following different interventions (e.g., fMRI (Farahani et al. 2019), EEG (He et al. 2019; Nickel et al. 2020a), MEG (De Pasquale et al. 2010), etc.). Somatosensory evoked potential (SEP) is the evoked cortical response following external stimuli. Several studies reported the ability of SEP to examine the functionality of neural pathways, cortical activity, and neuroplasticity (Manresa et al. 2015; Mouraux and Iannetti 2018). Moreover, the functional brain connectivity between different brain areas has been reported as a valid feature to assess the changes in the central nervous system (Lee et al. 2020a; Nickel et al. 2020b; Ta Dinh et al. 2019).

The focus of this thesis is to investigate the effect of TENS on alternation in cortical response and sensory perception. Moreover, the analgesic effect of TENS and induced changes in cortical activity is also investigated in this project.

CHAPTER 2. BACKGROUND

2.1. PHANTOM LIMB PAIN

Virtually, amputees feel non-painful sensations in the amputated limb as if the missing limb is still present. The brain continues to feel the removed limb, and these non-painful sensations are known as the phantom limb sensation. These sensations can be represented in various somatosensory experiences like touch, warmth, itching, and cold (Kooijman et al. 2000). Moreover, some patients also reported experiences that are kinesthetic sensations such as position, shape, and size of the removed limb. Besides, phantom limb sensation may include voluntary movements, such as grabbing an object, moving their fingers, or making a fist (Weinstein 1998), and involuntary movements of the amputated limb like developing a spasm in hand or occupying a posture (Ramachandran and Hirstein 1998).

Although phantom limb sensations are considered non-painful sensations, it is also reported that amputees feel pain in the amputated limb. Two kinds of pain related to the amputated limb exist: residual limb pain and phantom limb pain (PLP) (Ahmed et al. 2017). The pain felt in the stump of their removed limb is considered residual limb pain, while the pain perceived in the missing limb is addressed as phantom limb pain (PLP). The presence of PLP can be described as tingling, nagging, cutting, shocking, piercing, radiating, squeezing, tight or stinging, or any combination of these sensations, which can start as soon as after the amputation (Ehde et al. 2000; Nikolajsen and Jensen 2001). The perceived pain has also been reported as distally localized pain regardless of the site of the amputation (Nikolajsen et al. 1997).

Various physical or psychological factors might worsen or elicit PLP (Arena et al. 1990; Sherman et al. 1989). These factors include, but are not limited to, changes in weather, pressure on the residual limb, and emotional stress. Modulation of PLP is also affected by cognitive factors, such as coping strategies. Individuals who passive coping mechanisms are reported to be more affected by PLP and are known to report more interference (Richardson et al. 2007). It is also researched that the prevalence of PLP is more frequent after the traumatic amputation (Ramachandran and Hirstein 1998). Studies have shown that the PLP is less prevalent in children amputees, lower-limb amputees, patients with a congenital limb deficiency, while it is more common in adult amputees, amputees who have experienced surgical amputation, and patients who have undergone upper-limb amputation (Krane and Heller 1995; Melzack et al. 1997).

The PLP prevalence widely varies in the literature; however, generally, 50-80% of amputees reported PLP (Kooijman et al. 2000). Ephraim et al. performed a study with 914 amputees respondents and reported that 79.9% of the participants stated that they were experiencing phantom pain. Among those experiencing phantom pain, 38.4%

stated experiencing severe pain (higher than 7 on a 0-10 analog scale) (Ephraim et al. 2005). They found no meaningful difference in the rates of PLP based on etiology, level of amputation, and age. It was also reported that rates of upper-limp PLP were 83% consistent with the study population and the average pain intensity for all patients was 5.5 ± 2.6. Although no clear reason has been found for PLP prevalence discrepancies, some evidence such as response rates and bias in the study population has been suggested.

As mentioned earlier, the onset of PLP can immediately follow the amputation, or it can be years later (Schley et al. 2008). While the persistence of PLP has been reported as years or even decades, in more than 75% of cases, the onset of PLP is during the first few days after the amputation. There are differences in when PLP first occurs and how external variables impact the PLP onset. A study has stated that 47% of amputees experience PLP in the first 24 hours following their amputation (Jensen et al. 1983). Moreover, eight days following amputation, this percentage increased to 84% and then to 90% after six months. Ehde et. al., has reported 72% of individuals experienced PLP within six months of post-amputation (Ehde et al. 2000). They mentioned in half of the amputees, the PLP onsets occur approximately within a day, while the first PLP experience can still occur up to six months after amputation. Moreover, the incidence of PLP can be increased by environmental factors such as postoperative analgesia, pre-amputation pain, and smoking after amputation (Ahmed et al. 2017; Yin et al. 2017).

Few studies reported a gradual decrease in the intensity, severity, and frequency of phantom pain over time (Nikolajsen et al. 1997). However, no evidence has been found the association between time elapsed after the amputation and the occurrence of PLP. For example, a large-scale survey with several thousand amputees found that 70% of amputees experienced PLP even after 25 years of amputation (Sherman et al. 1984).

2.2. NEUROBIOLOGY OF PHANTOM LIMB PAIN

The neurobiological mechanism of PLP is not well understood, as the impact of amputations can be seen on different levels of the nervous system, showing that there are various compounding sources of pain. Although PLP was described first by theories focused on the psychological phenomenon, nowadays, different studies have been reported the underlying mechanism of PLP as the neurological nature (Flor et al. 2006; Raffin et al. 2016a; Seo et al. 2017). However, the development of PLP involved multiple mechanisms (Flor et al. 2006). These mechanisms comprise a complex system response from peripheral, cortical, and psychological origins(Hsu and Cohen 2013). The most important components in developing PLP are included in the peripheral nervous system (PNS) and central nervous system (CNS).

2.2.1. PERIPHERAL NERVOUS SYSTEM

Studies on the involvement of PNS in PLP suggest that phantom pain may be caused by atrophy of deafferented dorsal horn neurons and changes in the receptive fields of the spinal cord (Jensen et al. 1983). The deafferentation might be a consequence of amputation or another injury like brachial plexus injury. The changes in spinal cord receptive fields, also known as spinal reorganization, have also been recognized in functionally inactive regions (Hsu and Cohen 2013). Spinal mechanisms are important to consider because of the integration of sensory information in the spinal cord (Teixeira et al. 2015). Another argument for the peripheral basis of PLP is the positive correlation between stump pain and PLP. The prevalence of PLP has been reported more frequently in amputees with chronic stump pain than those without stump pain (Sherman and Sherman 1983).

One of the assumptions of the relationship between PLP and the peripheral nervous system can be explained by the neuromas. Following amputation, the location that causes pain is at the position of a severed nerve which is termed neuroma (Fried et al. 1991). The development of terminal neuromata starts to form within hours and is generally formed within 1-12 months after the transection of the nerve (Boutin et al. 1998; Fried et al. 1991). When a neuroma is formed because of the truncation of peripheral nerves, it leads to aberrant growth of regenerating axons. Such stump neuromas have ectopic discharge that has been proposed as an important peripheral mechanism (Sun et al. 2005). The neuromas show hyper-excitability after chemical and mechanical stimulation (Devor et al. 1993).

While some studies have reported PLP prior to the formation of the neuromas in the residual limb (immediately after amputation), PLP cannot be only explained by peripheral factors (Nyström and Hagbarth 1981). Similarly, congenital amputees also report PLP, and a study with two amputees reported that PLP continued even after blocking the formation of neuromata with lidocaine (Nyström and Hagbarth 1981). In line with this evidence, the role of central factors in developing PLP as another mechanism should take into account.

On the other hand, the mechanism of spinal nerves in pain has been reported as the reason behind the pain. Roots leading into/out of the spinal cord are mentioned as the dorsal root and the ventral root. Incoming sensory information (afferent) is linked to the dorsal root, while the ventral root is responsible for the outgoing or efferent motor information. Increased sensory input to the dorsal root has been reported to develop PLP in amputees (Vaso et al. 2014). Amputation results in axotomized (cut axons of) neurons which increase the dorsal root input and such aberrant nociceptive impulses can be perceived and translated by the brain as pain. Vaso et al. has reported PLP reduction following intraforaminal nerve block, which suggests dorsal root ganglion as an automatic generator of PLP. However, the exact reason behind PLP relief is unknown. The PNS theories behind PLP have been abandoned by many studies, while

no complete PLP relief was found following neuroma infiltration and nerve block (Birbaumer et al. 1997). Therefore, following the evidence mentioned earlier, a significant portion of PLP research is focused on exploring the origin of PLP in the CNS.

Conclusively, as the research in PLP has been moving towards the central nervous system instead of the peripheral nervous system, there still seems to be some involvement. The involvement might not involve the neuroma, but the role of the spinal nerve should take into account.

2.2.2. CENTRAL NERVOUS SYSTEM

Theories of PLP that discuss the involvement of central nervous system (CNS) have reported PLP as the result of maladaptive brain plasticity (Birbaumer et al. 1997; Flor et al. 2006). Specific brain areas within the somatosensory region are dedicated to certain body parts. However, cortical presentation of various parts of the body continually changes based on the pattern of afferent nerve activities (Sterr et al. 1998). The brain regions neighboring the sensory cortex would occupy a certain cortical area if no input was received to that certain region (e.g., following an amputation) (Karl et al. 2001). No desired cortical areas are activated if an amputee tries a specific movement of the phantom limb as the pre-assigned cortical area for that particular movement has moved to adjacent brain regions (Karl et al. 2004). This phenomenon is known as cortical reorganization and has been verified by several studies (Chen et al. 2013). Deafferentation of the somatosensory cortex (either by amputation or local cutaneous anesthesia) is the main cause for cortical reorganization (Björkman et al. 2009). Extensive experimental researches have shown that sensorimotor cortices are affected by brain reorganization in individuals with extremity amputation (Chen et al. 2002; Flor et al. 1995). The cortical reorganization in amputees can be maladaptive and associated with pain maintenance, where the PLP starts.

Brain plasticity is reported to include different brain areas such as the primary somatosensory cortex (S1) (Elbert et al. 1994; Yang et al. 1994), the secondary somatosensory cortex (S2) (Flor et al. 2000), the primary motor cortex (M1) (Karl et al. 2004; Lotze et al. 2001). The S1 represents the somatotopic of the contralateral side of the body and lies within the post-central gyrus. S1 receives nociception from the thalamus via thalamocortical afferents and is responsible for processing the sensory discrimination of peripheral source of nociception (Lithwick et al. 2013). S2 lies adjacent to the S1. It is involved in quantifying the nociceptive input, and it has been shown to include information on the intensity of pain (Vierck et al. 2013). M1 is located anterior to the S1 and posterior to the pre-motor cortex of the brain. It is one of the main areas involved in motor function and control of limb movements (Lotze et al. 1999). The pre-motor cortex lies within the frontal lobe as well. It is positioned anterior to the primary motor cortex and is responsible for planning movements and spatial guidance of movement (Halsband et al. 1993). While the trunk and the face

areas are next to the arm in M1, It has been shown that phantom limb sensation can occur when the trunk or face is stimulated (Knecht et al. 1996). MRI imaging also verified the cortical reorganization at M1. The phantom sensation is not only perceived following stimulation of the ipsilateral side, but also it caused some phantom sensations following contralateral side stimulation, which suggests the involvement of interhemispheric structures. Additionally, other brain regions in the lower level, such as thalamocortical activity, have been mentioned to contribute to the cortical reorganization in the somatosensory motor cortex (Flor et al. 1995).

Studies have suggested a positive correlation between the extent of cortical reorganization and the lack of phantom limb control ability. In line with this, less cortical organization has happened if the amputees felt that they could control their phantom hand movement (Raffin et al. 2016b). It has been reported that the level of PLP is associated with the extent of sensorimotor cortices reorganization.

2.3. ASSESSMENT OF SENSORY PERCEPTIONS AND PAIN

A variety of neuroimaging methods, e.g., electroencephalography (EEG) (Chen et al. 2013), functional magnetic resonance imaging (fMRI) (Weiss et al. 2000), and positron emission tomography (PET) (Strelnikov et al. 2015), have been used to demonstrate cortical remapping following limb amputation.

fMRI is a radiation-free, noninvasive imaging procedure. This hemodynamic technique calculates changes in cerebral blood flow (CBF) (Buxton 2013). Blood oxygen level-dependent (BOLD) fMRI is most commonly used to investigate cortical reorganization for its ability to link activation to specific cortical structures (Gore 2003).

Cortical activity differences between amputees and healthy subjects have been investigated in different studies. The majority of studies that investigated cortical reorganization using event-related BOLD fMRI, concentrated on the primary sensory cortex (S1) and primary motor cortex (M1) (Flor et al. 1995). For example, Lotze et al. investigated the activation locus for hand and lip gestures in amputees with PLP (n=7), amputees without PLP (n=7), and healthy subjects (n=7) (Lotze et al. 2001). In patients with PLP, reorganization of the hand and lip areas in M1 and S1 was observed, while no changes were found in other groups.

Using BOLD fMRI to research cortical differences includes several disadvantages that should be taken into account. The most important drawback is the measurement time. The hemodynamic response function (HRF) is an increase in oxygenated blood (particularly oxyhemoglobin) compared to a resting state, which is used in BOLD fMRI comparison. The underlying theory is that increased neuronal activity causes a causal, time-delayed rise in blood in a specific area. Since stimuli do not evoke immediate responses, this information explains fMRI's intensive time requirements.

Beyond the biological system's time dynamics, problems with a signal-to-noise ratio (SNR) play a greater role in long experimentation paradigms. To compensate for the low SNR, fMRI paradigms usually employ signal averaging, resulting in longer measurement times.

PET is a method of studying neuronal function that has been used to research cortical remapping and brain plasticity used in nuclear medicine (Strelnikov et al. 2015). PET uses the inserted radioactive substances (radiotracers) into the bloodstream that reveals how the brain functions by constructing a three-dimensional image. The tracer is a biologically active molecule selected based on the trait being studied; for example, the tracer H2 15O is used to study cerebral blood flow, while fludeoxyglucose (18F) is used to study glucose metabolism (Tai and Piccini 2004). While PET is a valuable tool for research and diagnosis, the use of radioactive isotopes has health implications (Karakatsanis et al. 2015).

Changes in bioelectrical potentials on the scalp can result in case nociceptive information is sent to different cortical/subcortical structures. EEG can accurately represent brain dynamics with high temporal resolution (lower than ms) as a noninvasive recording of brain electrical activity. Multiple electrodes are implanted on the scalp to record the brain's electrical activity across time. The EEG signal is mainly produced by pyramidal neurons in the cortex, and signals originating deep in the brain are less contributing (Crosson et al. 2010). EEG signal is mainly generated by the electrical activity of excitatory and inhibitory neurons in brain sources (i.e., a larger population of neurons with similar spatial orientations). These electrical activities are transmitted to the EEG electrodes through volume conduction (Nunez and Srinivasan 2009). The volume conduction sources are considered the spherical geometry of the head and different brain tissues with various conductivity (i.e., cerebrospinal fluid, dura, skull, and scalp) between brain sources and EEG electrodes (Haueisen et al. 2012). Therefore, the recorded EEG signals are generated from the activity of different brain sources, not specific brain sources. This mixing effect (so-called volume conduction) results in artificially correlated signals which should carefully consider in data analysis.

Brain function in response to experimental and clinical pain has been investigated using EEG recordings. The oscillatory dynamics of EEG have been shown as a biomarker to investigate the cortical activity. The most common frequency bands of EEG signals are categorized as δ (0.2-3 Hz), θ (4-7.5 Hz), α/mu (8-13 Hz), β (14-30 Hz), and γ (14-30 Hz). It has previously been demonstrated that the pain intensity level and spectral power of δ, θ, and β bands are correlated (Stevens et al. 2000). Another study has shown that the spectral power of the resting EEG of patients with chronic neuropathic pain was higher than healthy subjects (over the frequency range of 2-25 Hz). In addition, Sarnthein et al. reported spectral power reduction in neurogenic pain patients after thalamic surgery, which had returned to normal levels 12 months later, implying that EEG power is linked to the amount of neurogenic pain.

Moreover, Huber et al. used thermal stimulation to induce tonic experimental pain and found significant changes in EEG oscillation (Sarnthein et al. 2006).

Despite EEG disadvantages due to volume conduction and poor spatial resolution, it has many advantages over other neuroimaging techniques. Although fMRI and PET have time resolutions ranging from seconds to minutes, EEG has a distinct advantage over current fMRI and PET neuroimaging methods with high temporal resolution and low hardware requirements. Somatosensory evoked potentials (SEPs) and functional connectivity between various brain areas in the cortical and subcortical stages are powerful methods to examine the activations of the brain's underlying mechanisms in response to pain sing EEG. These methods will be addressed in the following paragraphs.

2.3.1. SOMATOSENSORY EVOKED POTENTIALS (SEP)

Evoked potentials are widely used to study the functionality of the somatosensory system, including nociceptive pathways (Arguissain et al. 2015; Dhillon et al. 2004; Mouraux and Iannetti 2018). Evoked potential are cortical responses following a brief stimulation (electrical, visual, thermal, etc.) applied to a specific body part and recorded at the scalp using EEG. Electrical potentials produced in sensory pathways at the cortical, spinal, and peripheral levels in response to peripheral stimulation are known as somatosensory evoked potentials (SEPs). SEPs have been used to study cortical reorganization associated with severity and perception of pain (Flor 2002, 2003). The loss of tonic inhibition in tactile afferent has been reported to correlate with chronic pain (Treede 2003). It can impact the SEPs to external stimulus in chronic pain patients, resulting in the cortical reorganization, as seen in phantom limb pain. As a result, the SEPs biomarkers can be utilized to study cortical reorganization in the nociception system after damage or lesions to the peripheral nervous system. The spatial and temporal characteristics of the modulated SEP have been reported as powerful tools to investigate brain dynamics. These characteristics are included but not limited to the amplitude, dipole, latency, and topography analysis of SEPs.

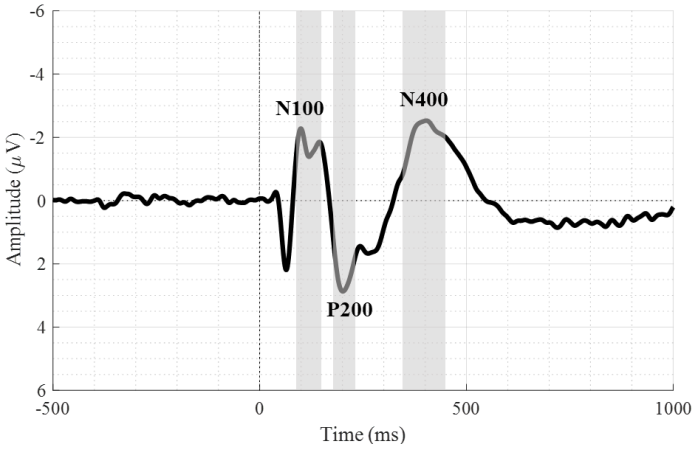

Figure 2.1. Illustration of group averaged somatosensory evoked potential recorded from C4 channel induced by electrical stimulation.

Evoked potentials are divided into early, late, and ultra-late components and are represented by their polarities (positive (P) and negative (N)) as well as latencies and amplitudes. The latencies of these components vary depending on the body site and stimulation modality. However, in laser evoked potential (LEPs), the intervals for early, late, and ultra-late components are <200 ms, 230-380 ms, and more than 800 ms, respectively (Hu et al. 2014; Truini et al. 2005). The late components are assumed to represent A-δ and C-fibres activity, respectively, and are reported to be closely related to nociception. The N1, N2, and P2 waves are the key components of interest when studying pain-related evoked potentials (Valeriani et al. 2012). The N1 wave is thought to represent the earliest nociceptive feedback to the cortex (negative wave in a approximate time window from 80-140 ms). At the same time, the N2/P2 peak-to-peak amplitude is the factor most related to nociception, with a greater N2/P2 amplitude associated with higher pain (N2 and P2 as positive and negative waves in a approximate time window from 200-300 ms). This late portion of the EP has been demonstrated to capture both the affective and sensory-discriminative pain components (Greffrath et al. 2007; Pazzaglia et al. 2016). Using EEG and MEG, a study investigated the sequence of activation in the cortex following peripheral stimulation and reported almost simultaneous SI, SII, and insular cortex activation (in parallel) (Kakigi et al. 2004). They also reported that these brain regions are correlated with pain discrimination which can be identified with the early and middle SEP waves. Moreover, the cingulate cortex and the medial temporal regions around the hippocampus and amygdala have been reported to activate following painful stimulation. These brain areas are more connected to the emotional and cognitive dimensions of pain.

Intracortical source localization

Many studies have been performed to understand the underlying brain source activity that generates the EEG. Different solutions have been suggested to transfer from the electrode domain (EEG signals recorded from scalp) to the source domain (brain activity of different brain regions). Solving inverse and forward problems are the main aspects of this transformation. Electrical activity from active neurons in the brain can represent and form electrical source activity, which is the starting point of the forward problem. Following the forward problem, EEG electrodes on the scalp recorded the electrical activity from configured sources. The forward problem has a direct solution in the case that the shape and distribution of brain sources are recognized with the high-temporal resolution, and the information of volume conduction and the conductive characteristics of the brain areas are considered with high spatial resolution. On the other hand, calculating the brain source activity from scalp electrodes (EEG signal) is considered the inverse problem (Rushton 2002), which has no straightforward solution. There is no unique solution for the inverse problem. The same EEG signal can be generated from different source configurations. Unique inverse solution (unique source localization) can only be obtained by EEG recording using the infinite number of electrodes. However, the brain source activity can be estimated from signals from scalp electrodes if physiologically and physically accurate prior limitations are addressed. Details such as the number and type of sources and source location have been considered as helpful constraints to solve the inverse problem (Malmivuo and Plonsey 2012). For example, Koles et al. have specified the skull and ventricles of the brain as the areas with no brain source activity, which resulted in eliminating many incorrect source configurations. While an incorrect combination of these constraints may give a solution that does not provide any physiologically meaningful information about the generators, these factors play an essential role in the proper inverse solution (Koles 1998).

Several algorithms have been suggested to estimate the brain source activity from recorded EEG signals. The main criteria in most of these algorithms is selecting the model with minimum overall source activity and providing the recorded EEG distribution (Hämäläinen and Ilmoniemi 1994). While Minimum-Norm Estimate has been previously used to handle the model selection, the deeper brain source activities are not estimated accurately in this method (Choi and Kim 2018; Luck 2005; Michel and Brunet 2019). Weighted Minimum-Norm Estimates has been proposed to address this issue, and it is implanted in the low-resolution electromagnetic tomography (LORETA) algorithm (Pascual-Marqui et al. 1994). LORETA has been widely used in different neuroimaging studies to estimate brain source activity from EEG signals (Jiang et al. 2019; Stefanie et al. 2011; Stern et al. 2006a). A Laplacian operator in LORETA is used for source localization based on the smooth spatial distribution (as the activity of neighboring neurons are correlated) (Choi and Kim 2018; Luck 2005; Michel and Brunet 2019). Using the Montreal Neurological Institute (MNI-305) template that limited the source distribution to cortical gray matter, the spectral

density of intracerebral brain volume is divided into 2394 voxels at 7 mm spatial resolution (Collins et al. 1998).

In order to analyze the brain activity in the source domain, the characteristics of the region of interest (ROI) should be specified based on the study objectives. The neuroimaging method, anatomical parcellation schemes, and the type of connectivity method have been reported as essential considerations for ROI selection (Rubinov and Sporns 2010). In analyzing the brain network, proper selection and the size of selected ROIs has to be considered properly. The node can be considered as an ROI in brain network functional connectivity. On a microscopic level, the individual node can be reflected by each neuron, while the connection between nodes (i.e., edges) represent by the synapses (Watts and Strogatz 1998). However, anatomically defined template maps represent the nodes on a macroscopic level. Using anatomical templates such as Brodmann areas or the Automated Anatomical Labeling (AAL) atlas has enabled researchers to conduct different comparable studies in both functional and structural networks.

2.4. BRAIN FUNCTIONAL CONNECTIVITY

Structural and functional connectivity are two main modes of brain connectivity. The presence of a physical link as a direct connection between two nodes is defined with structural (or anatomical) connectivity. At the microscopic level, the structural connection between neurons (synaptic strength) has been shown as a challenging problem. Structural connectivity can be visualized with diffusion-weighted imaging or diffusion-weighted imaging of the corticospinal tract. Moreover, diffusor tensor imaging (DTI) is a recently advanced imaging technique that represents axonal fibers interconnecting two regions (Alexander et al. 2007). Despite the useful information acquired through structural connectivity, the connectivity between regions can not be only explained by the anatomical connection. The structural connection does not indicate information transmission through that connection. Additionally, the transmission characteristics in a structural connection are subjected to change (i.e., either slowly or rapidly).

Therefore, anatomical connectivity can be complemented using functional connectivity as it assesses the interaction between two neural sources. Analyzing functional connectivity using EEG is a popular method for assessing cortical activity and has been widely used (Barzegaran and Knyazeva 2017; Bramati et al. 2019; Furman et al. 2018).

Brain regions involved in pain have been investigated in numerous studies, and as a result, an extensive brain network associated with pain, such as the pain matrix, has been suggested. The primary somatosensory cortex (S1), the secondary somatosensory cortex (S2), insular cortex, and anterior cingulate cortex have been mentioned to play an essential role in this pain matrix. Chronic pain has been

mentioned to include structural and functional abnormalities within and between these pain-related brain regions (Apkarian et al. 2005; Peyron et al. 2000; Stern et al. 2006b).

2.4.1. FUNCTIONAL CONNECTIVITY

Analyzing connectivity has been done in two different domains in EEG studies, i.e. in electrode domain or source domain. In the electrode domain, an individual scalp EEG electrode is considered as a node while the activity of specific brain area registered as a node in source domain. Analyzing the connectivity in the electrode domain can be highly affected by the strong correlation with neighboring nodes because of the volume conductance problem (Stam et al. 2007). Analyzing functional connectivity and network analysis with the nodes constructed in the source domain has been suggested to overcome the EEG volume conduction issue (Dos Santos Pinheiro et al. 2016).

Functional connectivity represents the correlation between spatially remote neurophysiological events (Fingelkurts et al. 2005). The correlation between two time series (i.e. recorded activity at the nodes) is defined as functional connectivity (fig. 2.2). The connections between nodes could be either in the electrode domain or source domain. It has been studied using EEG as neuroimaging methods. EEG-based functional connectivity analysis is usually performed by filtering the data in different frequency bands. Based on the spectral analysis, frequency bands of Delta (0-4Hz), Theta (4-8Hz), Alpha (8-13Hz), Beta (13-32Hz), and Gamma (32-60Hz) are prevalent suggested frequency bands to analyze functional connectivity. This Ph.D. thesis included functional connectivity and network analysis in **study II** to investigate the alternation in functional connectivity between pain and sensation related brain areas following TENS.

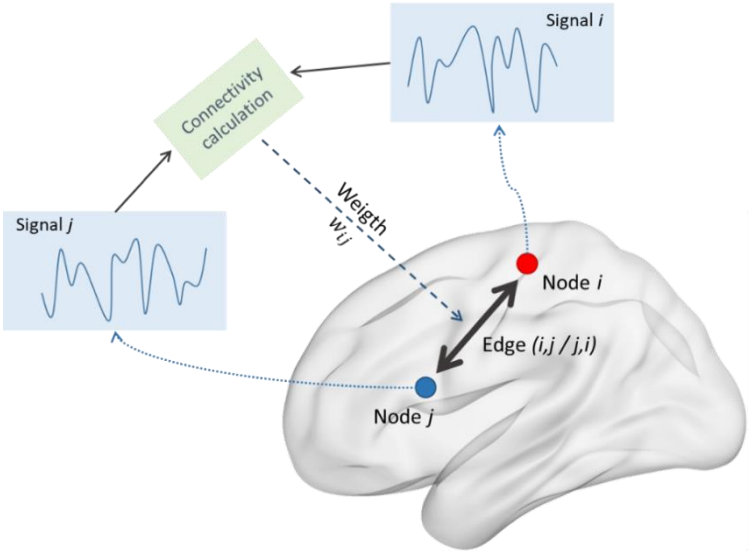

Figure 2.2. Example of the connectivity analysis of a graph from two brain signals. Node i and j can refer to brain sources or EEG electrodes, and signal i and j is the electrical activity of these areas. The estimated connectivity between two signals is considered the wight of the edge (connection) between two nodes.

Although, different methods have been suggested for source localization, the way that these methods deal with volume conduction issue play an important role. However, several methods have been developed to address and deal with the volume conduction influence on analyzing functional connectivity. The following paragraphs review some of these methods.

Coherence

The phase synchronization of EEG dynamic activity termed coherence has been widely used as an index of functional connectivity (Fries 2015; Miskovic and Keil 2015; Sun et al. 2004).Coherence is a simple connectivity measurement described by the correlation between two signals in the spectral domain. While coherence is considered as phase-based connectivity, it reflects the timing of activity with or between neural populations. Coherence between two signals (e.g., x(t) and y(t)) is defined by:

$$\boldsymbol{Coh_{xy}} = \frac{\left|\boldsymbol{S_{xy}(f)}\right|^2}{\boldsymbol{S_{xx}(f)S_{yy}(f)}} \qquad \textbf{(2.1)}$$

Where $S_{xy}(f)$ is the cross-spectral density between x and y, and $S_{xx}(f)$ and $S_{yy}(f)$ are the power-spectral density of x and y. It should be mentioned that while the coherence algorithm is affected by volume conduction artifact (Bastos and Schoffelen 2016) (Sakkalis 2011), the results must be interpreted with caution. It has been shown that coherence is highly influenced by inter-nodes distance (i.e., high coherence value for neighboring electrodes).

The volume conductance issue is considered as a synchronized activity, while the volume conductance issue instantaneously appears in all electrodes. Consequently, it should be taken into account that any amplitude-based or frequency synchronizations-based connectivity measurement is affected by volume conduction.

Phase Synchrony

Phase locking values (PLV) is another frequency-based connectivity method that evaluates the phase and amplitude synchrony between two time series from pairwise nodes. The PLV is calculated by calculating instantaneous amplitudes and phase of the signal following band-pass filtering of the signal. Florian et.al. suggested extracting the amplitude A(t,f) and phase Φ(t,f) information synchrony by convolving s(t,f) (the band-pass filter the signal s(t) around the frequency f) of each individual electrode with a Gabor wavelet (Florian et al. 1998).

$$\begin{aligned} \boldsymbol{C(t,f) = s(t,f) * G(t,f)\ with\ G(t,f)} \\ \boldsymbol{= \exp\left(-\frac{t^2}{2\sigma_t^2}\right)\exp(j2\pi ft)} \end{aligned} \tag{2.2}$$

$$\boldsymbol{A(t,f) = \sqrt{Re(C(t,f))^2 + Im(C(t,f))^2}} \tag{2.3}$$

$$\boldsymbol{\varphi(t,f) = \arctan\left(\frac{Im(C(t,f))}{Re(C(t,f))}\right)} \tag{2.4}$$

The average phase differences value across all trials defined the PLV.

$$\boldsymbol{PLV_t = \frac{1}{N}\sum_{1}^{N}(\exp(j\Delta\varphi(t,n)))} \tag{2.5}$$

PLV is close to 1 when two nodes are perfectly synchronized over trials, and PLV values close to 0 represent no synchronization. While PLV is defined based on the phase synchrony, a more precise connectivity assessment can be obtained through PLV compared to the coherence. Despite the advantages of PLV, the volume conduction issue can not be addressed in applying this technique.

Phase Lag Index (PLI)

Phase Lag Index (PLI) has been widely suggested as a connectivity measure to minimize the effect of volume conduction (Stam et al. 2007). The PLI is based on the non-zero lagged phase coupling and eliminates the volume conduction issue by not considering the amplitude or the phases (Yu et al. 2018). PLI represents the asymmetrical phase activity between two nodes. In line with this, the continuous phase variations between two sources should frequently be either smaller or greater than zero for a given time window. The following equation can mathematically explain the PLI calculation.

$$\boldsymbol{PLI = \langle sign[\Delta\varphi(t_k)]\rangle} \qquad \textbf{(2.6)}$$

The average of the sign of successive phase differences describes the PLI. The PLI values are between 0 to 1 as no coupling and perfect coupling, respectively. The direction of the coupling can be extracted by not using the absolute value of PLI. PLI is easy to interpret as the ideal coupling between two nodes is considered if one node is continuously in phase advance or phase delay with the other node. No connection between two nodes can be achieved by the constant but 0 mod π phase delays. While the volume conduction issue leads to phase synchrony between different nodes, the PLI technique eliminates this effect by discarding zero-phase activity between nodes. We used PLI in the second study to evaluate functional connectivity.

2.4.2. NETWORK ANALYSIS (GRAPH THEORY)

Moving beyond analyzing pairwise connectivity, considering the brain as a network of interconnected areas has been widely used in recent brain connectivity studies. In this view, the complex phenomenon is eventually supported by the organized activity between all brain regions (Bassett and Sporns 2017). Different methods have been suggested to analyze these networks, and among them, graph theory as a mathematic tool has been widely used and shown as a reliable technique (Lee et al. 2020a; Nickel et al. 2020b; Ta Dinh et al. 2019). As the graph presents a simplified view of a complex system, considering the brain network as a brain graph has been recently commonly discussed (Farahani et al. 2019; He et al. 2019; Medaglia 2017).

A functional brain network is a representation of the brain that is defined by nodes and their pairwise connections. As previously mentioned nodes can represent either electrodes or brain areas. Nodes are considered as brain regions in a functional brain

network, and their pairwise connection as edges denote the functional connectivity between two nodes. A brain functional network is obtained by a matrix including all the pairwise connections. The matrix of connection is created by connectivity calculation between all possible node combinations when each row represents a node, and each column illustrates the connectivity index of the selected node and all other nodes in the network. The functional connectivity can also include the casualty information as the direction fellowing information. Thus the network matrix could be non-symmetric (Bastos and Schoffelen 2016). Moreover, the edges can be weighted or unweighted. The connection strength or causal interactions are considered weighted edges. A weighted network can be binarized or unweighted using the thresholding technique, and the resulted binarized edges represent the presence or absence of a pairwise connection.

The higher-order structure of the brain networks has been commonly investigated using graph-theoretic measures. Topological characteristics of brain functional/structural networks have been widely investigated using graph theoretical approaches (Lee et al. 2020a; Nickel et al. 2020b; Ta Dinh et al. 2019). Specific pattern patterns of network structure have been mentioned in different naturally occurring networks such as the brain. Several complex network indexes can be utilized to examine the functional network and identify different aspects of local or global brain functional connectivity. For example, the sum of all edges to a selected node is defined as the node strength, which shows how other region's activity influences the desired brain region. In the following paragraphs, some selected network indexes used in this thesis to investigate the brain network alternation following TENS are briefly presented.

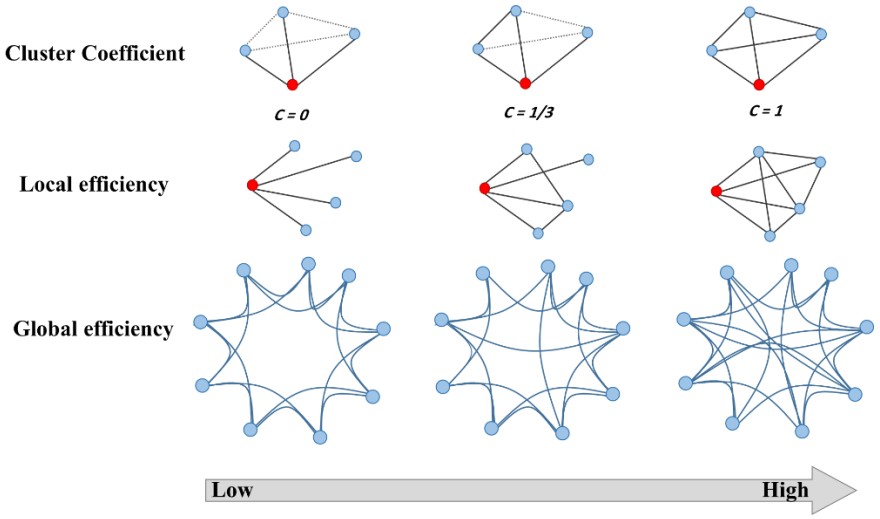

Figure 2.3. Illustration of three theoretical graph indexes for the network analysis used in this thesis.

Clustering coefficient

The extent to which nodes tend to cluster mutually is evaluated by the clustering coefficient. The fraction of connected triangles around a node shows the clustering coefficient. The brain networks can be considered as small worlds while different regions can function independently but are connected to the other regions(nodes) through hubs (Lee et al. 2020b). A high clustering coefficient shows the presence of local clusters developing specialized functional units. Imagine G is the weighted network, the clustering coefficient (Ci) for node i is defined as:

$$\boldsymbol{c_i = \frac{2}{d_i(d_i - 1)} \sum_{i,k} (\widetilde{\omega}_{ij} . \widetilde{\omega}_{jk} . \widetilde{\omega}_{ki})^{1/3}} \quad \textbf{(2.7)}$$

where $\widetilde{\omega}_{ij}$ is normalized to the maximum value in the network. $d_i(d_i - 1)$is the maximum value of edges when the subgraph of neighbors of node i is totally connected (Antoniou and Tsompa 2008). The global clustering coefficient is defined by averaging the local clustering coefficient for the entire network.

$$\boldsymbol{C = \frac{1}{N} \sum_{i}^{N} c_i} \quad \textbf{(2.8)}$$

Where N is the number of nodes in the network (Costa et al. 2007). The value for both C and c_i is between 0 to 1. A node with a fully interconnected cluster is depicted with $c_i = 1$ and $c_i = 0$ if there is no connection between node i and its neighbors..

Strength

In a weighted network, strength depicts the basic structural characteristics. Node strength is the sum of the weighted edges connected to the selected node (Guo et al. 2019). Consider w as the weighted network matrix, the strength can be formulated as:

$$\boldsymbol{S_i = \sum \omega_{ij}} \quad \textbf{(2.9)}$$

Where j indicate nodes neighbor node i. nodal strength reveals the strength of interconnectivity with other nodes (Mieghem 2010).

Efficiency

Network efficiency is an index of functional integration and estimates the efficiency of information flow within a network. The global efficiency is considered by the

average inverse of the shortest path length, which indicates the minimum connected distance of two nodes in the network (Bullmore and Sporns 2009; Harrington et al. 2015). While the global efficiency measure is not affected by short paths, it is admitted a very reliable index of functional integration (Achard and Bullmore 2007). The calculation of the global efficiency can be formulated as:

$$\boldsymbol{E_{global} = \frac{1}{N(N-1)} \sum_{j,k \in G_i} \frac{1}{L_{j,k}}} \quad \textbf{(2.10)}$$

Where N is the number of nodes in the network and $L_{j,k}$ is the average path length between node i and j in the network. The maximum global efficiency in the network is depicted by $E = 1$, and $E = 0$ shows no global efficiency.

From a local view, local efficiency indicates the ability of a node to flow efficient information. The local efficiency of a node is calculated as the inverse of the average shortest path between the selected node and all neighbors of that node (local subgraphs). Local efficiency is formally calculated as:

$$\boldsymbol{E_{local} = \frac{1}{N_{G_i}(N_{G_i}-1)} \sum_{j,k \in G_i} \frac{1}{L_{j,k}}} \quad \textbf{(2.11)}$$

Where N_{G_i} depicts the set of nodes in the subgraph G_i . High local efficiency in functional brain networks implies a topological organization characteristic of segregated neural processing (Drakesmith et al. 2015). Moreover, the tendency of the node to effectively share information within their neighboring nodes is revealed by local efficiency.

2.5. TREATMENT OF PLP

Common treatments used for PLP can be classified as noninvasive, invasive, and pharmacological methods. Below is a brief description of each category.

2.5.1. PHARMACOLOGICAL

It has been found that opioids are an effective treatment for relieving the symptoms of PLP (Alviar et al. 2016). This efficacy has been observed for intravenous and oral use of morphine to treat phantom pain (Huse et al. 2001). PLP relief using morphine was observed in up to 50% of patients with PLP (Wu et al. 2002). Despite the efficiency of opioids in PLP reduction, they have common side effects, including dizziness, tiredness, constipation, itching, sweating, nausea, urination difficulties, shortness of breath, and vertigo (Huse et al. 2001). Also, opioid has a higher potential for addiction, which is especially important for the veteran population with several other illnesses than other psychiatric disorders like post-traumatic stress illness

(Wilder et al. 2016). Many medical interventions have been reported, such as antidepressants, N-methyl D-aspartate receptor antagonists, β-blockers, neuroleptics, anticonvulsants, and muscle relaxants (Alviar et al. 2016). Despite many medicines or combinations of medicines that have been tried over decades, different results have been obtained. However, these medication has a temporary effect on PLP, and a type of treatment with long-effect is needed.

2.5.2. INVASIVE TREATMENT

Deep brain stimulation and stump revision have been reported as two invasive treatments for PLP (Tintle et al. 2012). While these treatments are invasive in the brain and stump, the potential side effects should be taken into account. Revision of stump for a prosthesis can be performed because of the deal with and manage skin scarring, bone shape, or chronic wounds that prevent the prosthesis from installation properly.

Deep brain stimulation requires applying electrical stimulation through the electrodes placed deep in the brain to stimulate specific brain regions or target specific cells to stimulate certain neurotransmitters (Farrell et al. 2018). Several neurological illnesses such as chronic pain, parkinson, and tourette syndrome have been reported to be treated by DBS (Daneshzand et al. 2018; Smeets et al. 2018). DBS is a successful chronic pain treatment when failing other treatments like medications as well as other conservative measures (Boccard et al. 2015). PLP reduction (up to 60%) has been reported by applying DBS in the peritoneal grey matter and the somatosensory thalamus sensory (Abreu et al. 2017). Like most treatments of phantom limb pain, a small population is included in such studies, hence, more research is needed to verify the efficiency of DBS as a treatment for PLP. In conclusion, chronic pain and these small phantom limb studies generally represent the promise of deep brain stimulation efficacy.

Spinal cord stimulation (SCS) has also been mentioned as a possible treatment for PLP reduction. In this method, mild electrical current is applied to the spinal cord through the electrodes placed in the dorsal epidural space (Eldabe et al. 2015). Although the underlying mechanism of SCS is not well known, two mechanisms have been proposed. First, alternation in the chemical transmission of the dorsal root following SCS and Second as activation of dorsal column nuclei (Smits et al. 2013). However, the results of SCS as a PLP treatment are mixed, and several side effects have been reported for using SCS (McAuley et al. 2013; Viswanathan et al. 2010). As mentioned before, studies with invasive treatment had small sample sizes, and further research with more PLP patients is needed to verify the effectiveness of these methods. However, results from invasive treatments have shown neurostimulation to be promising. In section 2.5, we report the efficiency of TENS as a noninvasive neurostimulation method on pain relief and neurorehabilitation.

2.5.3. NON INVASIVE TREATMENTS

Transcutaneous electrical nerve stimulation (TENS) and mirror therapy are two noninvasive options for phantom limb pain treatment. Later in this chapter, TENS will be addressed in detail.

Mirror therapy has been suggested as a noninvasive, low-risk, and effective treatment option for PLP in both upper-limb and lower limb amputees. The effectiveness of mirror therapy is not limited to the PLP; it has even been shown to benefit people with strokes and Parkinson's disease (Bonassi et al. 2016; Pérez-Cruzado et al. 2017). Mobility enhancement, motor recovery, and pain reduction have been reported following mirror therapy in people with these conditions. The use of mirror therapy has been shown to be successful in PLP relief. This effect was reported to link with a reduction in pain intensity and pain duration (Timms and Carus 2015). Mirror neurons are thought to be one of the key mechanisms for mirror therapy. Mirror neurons are assumed to be activated when a person sees their limb reflected in a mirror (Foell et al. 2014).

2.6. TRANSCUTANEUS ELECTRICAL NERVE STIMULATION

Case studies have shown that the potential of electrical stimulation of the residual limb using TENS or functional electrical stimulation (FES) in pain reduction. Studies have also been reported that TENS intervention as a noninvasive treatment can improve analgesic consumption and medication-related side effects (Katz and Melzack 1991; Tilak et al. 2016). The application of TENS as a tool for pain management in chronic pain patients has shown short-term pain relief. The sensory nerves are stimulated/excited through the electrical current applied by TENS.

Desensitization (i.e. suppress or normalize the responsiveness of the body to special sensations) induced by TENS has been shown to relieve PLP in a number of placebo-controlled trials and epidemiologic surveys (Baron 2006; Halbert et al. 2002). Tilak et al. has reported TENS as an effective pain relief technique for amputees suffering from PLP (Tilak et al. 2016). However, no study has revealed the long-term PLP reduction following TENS treatment. PLP reductions after a year of TENS therapy have been shown to comparable PLP placebo reductions in some trials. The application of TENS has also been found to be an effective option to relieve stump pain (Mulvey et al. 2013).

TENS provides different analgesic effects and cortical modulation depending on the characteristics of delivered electrical stimulation, including intensity and frequency (Schabrun et al. 2012). High-frequency, low-intensity stimulations (HF-TENS; >10 Hz, low and not painful intensity) and low-frequency, high-intensity stimulations (LF-TENS; < 10 Hz, strong and not comfortable intensity) are the most commonly used TENS stimulation pardigmes (Peng et al. 2019). HF-TENS stimulations lead Aβ-

fibers to depolarize, resulting in segmental analgesia via gate control mechanisms (Gozani 2019). The intense stimulations applied by LF-TENS, on the other hand, depolarize Aδ and C fibers and reduce pain by activating descending pain modulation pathways that originate in the brainstem (Willer et al. 1999). Both HF and LF-TENS induce analgesia by releasing endogenous opioids, while the analgesia of HF-TENS and LF-TENS are mediated by δ and μ opioid receptors, respectively (Leonard et al. 2010; Ng et al. 2003).

The various electrode designs also have a significant impact on the lasting effect of analgesia induced by TENS. Studies have shown that TENS electrodes stimulate the entire A-fiber spectrum with no preference. According to the gate control theory, conventional TENS affect Aβ-fibers, and inhibitory GABAergic interneurons which mediate the hypoalgesic effect (Melzack and Wall 1965). Longer-lasting hypoalgesia after tolerable painful TENS often requires Aδ fiber recruitment, but only for a short time.

Other types of electrical stimulation have also been shown to be successful. Peripheral nerve stimulation demonstrated a meaningful effect on pain and quality of life (Rauck et al. 2014). However, the trial lacked a placebo control group and had a limited number of patients. The effectiveness of TENS on other body parts than the residual limb, such as the contralateral limb and ears, has also been investigated (Katz and Melzack 1991; Tilak et al. 2016). In case studies, short-term trials, both of these approaches had a favorable impact, but neither was compared to placebo groups. A study with ten amputees reported PLP reduction and cortical reorganization following TENS-based sensory discrimination training (Flor et al. 2001). Sensory discrimination included random, meaningless electrical stimulation patterns of varying frequency, intensity, and position. With the hypothesis that distraction from pain actually decreases pain, amputees were asked to recognize various patterns. Although the long-term effects of the sensory discrimination method have not been reported, they reported a positive correlation between discrimination ability, cortical reorganization, and PLP reduction.

CHAPTER 3. OUTLINE OF PH.D. WORK

In the previous chapters, it was shown that chronic pain and sensation involved in complex mechanisms in the brain and different brain regions such as SI, SII, Insula, ACC, and mPFC play an important role in sensation. Brain reorganization at SI has been mentioned as one of the main causes of PLP, which can be assessed with SEP. Moreover, alternation in the brain network associated with pain and sensation has also been reported as a valid biomarker in pain studies. While different methods have been established to treat with PLP, neurostimulation has shown promising results in long-term PLP reduction. TENS has been reported as a noninvasive and effective treatment in chronic pain reduction and neurorehabilitation. Although several mechanisms on the role of the peripheral nervous system in TENS mechanism have been suggested, the underlying mechanism of TENS on the central nervous system with possible PLP relief is not well-known.

3.1. AIM

The objective of this Ph.D. thesis is to investigate the possibly altered brain mechanism and pain/perceptual response following the application of TENS.

The following research questions raised to address the thesis objectives:

Q1. How does somatosensory cortex activity alter following TENS, and how long does the TENS induced changes remain?

Q2. How does the TENS intervention affect the functional connectivity between the brain areas involved in sensation and pain processing?

Q3. To what extent does the TENS induce cortical alterations in amputees, and can the changes be associated with PLP reduction?

3.2. SOLUTION STRATEGY

Three different studies were designed and conducted to address the thesis questions. Brain activity was examined using EEG signals through different methods such as SEP and functional connectivity. However, our findings from the first two studies were compared and verified with the results from Study III on two amputees with PLP.

Study I. Zarei A.A, Faghani Jadidi A, Lontis E.R.R, Jensen W., Short-term Suppression of Somatosensory Evoked Potentials and Perceived Sensations in

Healthy Subjects Following TENS. IEEE Transactions on Biomedical Engineering, vol. 9294, Jan. 2021. (Published).

The objective was first evaluated on a large sample size of healthy subjects to provide an adequately large and homogeneous subject population. The Study I was conducted to evaluate the effect of TENS on perceived sensation and induced changes in SEP waves since the SEP waves have been reported as a verified biomarker for assessing pain and sensory processing.

Study II. Zarei A.A, Atashzar S.F, Faghani Jadidi A, Lontis E.R.R, Jensen W., Gamma-band Suppression of Functional Brain Connectivity Following TENS. Under revision in IEEE Journal of Biomedical and Health Informatics (submitted on 07 May 2021).

We investigated the brain functional network alternation following TENS in Study II.

Study III. Zarei A.A, Lontis E.R.R, Faghani Jadidi A, Jensen W., Cortical Alternation in Amputees with Phantom Limb Pain Following TENS. IEEE Transactions on Neural Systems and Rehabilitation Engineering, (Under preparation)

While the first two studies were conducted on healthy subjects, the induced changes following TENS in the brain and associated PLP relief were evaluated in PLP patients and compared with healthy subjects in Study III.

CHAPTER 4. METHODOLOGICAL APPROACHES

4.1. PROCEDURE AND STUDY DESIGN

A summary of the study design and the experimental procedure for each study are included in this section. The North Denmark Region Committee on Health Research Ethics (N- 20180049) approved all procedures in this thesis. The first two studies followed the same procedure as they were conducted on the same group of subjects. In Study III, almost the same experimental setup as the first two studies were used as the objective was to verify the results from the first two studies on PLP patients.

4.1.1. STUDY I AND STUDY II

Experimental setup

Forty healthy subjects (20 men) were recruited in these studies. Subjects were randomly assigned to either an intervention group (TENS) or a sham group. Four recording phases were defined to evaluate the effect of TENS intervention. A baseline recording before the TENS intervention (Pre), immediate after the intervention (Post0), 30 minutes after the intervention (Post30), and an hour after intervention (Post60) (Fig 4.1). Each recording phases consisted of EEG recording, reaction time recording, and perceived sensation recording.

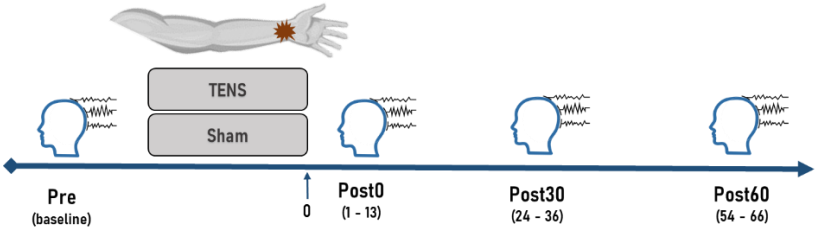

Figure 4.1. Overview of experimental procedure in Study I and Study II

TENS

Subjects in the TENS group received biphasic electrical pulses (100 Hz with 1 ms pulse width) on their left median nerve with 20 s on-time and 10 s off-time stimulation for 20 min. The intensity was adjusted to 80% of their discomfort threshold. The staircase was conducted to evaluate the sensation and discomfort threshold (Biurrun Manresa et al. 2018). Moreover, subjects in the sham group followed the same

procedure but with different intensity and on-time stimulation. The electrical current intensity was set to the individual sensation threshold, and the electrical stimulation onset was set for 1 minute (no stimulation for the next 19 min). However, subjects were orally instructed as follows: "For the next 20 min, the electrical stimulation will be delivered to your median nerve. The perceived sensation corresponds to no/weak sensations to intense sensations" (Zarei et al. 2021).

Data Recording

The EEG data were recorded using a 64-channel EEG cap amplified by a BrainAmp MR plus amplifier (Brain Products, GmbH). EEG data were recorded with a sampling rate of 5 kHz while the reference electrode was placed at FCz electrode. 80 double pulses electrical stimulation with 6-8 s inter-stimulation interval was applied to the left median nerve to record the SEP. Electrical pulses were generated by DS5 current stimulator (Digitimer, UK). The electrical pulse intensity for SEP was adjusted to twice the individual sensation threshold (not muscle contraction). Reactions to the stimulation were recorded while the subjects were asked to push a button (held in the right hand) as soon as they felt each electrical stimulation. The intensity of the perceived sensation and the area of the perceived sensation on the hand were recorded following forty SEP responses for each recording phase.

4.1.2. STUDY III

Experimental setup

Two patients with PLP participated in this study. The first patient was lower amputee and the second patient had complex regional pain syndrome (CRPS) following brachial plexus injury in left hand. Unfortunately following technical problems happened at the recording site, the data from the first patient (amputee) was largely contaminated by noise and was not possible to processed EEG data. The second subject was 55 years old and got injured 34 years ago (in 1987) following car accident. The subject could not feel anything from the elbow to the fingers in his left hand.

TENS

TENS was applied to two predefined referred sensation areas for 30 minutes with the frequency of 20Hz and the pulse width of 600µs and 400µs with the intensity of 22mA. The intensity was adjusted to the patient's comfort. The effect of TENS on cortical activity and pain/sensation was evaluated before and immediately following the intervention

Data Recording

While the subject has lost 90% of his motor and sensation function in the left hand, SEPs were recorded by delivering electrical pulses to median nerve in both intact and the injured hands. The effect of TENS on cortical activity was assessed by comparing the SEP results following TENS with the baseline SEPs from both hands. First, the functionality of the median nerve from the left hand (injured hand) was compared

with the right hand (intact hand) at the baseline. Next, the changes in SEP results in each hand was compared with the correspond baseline activity to show the effect TENS.

4.2. DATA ANALYSIS

4.2.1. SOMATOSENSORY EVOKED POTENTIALS (STUDY I – STUDY III)

Time Domain

The effect of TENS on somatosensory evoked potentials was investigated by evaluating the SEP waves. N100, P200, and N400 SEP components were extracted using global field power in different time windows. The amplitude and latency of the extracted SEP components were compared between TENS and the sham group for up to an hour following the intervention.

Time-Frequency Domain

Since the dynamic activity of SEPs has been reported as a biomarker in pain/sensation assessment, the time-frequency activity of SEP phases at Pre and Post were compared between TENS and the sham group. Event-related spectral perturbation (ERSP) was utilized to analyze the time-frequency activity, and electrodes with significant changes following the intervention were defined for all SEP waves.

4.2.2. FUNCTIONAL CONNECTIVITY (STUDY II – STUDY III)

The off-line data processing in Study II was conducted in BrainVision Analyzer 2.2 software (Brain Products® GMBH) and Matlab 2020b. The raw EEG data was preprocessed, and the extracted epochs from the electrode domain were transferred to the source domain using the LORETA algorithm. Eight brain areas were selected to extract the source activity; the primary sensory cortex (SI, contralateral and ipsilateral to the stimulation site), the secondary sensory cortex (SII, contralateral and ipsilateral to the stimulation site), the medial prefrontal cortex (mPFC), the anterior cingulate cortex (ACC), and the anterior insula (contralateral and ipsilateral to the stimulation site). The time series activity of the selected areas were considered for functional connectivity analysis.

Functional connectivity method

Analyzing the functional brain connectivity in different frequency band have been reported previously (Tøttrup et al. 2020). The source activities were further filtered in six classic frequency bands [δ: 0.5-4, Hz θ: 4-8 Hz, α: 8-13 Hz, β: 14-40 Hz, γ: 40-90 Hz, and 0.5-90 Hz. The analytic signals, extracted by applying the Hilbert-transform, were used to calculate the functional connectivity. PLI was used to calculate the functional connectivity for all pairwise connections (between eighth ROIs, a total of 28 edges) at each frequency band (6 frequency bands), SEP phases (Pre, Post, Post30, Post60), and both TENS and sham group. The PLI index is between 0 to 1, indicating

the range between a weak connection to a strong connection between two nodes, respectively. The functional connectivity network at each frequency band was then considered for network analysis.

Network Analysis

Several graph-theoretical indexes have been suggested to analyze the brain functional connectivity network. The functional integration and functional segregation of the information in the network were analyzed using global efficiency and global cluster coefficient, respectively. Overall node network connection strength was also evaluated by global strength. These global characteristics of the network were examined across all six frequency bands for Pre and Post time phases between the TENS and Sham group. Moreover, the local characteristics of the brain network were analyzed by local efficiency, local cluster coefficient, and nodal strength. Brain Connectivity Toolbox (BCT) (Rubinov and Sporns 2010) was used for graph analysis.

CHAPTER 5. SUMMARY OF MAIN FINDINGS

5.1. SUMMARY STUDY I

Study I aimed to investigate the effect of TENS on the sensory-motor cortex, the modulation of somatosensory evoked potentials, and evoked sensation.This objective was evaluated on forty healthy subjects.

5.1.1. CORTICAL RESPONSE

To analyze the altered cortical activity following TENS on the sensory-motor cortex, the time windows for different SEP waves extracted using global field power (fig ***).

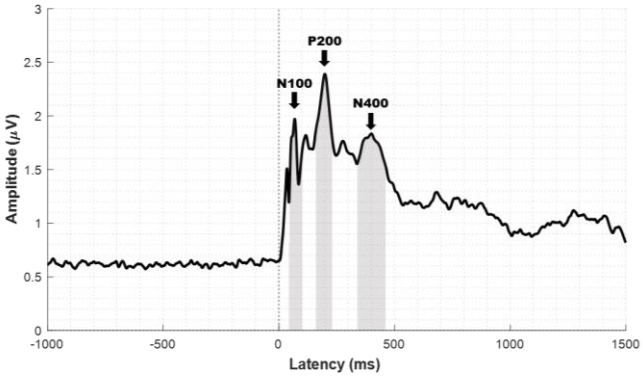

Figure 5.1 Grand-average global field power of the SEPs following double pulse surface electrical stimulation. Three SEP peaks can be mentioned. First, the N100 component as a negative peak in a time window from 80 to 140 ms. Second, a positive peak in the time window from 180 to 240 labeled as the P200 component. Third, the N400 component as a negative peak appears in a time window from 350 to 450 ms.

Results from ANOVA on the Cz channel revealed significant suppression of the N100 wave amplitude in the TENS group for at least up to an hour. Moreover, an inhibition in the amplitude of the P200 component following TENS was found up to 30 minutes following the intervention. However, no meaningful changes were found in the N400 wave amplitude and the N100, P200, and N400 latencies following TENS. Considering other channel locations, several channels with SEP waves amplitude affected by TENS were found in the TENS group. In contrast, no channel with statistically significant changes in the sham group was found.

5.1.2. DYNAMIC ACTIVITY

The time-frequency maps of the Cz channel extracted by ERSP were extracted and compared between Pre and Post in both TENS and sham groups. Results from ANOVA with false discovery rate (FDR) correction revealed significant suppression in thata and alpha band power following TENS. A significant decrease in alpha band was found at electrode locations Cz and C2 for the N100 time window and at electrode locations C2, C4, and Cp2 for the P200 wave.

5.1.3. PERCEIVED SENSATION

Reported perceived sensation and the area of evoked sensation following for each subject were individually normalized to the baseline. Non-parametric statistical analysis showed a significant effect of TENS on the perceived sensation at least up to an hour following the intervention (fig. 5.2). These findings were associated with a decrease in the area and the quality of evoked sensation on the stimulated hand.

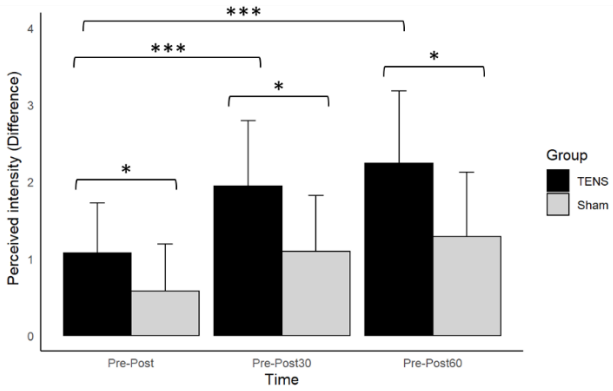

Figure 5.2. Mean ± standard deviation of changes in perceived sensation (normalized to the baseline recording) in TENS and sham group.

5.1.4. BEHAVIOURAL RESPONSE

The reaction time to each double pulse stimulation in SEP phases has been considered as a behavioral response (May et al. 2017). Moreover, the number of detections (with a reaction time of 150 ms <RT< 650 ms) at each SEP phase (Pre, Post0, Post30, and Post60) for each participant and group were analyzed (fig. 5.3). Statistical analysis results revealed no significant changes in reaction time and the detection rate following TENS compare to the sham group.

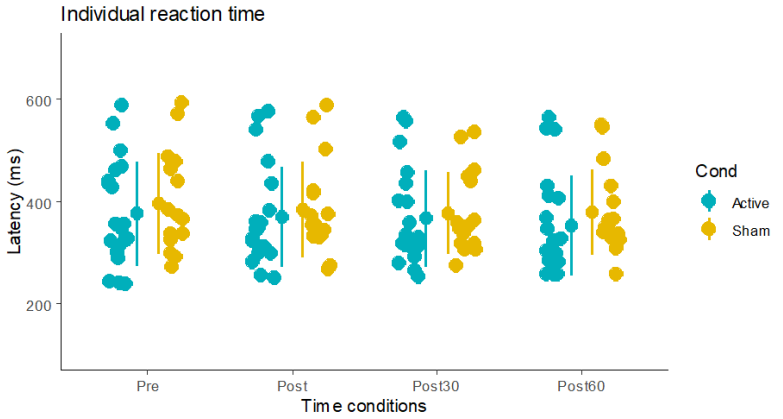

Figure 5.3 Individual reaction time and group-level mean ±std for each group and time conditions

5.2. SUMMARY STUDY II

The aim of **Study II** was to investigate the possible altered changes in the functional brain connectivity across the brain regions involved in sensation and pain processing. The mentioned objective was evaluated by analyzing the functional connectivity across different brain region sources localized by the LORETA algorithm.

5.2.1. FUNCTIONAL CONNECTIVITY

The functional connectivity (edge) of 28 pairwise connections (across eight ROIs) was measured at four time-points (SEP phases) across six different frequency bands for the TENS and sham groups. The functional connectivity for all connections followed an increasing pattern compare to the sham group in all frequency bands. However, a significant increase was found in the functional connectivity between right SI (contralateral to the stimulation site) and ACC and between right SI and mPFC in the gamma band, which lasted at least up an hour following TENS.

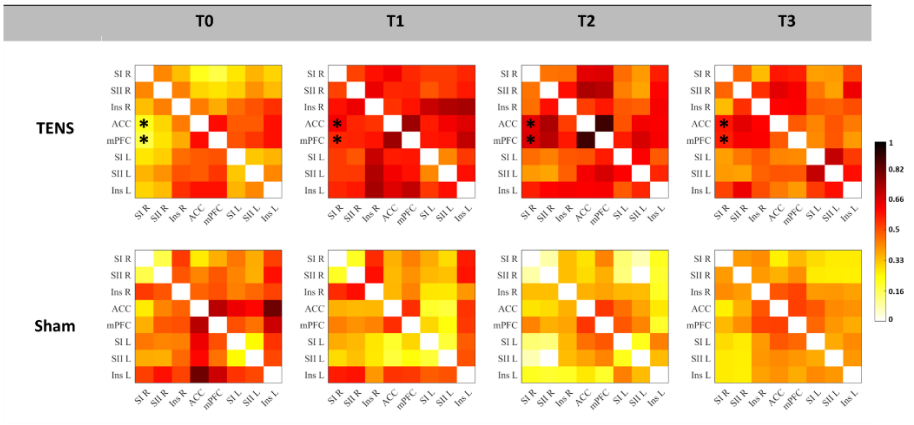

*Figure 5.4. The normalized, mean functional connectivity matrices calculated for the TENS (first row) and Sham (second row) groups at the four different time phases (four columns) in the gamma frequency band. Connections with significant changes (i.e., SI_R to ACC, and SI_R to mPFC) are highlighted with *.*

5.2.2. NETWORK ANALYSIS

Global and local characteristics of these networks were analyzed using well-known graph theory indexes such as clustering coefficient, efficiency, and strength. First, the changes in the global indexes of the network at each frequency band were measured for each group. Results showed that the global cluster coefficient, global efficiency, and global strength in the gamma band have significantly increased following TENS compare to the sham group. Three mentioned graph theory features were then measured for gamma-band at each node as local indexes. Results from local efficiency in right SI, right insula, ACC, and mPFC showed a significant difference between TENS and sham group. Also, analyzing the local cluster coefficient revealed that ACC, mPFC, and left SI have a significantly higher cluster coefficient in the TENS compared to the sham group. Finally, the local strength at ACC and left SI was found to increase following TENS intervention.

5.3. SUMMARY STUDY III

5.3.1. SOMATOSENSORY EVOKED POTENTIAL

As the patient reported no sensation in his left hand following brachial plexus injury, it was expected to record no SEP activity. Results from the baseline recording at the injured hand showed no SEP. Additionally, no changes in the magnitude of SEP waves at the left hand (injured hand) were found following the TENS intervention. However, in the right hand (intact hand) the magnitude of N100 and P200 waves decreased by 1.86 μv (from -4.86 to -3 μv) and 3.38 μv (from 7.32 to 3.94 μv) respectively following TENS intervention.

5.3.2. FUNCTIONAL BRAIN CONNECTIVITY

The PLI values for all pairwise connections of 8 ROIs (as defined in **Study II**) in six frequency bands were extracted. While the data has been recorded from a subject, the extracted PLI value in **Study III**, compared to the distribution of those functional connectivity results in **Study II**, which had significant change following TENS (i.e., SI-ACC and SI – mPFC in gamma band). This analysis revealed that the functional connectivity between SI-ACC and SI-mPFC increased following the TENS intervention and followed the trend as observed in the healthy subjects (study I and study II)

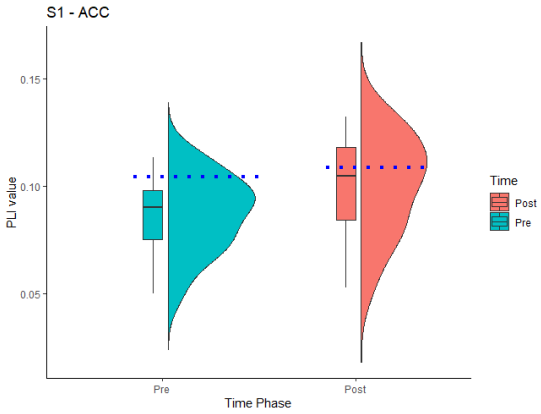

Figure 5.5 Average PLI value of the connection between SI-ACC for Pre and Post time phases illustrated by blue dash line. The half violin plot and the bar-chart showing the distribution of the same index in healthy subjects in the TENS group.

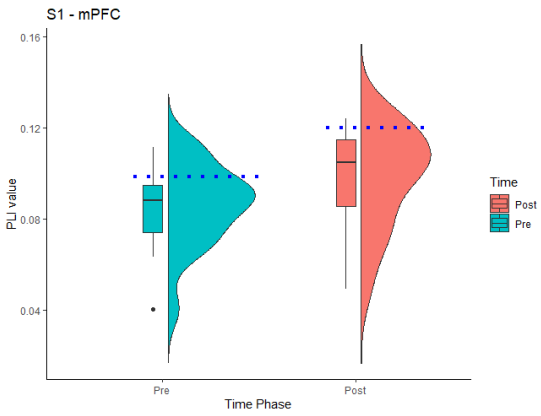

Figure 5.6 Average PLI value of the connection between SI-mPFC for Pre and Post time phases illustrated by blue dash line. The half violin plot and the bar-chart showing the distribution of the same index in healthy subjects in the TENS group.

5.3.3. PAIN LEVEL

The induced sensation by TENS was reported to be perceived in the entire hand, while the subject reported a pleasend feeling of the perceived sensation. He reported that his pain level could not get lower than 2 on the VAS scale. In addition, he mentioned the frequency of his pain spikes every 2-3 minutes, which lasts around 5 seconds with a pain level of 5. Following the TENS intervention, the subject reported that his pain level decreased from 4 to 2 on the VAS scale (from 0 to 10) and remained stable with no spikes (variations).

To conclude, results from **Study III** showed a reduction in the magnitude of N100 and P200 SEP waves and an enhancement in the functional connectivity in SI-ACC and SI-mPFC, which are associated with PLP relief. These results might be used as a possible feature for the effect of TENS on PLP reduction. However, our finding needs to be verified by a study with a large number of PLP patients.

CHAPTER 6. DISCUSSION AND CONCLUSION

6.1. Q1. HOW DOES SOMATOSENSORY CORTEX ACTIVITY ALTER FOLLOWING TENS, AND HOW LONG DOES THE TENS INDUCED CHANGES REMAIN?

In **Study I**, some evidence has been provided to demonstrated the effects of TENS on the cortical activities of SI. Induced changes in SEP components have been considered as a feature in studies in pain and sensation. Several studies have reported an increase in SI activity and reorganization at SI in chronic pain patients (Flor et al. 1997a; Wand et al. 2011; Zhao et al. 2017). In **Study I** the effect of TENS on cortical activity was found to suppress SI activity which may be beneficial for chronic pain reduction, including PLP and low back pain, as cortical facilitation has been reported in these patients. The hypothesis, that chronic pain increases due to sensorimotor disturbance, and decrease if the sensorimotor congruence artificially restored, supports this idea (Daenen et al. 2012; Mccabe et al. 2007; Perry et al. 2014; Thieme et al. 2016)s.

The early stage of sensory processing can be represented by the N100 wave which is independent to conscious awareness of the external stimuli. The origin of the N100 is believed to be SI and SII activity (Garcia-Larrea et al. 2003). Furthermore, memories of pain is associated with N100 magnitude enhancement in chronic pain patients (Angelakis et al. 2004; Flor et al. 1997b). It is also reported that the brain region representing P200 is correlated with ACC activity, which is involved in for conscious perception (Zeng et al. 2006). For example, the analgesic effect of TENS in has been reported to correlate with a reduction in P200 amplitude (Peng et al. 2019). Our findings indicate that the amplitude of P200 wave may be considered a feature for sensory processing following TENS application.

Moreover, studies have shown an increase in lower different frequency bans (i.e. delta, theta, and alpha) in chronic pain patients. Results from our study showed a significant suppression in delta theta and alpha band following TENS which can be supported by organization of pain-related cortical regions (Veldhuijzen et al. 2006; Vuckovic et al. 2014).

6.2. Q2. HOW DOES THE TENS INTERVENTION AFFECT THE FUNCTIONAL CONNECTIVITY BETWEEN THE BRAIN AREAS INVOLVED IN SENSATION AND PAIN PROCESSING?

Q2. How does the TENS intervention affect the functional connectivity between the brain areas involved in sensation and pain processing?

Study II investigated the changes in functional brain connectivity caused by TENS intervention. Results from **Study II** showed a significant effect of TENS intervention on brain functional connectivity compared to the sham intervention. Moreover, our study demonstrated that functional connectivity between SI-ACC and SI-mPFC changed following TENS intervention in gamma band occurring simultaneously with reduced perceived sensation. In addition, we found that the increment of functional connectivity existing between SI-ACC and SI-mPFC in the gamma band might be considered as a feature to show the influence of TENS on sensation and pain perception. These results can lead to a better understanding of the underlying mechanism and analgesic effect of TENS on sensory/pain sensation and how cortical actitivty alter following TENS.

The anatomical and functional connections between SI and ACC have been previously investigated using optogenetics and fluorescent tracing methods. Singh et al. improved the understanding of the sensory processing and neuropathic pain at the cortical level by reporting a direct projection from the SI to the ACC following noxious stimuli (Singh et al. 2020). Additionally, it has been shown that gamma oscillations can be used as a indicator of brain functional connectivity (Ta Dinh et al. 2019). It has been reported that gamma power increased at SI and the prefrontal cortex following noxious stimuli (Ploner et al. 2017) and at SI following non-noxious stimulation (Rossiter et al. 2013). The decrease of the gamma oscillation power which results following the application of TENS showes gamma band power alternation as possible evidence for the analgesic effect of TENS on pain.

Moreover, in **Study II**, a comparison was made in terms of an alteration in both global and local brain network metrics. Ta Dinh et al. in a recent study using EEG nuroimaging showed that a gamma-band global network could be reorganized in patients with chronic pain (Ta Dinh et al. 2019). They suggested a positive correlation between the brain network global efficiency reduction and chronic pain. Consistent with their results, our findings also show that the application of TENS results in enhancement of global efficiency and other network parameters including cluster coefficient and strength generally in all frequency bands with a significant increase in all three network parameters in the gamma-band. In addition, the suppression in global metrics of functional connectivity of involved sensory processing brain network in gamma-band has been reported in their study. As the results from **Study II** illustrated an enhancement in these global indexes following TENS, this study suggests global indexes of brain network as possible features to investigate the role of the central nervous system in the analgesic effect of TENS.

Moreover, results from local network analysis in **Study II** showed a number of significant alternations in local characteristics of the brain network. Three local indexes as local efficiency, local cluster coefficient, and local strength (nodal strength), have shown more changes in all eight ROIs in the TENS group that was not found in the sham group. However, a significant increase following TENS in

contralateral SI, insula, and ACC found in local efficiency. Previous studies showed that insula and ACC play an important role in sensory (Liberati et al. 2016) and pain (Mazzola et al. 2012; Schweinhardt et al. 2006; Singh et al. 2020) processing. Moreover, functional and effective connectivity between insula and other brain areas such as sensory, motor, and limbic area have been reported in earlier studies (Mesulam and Mufson 1982; Mufson and Mesulam 1982). Therefore the results of **Study II** indicates that another evidence for the mechanism of TENS on CNS can be the SI, insula, ACC, and mPFC elevation in regard to local efficiency.

Results of local strength showed a significant increase in the ipsilateral SI and ACC. It has been shown that somatosensory cortex activity can be altered by TENS in both ipsilateral and contralateral hemispheres (Peng et al. 2019). Also, studies have revealed the advantages of the application of TENS on the intact hand for PLP release (Giuffrida et al. 2010; Sabino et al. 2008). Moreover, inter-hemisphere functional connectivity has been reported to decrease in SI following amputation (Makin et al. 2015). Conforming to the previous studies in regard to the impact of the TENS on the hemisphere ipsilateral to the stimulation side, results from **Study II** showed a significant increase in the nodal strength and cluster coefficient of the ipsilateral SI which could be a possible evidence for the impact of TENS intervention on sensory perception and pain reduction.

6.3. Q3. TO WHAT EXTENT DOES THE TENS INDUCE CORTICAL ALTERATIONS IN AMPUTEES, AND CAN THE CHANGES BE ASSOCIATED WITH PLP REDUCTION?

In **Study III**, it was shown that the application of TENS on referred sensation areas would suppress the phantom limb pain level, and this suppression was associated with the reduction in the amplitude of somatosensory evoked potentials (N100 and P200 waves). Moreover, the functional connectivity in SI-ACC and SI-mPFC altered after TENS intervention and followed the same changes as investigated in **Study II**.

It has previously been shown that PLP is associated with SI reorganization (Flor et al. 2006; Jiang et al. 2015). Previous research has also reported alternations in SEP following amputation (Zhao et al. 2016). In line with this, the SEP suppression resulted following TENS application in **Study III**, might be possible evidence of the underlying mechanism of analgesic effect of TENS on PLP. Moreover, activation of other brain areas such as insula and ACC have been reported in PLP (Willoch et al. 2000). It is also reported that altered ACC activity following invasive stimulation leads to PLP relief (Nardone et al. 2014). The analysis of functional connectivity in this study revealed that the PLI values of SI-ACC and SI-mPFC have increased, which could be a possible mechanism for the induced PLP reduction by TENS. Although the results of **Study III** followed the same changes as **Study I and II**, further study with

a larger number of amputees with PLP is needed to validate the cortical induced changes caused by TENS intervention.

6.4. CONCLUSION

This Ph.D. thesis has presented three studies to investigate the possibly altered brain mechanism and pain/perceptual response following the application of TENS. Associated cortical changes with the pain/sensation reduction following TENS were examined by analyzing somatosensory evoked potentials and functional connectivity. Possible brain features such as suppression in the magnitude of N100 and P200 waves and enhancement in the functional connectivity and pain-related functional connectivity network index affected by the application of TENS were found. Significant reduction in the perceived sensation and the area where the sensation was felt were found following TENS intervention in the healthy subjects. In addition, PLP level and pain spikes reduction were found in our case study with a complex regional pain syndrome patient with PLP. There is now some evidence on the effect of TENS on cortical activity associated with pain reduction; however, to verify these results, clinical study with a large number of PLP patients should be considered as the focus of future work.

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