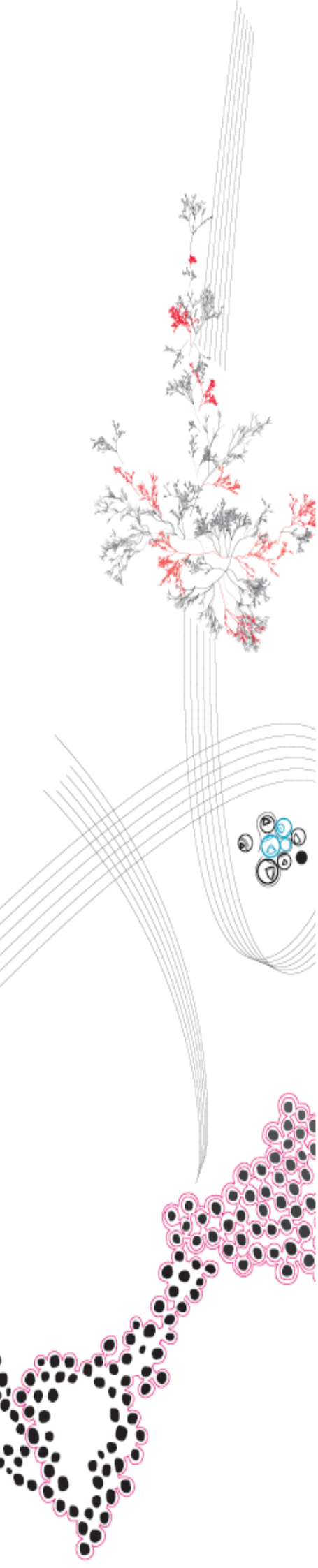

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Biomedical Signals and Systems

Enriched Stimulation Protocol for Improved Observation of Nociceptive Evoked Potentials during NDT-EP Method

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Preface

Working on this thesis was a pleasure, and a fulfilling and illuminating experience altogether. I am exceedingly grateful to my supervisors, N. Jansen MSc, Dr.ir. J.R. Buitenweg, and Dr. C.G. Zeinstra, for providing me with the opportunity to undertake this study, and for guiding me in learning and honing the skills needed for such research. I am also extremely thankful to my supervisors as well as my family and friends, for the immense support and encouragement they showed me during the more unfavourable times, thereby enabling me to complete this endeavour. Lastly, I owe thanks to the subjects who volunteered their time and energy to participate in the study.

This thesis is dedicated to the innumerable people who suffer silently from hidden or invisible disabilities like chronic pain, chronic fatigue, and a multitude of other neurological and mental disorders, and who are looking to the scientific community to find solutions for their hardships.

Summary

Chronic pain disorders affect innumerable people all across the world. Such pain manifests as pain lasting long periods over months or even years, and tends to adversely affect the quality of lives of those suffering from it in various ways. As of yet, there is no effective treatment for chronic pain nor any appropriate diagnostic method, as its exact mechanism is not well-understood. As chronic pain is thought to occur due to disturbed processes in the central nervous system, research into the characterisation of the central nervous system, especially the part of the sensory system that is involved in pain-processing, called the nociceptive system, is crucial.

Such research has its own obstacles. For instance, electrical activity on the scalp, caused by painful electrical stimuli, is measured with EEG, and the evoked potentials produced by the stimuli can be analysed to understand how the stimulus properties affect these evoked potentials. However, the measured potentials also include a component resulting from the novelty of the stimulus itself, and from the task of detecting the stimulus and indicating that it has been detected. This component is called the novelty component, and it must be removed if any characterisation of the relationship between stimulus properties and the evoked potentials produced by the stimulus is to be made.

In this study, a new repetitive stimulation protocol was designed with the purpose of removing the undesirable novelty component from the measured evoked potentials. The protocol was tested by obtaining EEG data from subjects, which was recorded during experiments done using this stimulation protocol. The evoked potentials and scalp topography induced by the stimuli were studied to see if it could be concluded that the novelty component was removed, as intended.

The results showed that the protocol designed for this study is a promising way of removing the novelty component from the evoked potentials. With some modifications and improvements, the repetitive stimulation protocol could be useful in obtaining evoked potentials that contain only the component corresponding to the physiological response of the central nervous system to the painful stimuli. For example, changing the method by which the subjects indicate that they felt the stimuli was found to be potentially useful in improving this protocol. More research is needed in order to confirm the utility of the protocol, and in order to improve it to suit its purpose.

Contents

List of acronyms

2IFC	two-interval forced choice
ACC	anterior cingulate cortex
CNS	central nervous cystem
DP	double pulse
DRG	dorsal root ganglion
EEG	electroencephalogram
EOG	electrooculogram
EMG	electromyogram
EP	evoked potential
ERP	event related potential
FBSS	failed back surgery syndrome
fMRI	functional magnetic resonance imaging
GCP	Good Clinical Practice
GFP	global field potential
GLM	generalised linear model
GN	go/no-go
IASP	International Association for the Study of Pain
ICA	independent component analysis
IES	intra-epidermal electrocutaneous stimulation
IPI	inter-pulse interval
MEG	magnetoencephalography
MN	microneurography

NDT	nociceptive detection threshold
NFR	nociceptive flexion response
NGF	nerve growth factor
NoP	number of pulses
NSP	Nociceptive and Somatosensory Processing
OFC	orbitofrontal cortex
PET	positron emission tomography
PFC	prefrontal cortex
PNS	peripheral nervous system
PSP	post-synaptic potential
PW	pulse width
QoL	quality of life
QP	quadruple pulse
SNR	signal-to-niose ratio
SP	single pulse
TMSi	Twente Medical Systems internation B. V.
WMO	medisch-wetenschappelijk onderzoek

Chapter 1

Introduction

1.1 Context

1.1.1 Chronic Pain

Chronic pain is generally defined as pain lasting for more than 3 months [1, 2], and is often considered to be pain that persists beyond the normal healing time, and/or pain that has no apparent cause. It is a debilitating condition that has diverse underlying mechanisms, and affects a significant proportion of the human population. Estimates tend to vary but suggest that roughly 20% of the European population experience chronic pain [3]. Several studies conducted across multiple European countries have shown that approximately 1 in 5 people suffer from chronic pain, with a majority of them reporting recent, frequent, and moderately severe pain [4, 5]. The worldwide prevalence of chronic pain seems to be similarly high [6].

This affliction has serious negative impacts on the life of the people suffering from it, leading to a much lower quality of life (QoL). Apart from the reduced capacity to perform certain tasks in daily life, a lot of patients also report problems with social interaction and relationships, and also adverse effects on their employment status [5, 7, 8]. One of the European studies showed that 19% of chronic pain patients had lost their job as a result of their pain, while 16% had to change job responsibilities, and 13% had to change jobs entirely. More often than not, sleep and mental health are also compromised due to chronic pain. In the same study referred to previously, 21% of the chronic pain sufferers had developed depression due to their pain [5].

In general, chronic pain places a heavy economic and social burden on society, and yet, adequate treatment for chronic pain is sorely lacking. In one survey, a staggering 64% of patients taking prescription medication for pain indicated that their pain was not adequately controlled by the medication [3]. Thus, it is apparent that better treatment for chronic pain needs be to be found.

1.1.2 Current Research

In order to develop treatment for chronic pain, the underlying mechanisms of pain and chronic pain need to be understood first. Currently, our understanding of these mechanisms

is rudimentary, and thus, research has to focus on this aspect as well. Central sensitisation, which can be described as an increase in the excitability of neurons in the central nervous system (CNS), is thought to be responsible for an increase is responsiveness to mildly noxious or innocuous stimuli, for the lowering of pain threshold, and for the spread of hypersensitivity to regions beyond injured tissue [9, 10].

In pain research, intra-epidermal electrocutaneous stimulation (IES) of the skin is used to detect nociceptive detection thresholds (NDT), while electroencephalography (EEG) is used as an objective measure of nociception-related activity in the central nervous system. The induced activity, called evoked potentials (EP), are seen to be sensitive to changes in the stimulus parameters, such as number of trials and number of pulses [11, 12]. Studying the extent and exact nature of this dependence could lead to a better characterisation of the nociceptive system, which could, in turn, be useful for coming up with better diagnosis, treatment and management of chronic pain.

Unfortunately, at present, the EPs found in such studies are heavily affected by the presence of a novelty/task-related component that is induced due to the detection of the stimulus itself. This component is the result of the engagement of the subject's attention due to changes in the environment, especially the processing of a novel stimulus. As this component is not related to the stimulus parameters, it presents as an impediment to understanding the encoding of stimulus parameters in the EPs. Hence, a method that removes this component from the measurements needs to be developed.

1.2 Research Objective

The main purpose of this study is to devise and test a way to possibly remove the novelty component from the nociceptive EPs obtained during NDT-EP experiments. To this end, the objectives of this thesis are:

- to design a new stimulation protocol that could help measure EPs unaffected by the novelty component.
- to analyse the EPs resulting from the new stimulation protocol and investigate whether the novelty component is removed.

1.3 Strategy

As noted above, the main goal of this study is to offer a possible method to remove the novelty component from evoked potentials. In this study, a new stimulation protocol that could achieve this goal is developed, based on past literature on experiments using the NDT-EP method and various types of stimulation.

This new modified protocol is implemented and and is subsequently tested, by conducting NDT-EP type experiments on a small group of subjects, while using the newly developed stimulation protocol for stimulation. The sets of EPs and other EEG results obtained from

these experiments (upon processing the EEG data) are analysed to see if inferences can be made about the effect of the stimulation protocol on the EPs, more specifically about the presence/absence or removal of the novelty component in the EPs. Based on the analysis and conclusions, recommendations for further research are also made.

Chapter 2

Background

2.1 Human Nervous System

This section explains some of the basic physiology of the human nervous system. The components of the nervous system and the propagation of signals within it are important to know in order to understand the mechanism of pain sensation. Most of the following information is well-known and has been gathered from various textbooks [13, 14].

2.1.1 Structure and Components of the Nervous System

The fundamental unit of nerve tissue is a *neuron*, which is made up of the cell body, an axon, and dendrites or dendritic branches, and which facilitates long-distance electrical signalling and inter-cellular communication in the nervous system via *synapses*. The dendrites receive information from other cells, while the axons relay information to other parts of the body. Figure 2.1 shows the structure of a neuron.

Axons are specialised for relaying information over long distances, and may thus vary in length from a few millimetres to the order of metres, depending on the type of the neuron. For instance, the axons that run along the leg from the spinal cord to the foot are about a metre long. *Dendrites*, or dendritic processes, are often found in the form of elaborate branching (called *arborisation*) and are the primary targets for synaptic input which they receive from the axon terminals of other neurons. The size and the branching of dendrites vary greatly throughout the body and influence the information-processing capacity of the neuron they are a part of. On one end of the spectrum, neurons that lack dendrites will be innervated by only one or two axons from other neurons, causing a more faithful relay of information. As the complexity of the branching of the dendrites becomes more and more elaborate, they are innervated by a commensurately large number of neurons, thus allowing for greater integration of information. The number of inputs to a neuron is a measure of its convergence while the number of targets innervated by the neuron reflects its degree of divergence.

There is no physical continuity in the synaptic contact made by an axon of a presynaptic neuron on dendrites of postsynaptic receptors even though they are immediately adjacent to

each other, and the communication between has to take place with the help of neurotransmitters. These neurotransmitter molecules travel across the extracellular space between the pre- and post- synaptic elements called the *synaptic cleft* (Figure 2.1. This synaptic cleft is not simply empty space but contains proteins that aid with the binding, diffusion and degradation of neurotransmitters and other molecules. Note that the above description applies to chemical synapses; electrical synapses also exist, wherein open fluid channels conduct electricity from one cell to the next.

Apart from neurons, another cell type, called the *glia*, are also present in the human nervous system. The glial cells do not directly participate in neural signalling, but help maintain the functions of the nervous system by playing a supporting role. Their main functions are to maintain a chemical environment suited for neuronal signalling, to lay down a laminated wrapping called myelin around some axons, and to scavenge and remove cellular debris from, for example, site of injury. In addition to these, glial stem cells in adult humans proliferate and generate new glial cells or even neurons.

Neurons never function in isolation; they are also often found in clusters, such as in the form of ganglia, which are local accumulations of neurons and glia, while axons are often bundled together in the form of nerves. Neurons usually function in ensembles called neural circuits, within which the arrangement of neurons can vary widely based on the served function, but which share some common characteristics. There are three fundamental components of a neural circuits, namely, the afferent neurons, the efferent neurons, and the interneurons. *Afferent* neurons carry information from the periphery towards the brain or spinal cord, while *efferent neurons* carry information away from the brain or spinal cord. *Interneurons* are local circuit neurons that conduct the flow of information between sensory and/or motor neurons.

Overall, the human nervous system can be divided anatomically into two sets of components. The central nervous system (CNS), consists of the brain and the spinal cord, while the peripheral nervous system (PNS) is comprised of the sensory neurons that link sensory receptors in the surface of the body to the relevant neural circuits within the CNS. Apart from this, the nervous system can also be classified based on the function they serve, called neural systems. The various sensory systems acquire and process information from the environment, while the motor systems generate movement in response to the gathered information. Of these, the sensory system concerned with the sensation of pain is of primary relevance in this thesis.

2.1.2 Neural Signalling

Information is encoded and transferred in the nervous system by neurons using different types of electrical signals, which are generated due to differences in the concentrations of (mainly potassium and sodium) ions across nerve cell membranes, which are in turn selectively permeable to some of those ions. The permeability of a membrane changes due to opening or closing or ion channels, causing a flow of ions from one side to another, which results changes in the ionic concentrations of either side of the membrane. This ionic

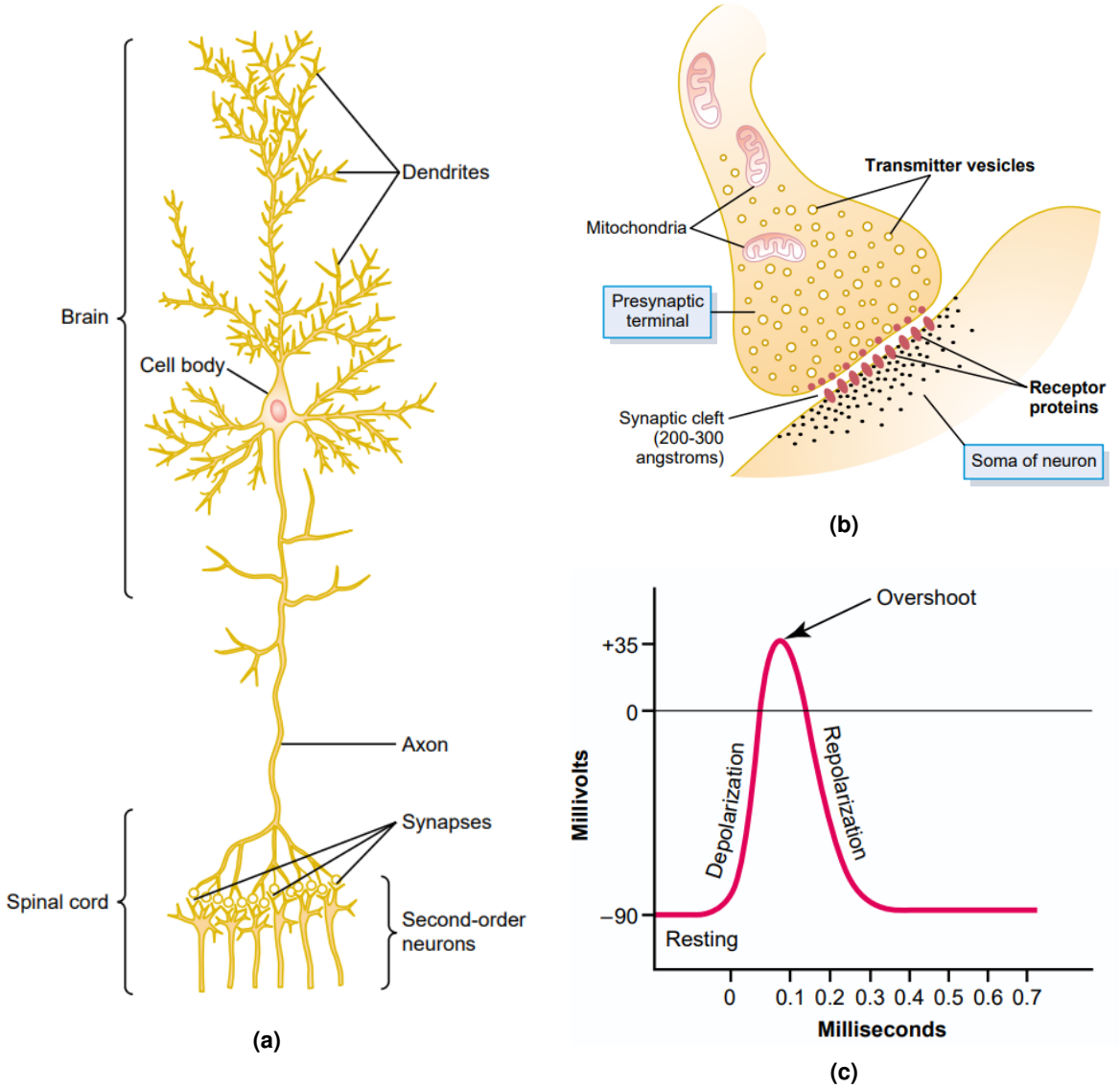

Figure 2.1: (a) Structure of a large neuron in the brain, (b) Physiology of a synapse, (c) Typical action potential showing a sharp and quick change in membrane potential. Reproduced from [14]

movement ensures electric activity despite neurons not intrinsically being good conductors of electricity.

When at rest, neurons generate a constant negative voltage across their membranes, called the *resting membrane potential*, which is usually a fraction of a volt in amplitude (-40 to -90 mV) [13]. The first step in generating any kind of sensation is a transient change in the resting membrane potential. These are the *receptor potentials*, wherein the activation of sensory neurons due to external stimuli like light, sound or heat, causes the resting potential to change for a fraction of a second. The magnitude of receptor potential varies in proportion to the magnitude of the sensory stimuli that generate them. Another type of electrical signal generated in the nervous system is the *synaptic potential*, which facilitates the exchange of information in complex neural circuits. The synaptic potential is generated at the synaptic

contact between two neurons and allows the transmission of information between those two neurons. The amplitude of such a synaptic potential depends on the number of synapses activated, the strength of each synapse, and the amount of previous synaptic activity.

Long range information transfer in the nervous system as well as from the nervous system to target organs occurs mainly via special electrical signals generated by neurons along their long axons, known as *action potentials*. It is seen that when the negative resting membrane potential is changed so as to make it more negative (hyperpolarisation), this change in potential is propagated through some distance; however, the magnitude of the change gradually reduces and dies out. On the other hand, if a potential that is more positive than the resting potential is injected instead (depolarisation), then at a certain level called the threshold potential, an action potential occurs. Such an action potential is a sharp and brief ($\sim$1 ms) change of the transmembrane potential from negative to positive, and occurs in the following three successive stages:

Resting Stage This is simply the resting membrane potential before the start of the action potential. The membrane is said to be *polarised* in this state.

Depolarisation Stage In this stage, the membrane becomes highly permeable to sodium ions, and a large number of these ions flow into the axon. This leads to *depolarisation*, which is a rise in the membrane potential in the positive direction. The potential reaches zero and may even cross over to the positive side.

Repolarisation Stage A few milliseconds after the membrane becomes more permeable to sodium ions, the sodium channels begin to close, but potassium channels open more than usual. This cause a rapid diffusion of potassium from the inside to the outside of the axon, which re-establishes the normal (negative) resting membrane potential, in a process called *repolarisation*.

An important property of the action potential is that it is independent of the current that evokes that potential. The action potential will either not exist (if the membrane potential caused by the applied current is less than than the threshold), or it will have a standard magnitude, once the membrane potential is higher than the threshold required to generate the action potential. However, if the magnitude or duration of the stimulus is increased sufficiently, there will be multiple action potentials occurring one after another, with the number of action potentials increasing with any increase in the amplitude of the injected current. Thus, the frequency, and not the amplitude, of the action potential encodes the intensity of the applied stimulus. The action potential also boosts spatial spread and will remain constant across the entire length of an axon rather than reduce gradually. This enables the nervous system to overcome the inherent bad conductivity and leakiness of neurons.

As action potentials are the basis of and also serve as an indicator of information transfer in the nervous system, they are an important measure in a variety of clinical treatments as well as in clinical research. Characterising the sensory system especially requires studying the action potentials generated as a result of applied external sensory stimuli.

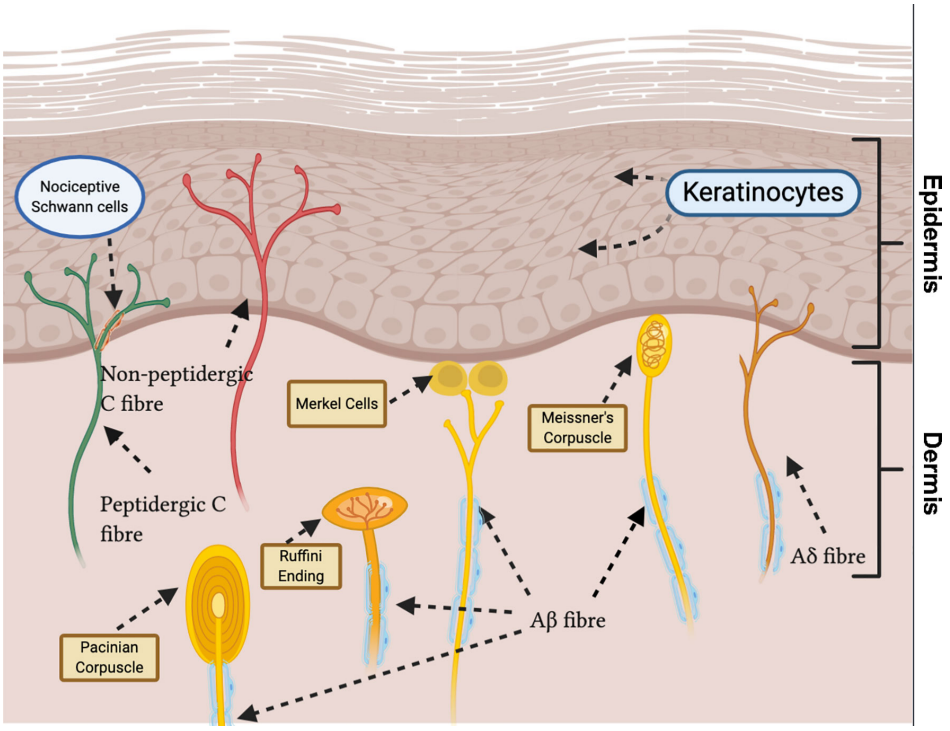

Figure 2.2: Receptors located in various layers of the skin. Nociceptive fibres terminate in the epidermis, while non-cociceptive nerves terminate in the dermis. Reproduced from [15]

2.1.3 Sensation and Sensory Processing

Sensation can be defined as the process of encoding and perceiving external and internal stimuli which can be auditory, visual, tactile, and so on. The sensory system that mediates senses such as touch, vibration, pain, itch, etc. is known as the somatosensory system. The somatosensory system consists of several subsystems, namely, (1) one that mediates the sensation of fine touch, pressure and vibration, (2) one that is specialised to detect the position of our limbs and other body parts, and (3) one that encodes information about painful or harmful stimuli as well as changes in temperature and non-discriminative touch.

The differentiation in the reception of different sensations happens at the skin, due to the various receptors that terminate in the different layers of skin. The location and depth of the receptors in the skin can be seen in figure 2.2, and are explained below:

Free Nerve Endings Free nerve endings run through the uppermost layer, just under the skin surface, and are usually found on thermoreceptors, receptors that detect changes in temperature, or on nociceptors, which are receptors that transduce pain.

Meissner Corpuscle These are found in large amounts in the finger pads right next to the ridges in the epidermis. They are rapidly-adapting, and together with Merkel discs, help understand texture, and elevation of as low as 5 μm.

Merkel Cell-Neurite Complex These are slow-adapting complexes that discharge in response to sustained pressure, such as when holding an object, and are also sensitive to edges, and thereby to shape, texture, etc with the help of Meissner Corpuscles.

Ruffini Corpuscle These are slow-adapting endings that are located in the dermis, and respond to shear stress, also called *drag*, and stretch.

Pacinian Corpuscle These are rapidly-adapting subcutaneous endings that respond to vibrations, especially bone vibrations, as they lie so close to the bone.

The information obtained by the peripheral nerves is transmitted via pathways in the spinal cord to the brain, where these signals terminate in the primary and secondary somatosensory cortices. Upon receiving these signals, several different areas in the brain involved in the cognitive processing of those signals are activated, based on the location of the incoming stimulus.

2.2 Nociceptive System

As the main topic under investigation in this thesis, and in the overarching research of the Nociceptive and Somatosensory Processing (NSP) group is chronic pain, the mechanism of pain sensation needs to be understood. Hence, the known information is given here in brief. The subsystem of the somatosensory system that is involved in the perception of painful sensations is called the *nociceptive system*. This is a specialised system that is separate from tactile and other sensory systems, and will be described next.

2.2.1 Physiology of Pain

Pain is an unpleasant sensory experience that occurs as a protective mechanism when there is damage in the body. The perception of harmful stimuli that cause such damage occurs via dedicated receptors and pathways in the nervous system. The different stages of pain perception in the nervous system are explained next.

Peripheral Nerve Fibres

Nociception begins in the periphery with a stimulus reaching the nociceptive nerve fibres. *Nociceptors* are the free nerve endings that initiate pain, and are found throughout the body in the superficial layers of the skin (Figure 2.2) as well as certain internal tissues. These receptors transduce mechanical, chemical or thermal pain stimuli into receptors potentials that trigger afferent action potentials.

Generally, nociceptors are categorised according to the properties of the axons associated with them; mainly based on the diameter or conduction speed of the axons. In contrast with the receptors that are responsible for sensations from innocuous stimuli, nociceptor axons are of smaller diameter, are either only lightly or not myelinated, and conduct relatively slowly. The Aδ fibres are a group of myelinated axons that conduct at speeds of 6-30 m/s, while the C fibres are unmyelinated axons that conduct at speeds of 0.5-2 m/s [14]. When a harmful stimulus is acting upon any tissue, at first, there is a sensation of sharp, localised

pain (called fast or first pain) due to signals transmitted by the Aδ fibres, after which a delayed, diffused and persistent sensation of pain (called slow or second pain) is induced due to transmission by the C fibres.

It is worth noting that pain is not elicited because of axons related to non-painful stimuli discharging at higher rates; in fact these axons do not discharge at a higher rate in response to painful stimuli at all. However, the nociceptive axons begin to discharge when the strength of a stimulus reaches a certain level.

Central Pain Pathways

The nociceptors described above arise from cell bodies in the *dorsal root ganglion* (DRG); these cells also send another axonal process into the spinal cord or brain-stem. When nociceptors are innervated, they carry the signal to the back of the spinal cord (known as *dorsal horn*) via the DRG.

A representation of the pain pathways of the human nervous system is shown in Figure 2.3. Within the dorsal horn, this first order neuron that carried the signal there relays the signal to a second-order neuron that crosses over to the opposite side of the spinal cord and enters the spinothalamic tract. The second-order neuron ascends through the spinal cord and brain-stem, and terminates in the thalamus of the brain. In the thalamus, third-order neurons receives the signal from the second-order neuron and relays it to the primary somatosensory cortex and other relevant regions. This is called the *ascending pathway*. Note that as the second-order neuron crosses over to the other side (contralateral side) of the body, the sensation of a painful stimulus on the left side of the body occurs in the right side of the brain, and vice versa [17].

In the dorsal horn, the signal from the first-order neuron is relayed to the second-order neuron with the release of a specific chemical called *substance p*. In the descending pathway of pain perception, a neuron travels down the length of the spine and ends in the dorsal horn, where it releases chemicals (endogenous opioids) that inhibit the release of substance p. These chemicals play the role of inhibiting the communication between the first- and second- order neurons, thereby controlling the pain signals going up the ascending pathway.

Pain Centres in the Brain

Human functional brain imaging shows that several regions in the brain are activated in response to painful stimuli, including the somatosensory, insular and cingulate areas, as well as frontal and parietal areas. There areas are all together sometimes referred to as the *pain matrix* [18]. The locations of and inter-connections between the various brain regions involved in pain perception are shown in Figure 2.4.

Sensory information about noxious stimuli is transmitted to the primary somatosensory cortex (SI) and the secondary somatosensory cortex (SII) from the peripheral nerves through the thalamus. The location of activation within the SI is related to the location of the painful

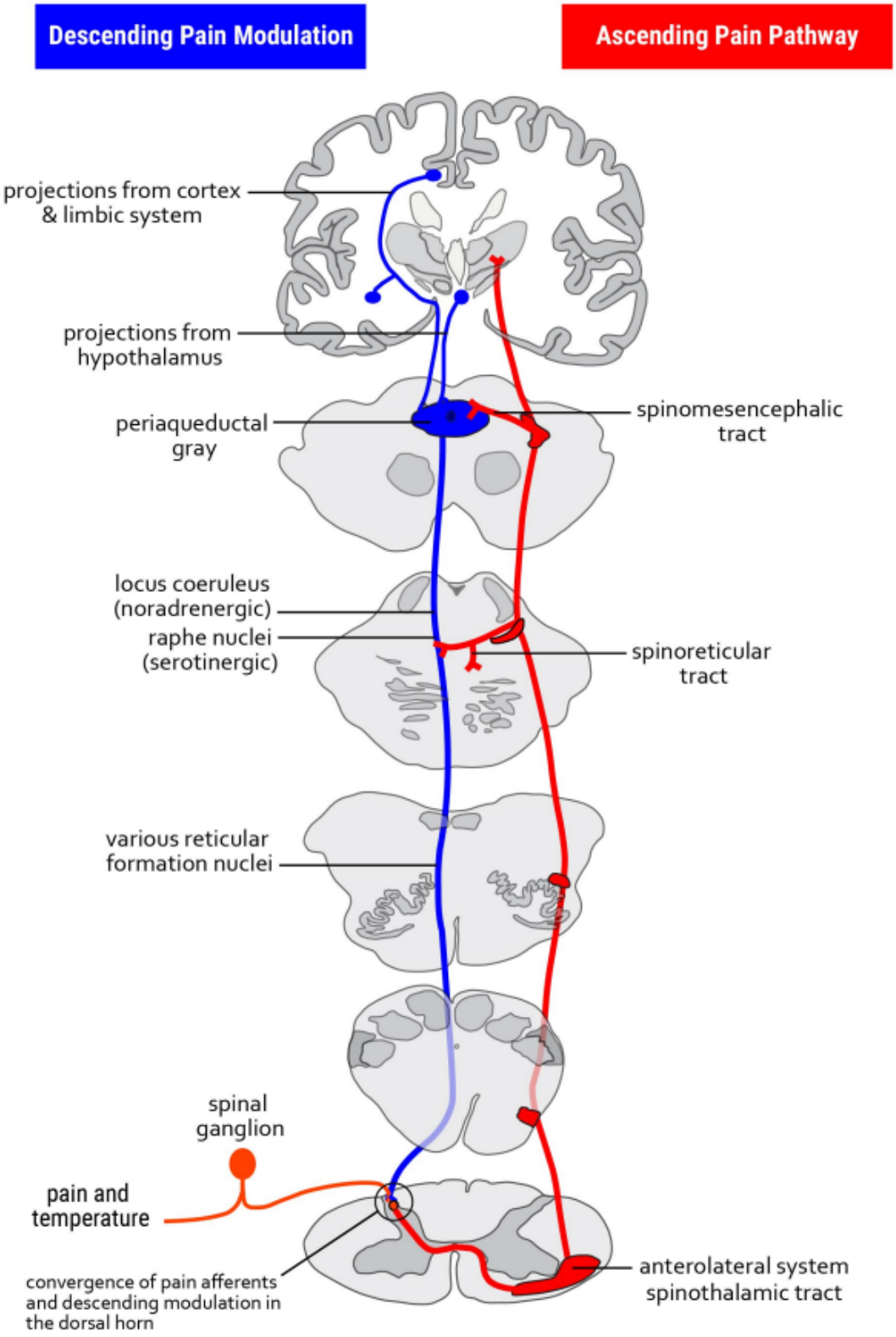

Figure 2.3: Illustration of ascending pain pathway and descending pain modulation pathway, as described in literature. Design & Artwork: The HIVE, UBC [16]

stimulus on the body (somatotopical organisation), and the SI is thus included in the discrimination of the location and intensity of pain [19].

The amygdala, which integrates emotional and instinctive behaviour, determines whether the stimulation is comfortable or uncomfortable, as well as whether the discomfort is negative or affective. When the pain is experienced again, memorised information regarding the nociception is retrieved by the amygdala, and negative emotions such as fear, anger, anxiety, freezing, escape responses, etc. are induced. The insular cortex is in contact with the amygdala and receives input from the thalamus and SII. Thus, it is involved in both the sensory aspect and the emotional aspect of pain. It is believed to be involved in the avoidance of painful situations, the prediction of pain, and also in placebo analgesia. The anterior cingulate cortex (ACC) is the central region of the descending pain-inhibitory system, and is itself divided into various parts. It too is involved in the avoidance of pain, and also in controlling emotional arousal [20].

Apart from these, the prefrontal cortex (PFC) and the orbitofrontal cortex (OFC) are also considered to be part of the pain matrix. They are both involved in the emotional aspect of pain, and in decision-making and in the emotion and reward system [20].

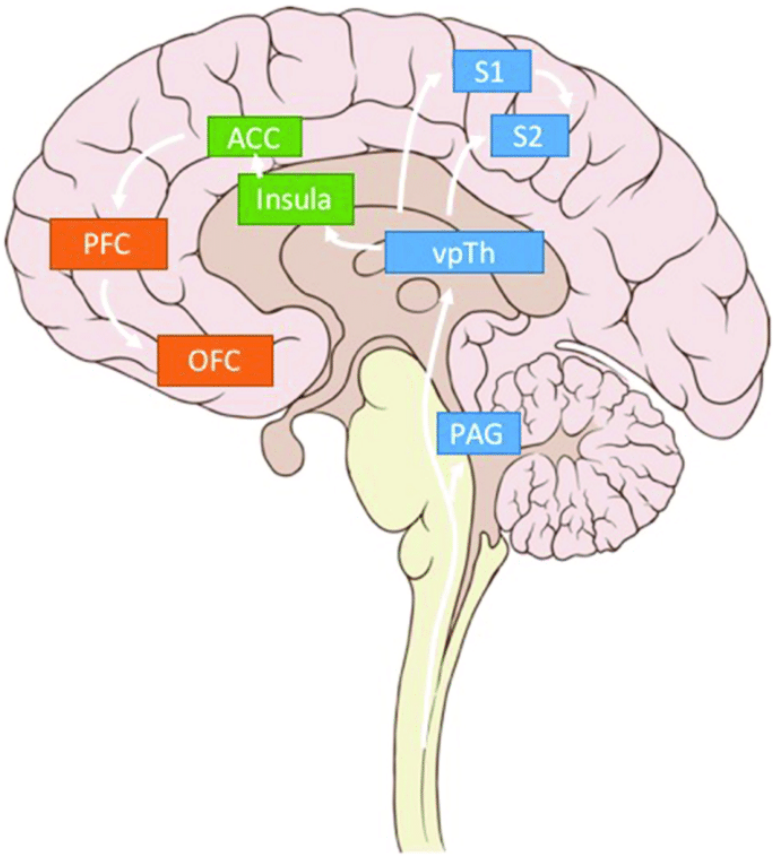

Figure 2.4: Pain related brain regions of interest, related to somatosensory (blue), affective (green), and cognitive (orange) pain processing. Reproduced from [21]

2.2.2 Bio-behavioural View of Pain

There is a growing belief that pain is a multidimensional experience with physiological, cognitive, behavioural and social components, all interwoven in a complex manner. All these facets of pain and the inter-relations between them need to be understood, assessed and treated with equal importance. Some of the characteristics of the cognitive-behavioural perspective towards pain are as follows [22]:

- People are active processors of pain, and not passive reactors.
- Thoughts can influence physiological process, the environment, as well as mood and behaviour, and vice versa.
- People can learn more adaptive ways of feeling, thinking and behaving.

A few of the important ways in which cognitive factors may influence the perception of pain are described next (as described in [22]. Note that this section is not exhaustive and many more factors may influence pain perception.

Beliefs about Pain

A person's beliefs about pain are hypothesised to affect pain perception and functioning during pain in two ways. Firstly, there is a direct influence on mood, which can in turn affect muscle tension, and hormones and neurotransmitters. Secondly, there is an indirect effect, mainly on coping effort, such as the use of medication or alcohol to relieve the symptoms.

Apart from beliefs about pain perception, a person's beliefs about their own capability of controlling pain also affect how they process painful stimuli. A stronger conviction of pain control and coping thoughts can lead to reduction in pain and more tolerance, while catastrophising thoughts (see section 2.2.2) lead to lower pain tolerance and higher ratings on pain perception.

Attention to Pain

An increased attention to pain is seen to enhance the painfulness of the nociceptive stimulus. Conversely, distraction from pain leads to reduction in the magnitude of pain intensity, evidenced by lowered activation of in several regions of the brain thought to be involved in pain processing.

On the other hand, the presence of pain may bring attention to several bodily signals and lead to hyper-vigilance. The person may notice and also focus on multiple symptoms other than the pain itself. There have been documented cases of people with chronic pain reporting several other bodily symptoms other than the pain.

Catastrophising

Catastrophising is a coping strategy that influences the level of pain, pain-related function and occurrence of depressive symptoms months after the pain-inducing event has happened. As such, it plays in role in pain-related disability as well as treatment outcomes.

Several studies show that in the case of post-surgical pain, catastrophising thoughts were positively correlated to the pain severity, use of medication and a generally poorer quality of life, as well as the development of chronic pain. In chronic pain patients, studies showed that significant variance in pain and disability were accounted for by the incidence of feelings of helplessness and catastrophising; catastrophising also led to poorer response to rehabilitation in some studies. On the other hand, a reduction in catastrophising was found to be significantly related to an increase in pain tolerance, and a decrease in pain intensity as well as physical and psychosocial impairment.

2.2.3 Other Important Phenomena

Several phenomena and properties in pain perception need to be taken into consideration during chronic pain research. Some of the relevant phenomena are described next.

Placebo Analgesic Response

A *placebo* response is said to have occurred when the analgesic response is due to psychobiological effects of the treatment process rather than due to any active property of the treatment itself. There is usually the presence of sensory cues that are associated with effective treatment or pain relief in the past, as well the expectation of pain relief [22].

Studies have shown the altered transmission of signals in pain pathways in response to placebo. For instance, activation of pain-modulation circuits and area in the brain important for pain-modulation, and also the activation of endogenous opioid and dopamine systems have been observed [22].

Salience Detection

The human brain is constantly bombarded with sensory information; it is important that the salient information be identified from amongst all the input, in order to guide behaviour, response, etc. A network consisting of various parts of the brain is considered to be responsible for detecting the behaviourally relevant stimuli that are received [23].

The *saliency network* helps isolate any auditory, visual or tactile (as well as nociceptive) information that the body receives from the unimportant background noise [24]. For instance, it may bring attention to painful stimuli that are unexpected or novel and guide behavioural response in planning an action to avoid these stimuli. As seen before, attention itself plays a role in the perception of severity of pain. Thus, dysfunction in the saliency network could influence the development of pain disorders, if certain stimuli are detected as more relevant

than they need to be. In some previous studies, patients with pain disorders have been known to show structural and functional abnormalities in their salience system [25].

Spacial and Temporal Summation

The intensity of the pain being experienced is an important characteristic and is conveyed to the brain in terms of signal intensity. The gradations of signal intensity are transmitted in two different ways by nerve fibres, via mechanisms called spatial summation and temporal summation [14]. In *spatial summation*, a signal of increasing intensity is transmitted by using more and more parallel nerve fibres. On the other hand, *temporal summation* is the phenomenon by which a signal of higher strength is transmitted by increasing the frequency of the nerve impulses in each fibre. Figure 2.5 shows a visual representation of how these phenomena occur.

In temporal summation, repeated and equal-intensity noxious stimuli at a specific (high enough) frequency cause an increase in the intensity of pain perceived due to those stimuli. This happens due to the post-synaptic potential (PSPs) evoked in spinal neurons and other cells in the CNS by input to the various sensory neurons. Figure 2.5 shows the mechanism by which temporal and spacial summation takes place. When two stimuli of the same amplitude are applied one after another to a single sensory neuron, recordings from a spinal cord neuron will show two PSPs of the same amplitude with an interval between them that is equal to the interval between the applied stimuli, corresponding to the two action potentials produced by the two stimuli. However, as described in section 2.1.2, the CNS neuron will have a re-polarisation stage during which the potential drops slowly back to the resting state. If the interval between the two applied stimuli is reduced such that the second action potential is produced before the re-polarisation stage of the first action potential is over, temporal summation takes place. The depolarisation associated with the second action potential adds to the re-polarisation associated with the first action potential, producing a summated PSP in the spinal neuron. If the two stimuli applied in extremely quick succession, the evoked PSP could ideally have double the amplitude as the PSP evoked by each of the (equal) stimuli. However, in reality, the strength of this summated PSP tends to be lower than twice the amplitude of the single PSP. This mechanism can be extended to three or even more action potentials with similar temporal summation effects.

On the other hand, if equal stimuli are apples simultaneously to two separate sensory neurons that are connected to the spinal neuron, their PSP strengths are summated in the same way as for temporal summation. This results, once again, in a higher PSP that is (less than) twice the PSP resulting from a single stimulus. This effect can also be extended to multiple more sensory neurons that are stimulated at the same exact time.

Habituation

Learning processes like habituation and sensitisation can affect the perception of pain too. *Habituation* can be defined as the reduction in the intensity of the physiological and be-

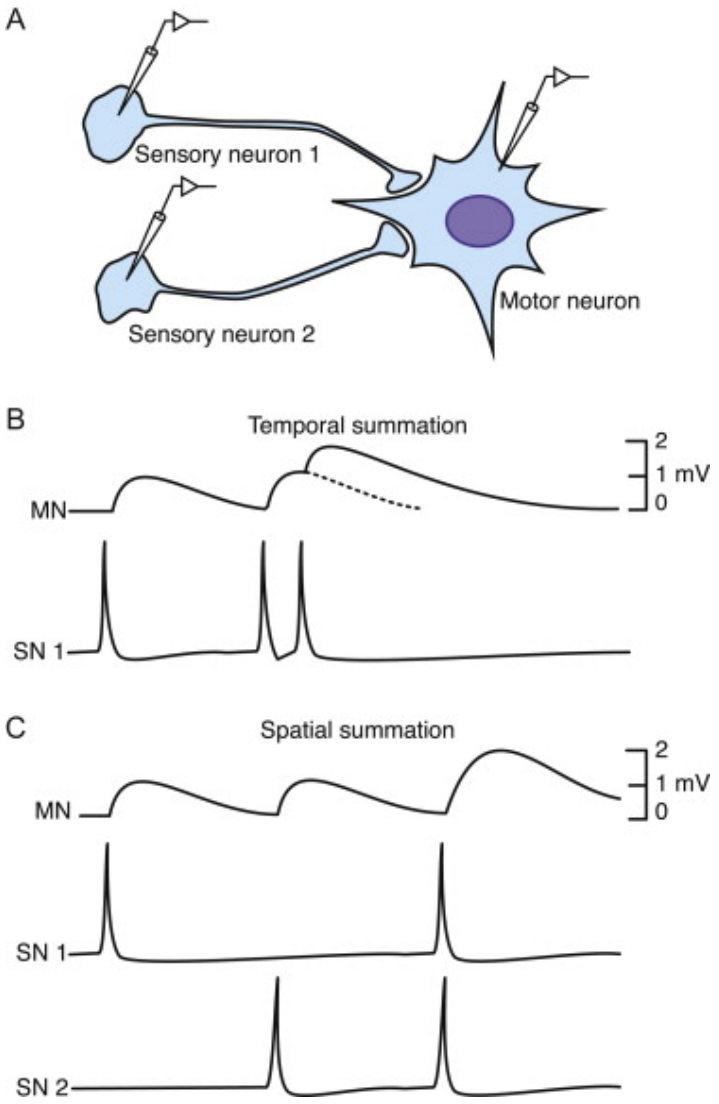

Figure 2.5: (A) Stimuli are applied to two sensory neurons, and the post-synaptic potential in a spinal cord neuron, in this case, a motor neuron, are measured. Illustration of (B) temporal summation, and (C) spatial summation. In both cases, post-synaptic potentials caused by separate equal stimuli get added and hence have much larger amplitude. Reproduced from [26]

havioural response when the same stimulus is presented multiple times, while sensitisation (explained in more detail in section 2.3.3) is the increase in such a response.

Habituation occurs only in response to the repeated sustained stimulation by the same noxious stimulus. The habituated response to the stimulus, e.g. in terms of potentials evoked in the brain, is lower compared to the response to the stimulus when it was first applied. However, any change in the stimulus causes dishabituation, whereby the lowered response is now increased as the stimulus is not the same. This is seen even in cases of new stimuli that would normally not cause a significant response on their own; they still cause a change (increase) in the habituated response [27]. Factors such as the intensity, frequency and duration of the stimulus can greatly affect the process of habituation. Sensory information in the form of anticipation of the pain can also increase the rate of habituation.

There may be various mechanism for habituation, either central or peripheral or both. In cognitive models, a cognitive representation of a stimulus's features is built as a result of repeated exposure to that stimulus [28]. Response to that stimulus is inhibited when this cognitive representation matches the actual stimulus, but when a repeated stimulus changes, the response is not inhibited anymore due to the mismatch between the stimulus and its cognitive representation. In the molecular mechanism, calcium channels close progressively (although the cause of this is unknown), due to which smaller-than-normal amounts of calcium ions can diffuse into the habituated terminal. Therefore, a smaller amount of sensory terminal transmitters is released, which causes the habituation [14].

Habituation to repeated stimuli on chronic pain research has been observed in several past studies. Understanding the underlying mechanisms of habituation may play a cruicial role in chronic pain research. It is generally seen as one of the key processes that may form a protective mechanism that may prevent chronification of pain [28]. Studies have shown that healthy subjects to get habituated to painful stimuli while chronic pain patients tend to get sensitised to the same stimuli [22, 28].

2.2.4 Nociception vs Pain Perception

According to the International Association for the Study of Pain (IASP), nociception refers to the neural encoding of any impending or actual tissue damage (from noxious stimulation), while pain refers to the subjective (emotional) experience of the unpleasant sensation associated with actual or impending harm to oneself [29]. Thus, nociception is the detection of noxious stimuli and the physiological response to this detection. However, the perception of pain itself is much more than simply this physiological process, as explained in the earlier section about the bio-behavioural view of pain.

There is a strong psychological element to pain perception, such as due attention, beliefs, etc., as described earlier, and demonstrated by the observation of phenomena such as the placebo effect, and by the observed effects of manipulation of attention and emotion of the subjects of studies [22]. Another example of this connection is battleground analgesia; this is a life-preserving mechanism, where soldiers feel no or little pain despite sustaining horrific injuries. More relevantly, some patients with severe chronic pain seem to have no apparent physical cause for their suffering, or suffer disproportionately compared to the the actual sustained injury [24]. While it is possible that nociceptive stimuli might evoke a response automatically as part of a coordinated defensive response, regardless of the awareness or conscious experience of pain, some research on the nociceptive flexion response (NFR) has indicated that reflexive responses mediated at the spinal cord level are affected by psychological factors such as expectation [30].

Due to this, the distinction between pain perception and nociception is of significance in research dealing with pain, where EEG measurements are taken in response to noxious stimulation, thus concerning itself with nociception. However, psychometric measures are often used as well, which depend on the perception of the painful stimuli rather than simply the nociception. Thus, while many studies regarding the sensitivity to noxious stimuli exist,

few have studied the response to actual pain, and most research in this field has failed to distinguish nociception from pain [30].

It is important to note that the difference between nociception and pain perception may have significant influence on the inferences drawn from the results of this study.

2.3 Clinical Abnormalities in Pain Perception

Undesirable changes occurring in the nociceptive system can lead to abnormal perception of pain, and thus, to pain disorders. This can manifest in several forms, and can have various causes, some of which will be described in this section.

2.3.1 Definition and Classification of Pain

The IASP defines pain as ”an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage” [29]. It is worth noting that this definition does not require the presence of injury or ailment for pain to exist. Pain is the phenomenon that arises a complex combination of a variety of biological factors (like medical comorbidities or sensory changes), psychological factors (like depression, anxiety, or cognitive distortions), and socio-environmental factors (such as social support and access to treatment) [20].

There are various ways in which pain can be classified [20, 29, 31]. One way is to differentiate based on duration:

Acute Pain This is pain that lasts for a relatively short duration, typically from a few minutes to three to six months. Such pain is often the result of some injury or illness and hence, subsides after the injury heals or the illness is cured. However, acute pain may develop into chronic pain if healing does not occur properly.

Chronic Pain This is pain that lasts for longer than three months, although it can be constant or intermittent. Chronic pain could be due to improper healing or long-term health conditions or other issues that will be discussed in more detail in section 2.3.2

Pain may also be categorised based on it pathophysiology, in the following way [20, 29]:

Nociceptive Pain This is pain caused by actual or potential/threatened damage to non-neural body tissue or due to disease, while the somatosensory system is functioning normally. It can be acute or chronic, and occurs in the skin, muscles, joints, tendons or bones, where the pain receptors are present. The activation of these receptors causes nociceptive pain.

Neuropathic Pain This is pain caused by injury to or lesions of the central or peripheral nervous system, which implies the presence of dysfunction of the somatosensory system. Such pain is disproportionate to the actual signs of damage, and can affect sensitivity to touch, heat/cold, as well mobility issues, thus making everyday tasks hard to perform.

Nociplastic Pain This type of pain is centrally-induced pain caused by altered nociception in the absence of any clear evidence of tissue damage, or of disease or lesion of the somatosensory system. While the mechanism underlying nociplastic pain are not well-understood yet, it is an important type of pain to consider as it does not usually respond to peripherally-directed treatments and therapies (such as opiiods and anti-inflammatory drugs), and is also thought to play a role in the development of chronic pain.

2.3.2 Chronic Pain Syndrome

Acute pain, which occurs in response to tissue damage and other related inflammatory processes, serves a specific purpose in mainly survival and healing. Chronic pain, however, persists after the acute danger/damage has passed and thus serves no evolutionary purpose, but ends up becoming a burden. It is often associated with maladaptive pathophysiological and anatomical changes, such peripheral and central sensitisation, as well as the development of new neural connections, and certain brain alterations [33].

2.3.3 Sensitisation

After certain injuries, stimuli in the injured region that were normally only slightly painful could be perceived as significantly more painful than they would have been perceived before. This phenomenon is called *sensitisation* and plays a central role in the development of chronic pain. Sensitisation may include a drop in the pain threshold or an increased response to sub-threshold stimuli [29].

Sensitisation that manifest as an abnormally painful reaction to normally less painful stimuli, especially repeated stimuli, is called *hyperalgesia*. When there is pain due to a stimulus that would not normally cause pain, it is called *allodynia* [29].

Such sensitisation may occur due to changes in sensitivity at the peripheral level as well as in the central system. Both peripheral and central sensitisation can occur via several different mechanisms, the details fo which are not relevant to this thesis and will not be described here. The two types of sensitisation are described next.

Peripheral

When tissue is damaged, an ”inflammatory soup” of substances such as extracellular protons, histamine, serotonin, nucleotides, nerve growth factors (NGF), etc. is released in response. All of these substances can interact with the ion channels of the nociceptive fibres or nociceptors, resulting in peripheral sensitisation, which is an augmentation in the response of those fibres. The release of all these and other chemicals at the site of injury is presumed to be the protection of the injured area, the prevention of infection, and also better/faster healing [13].

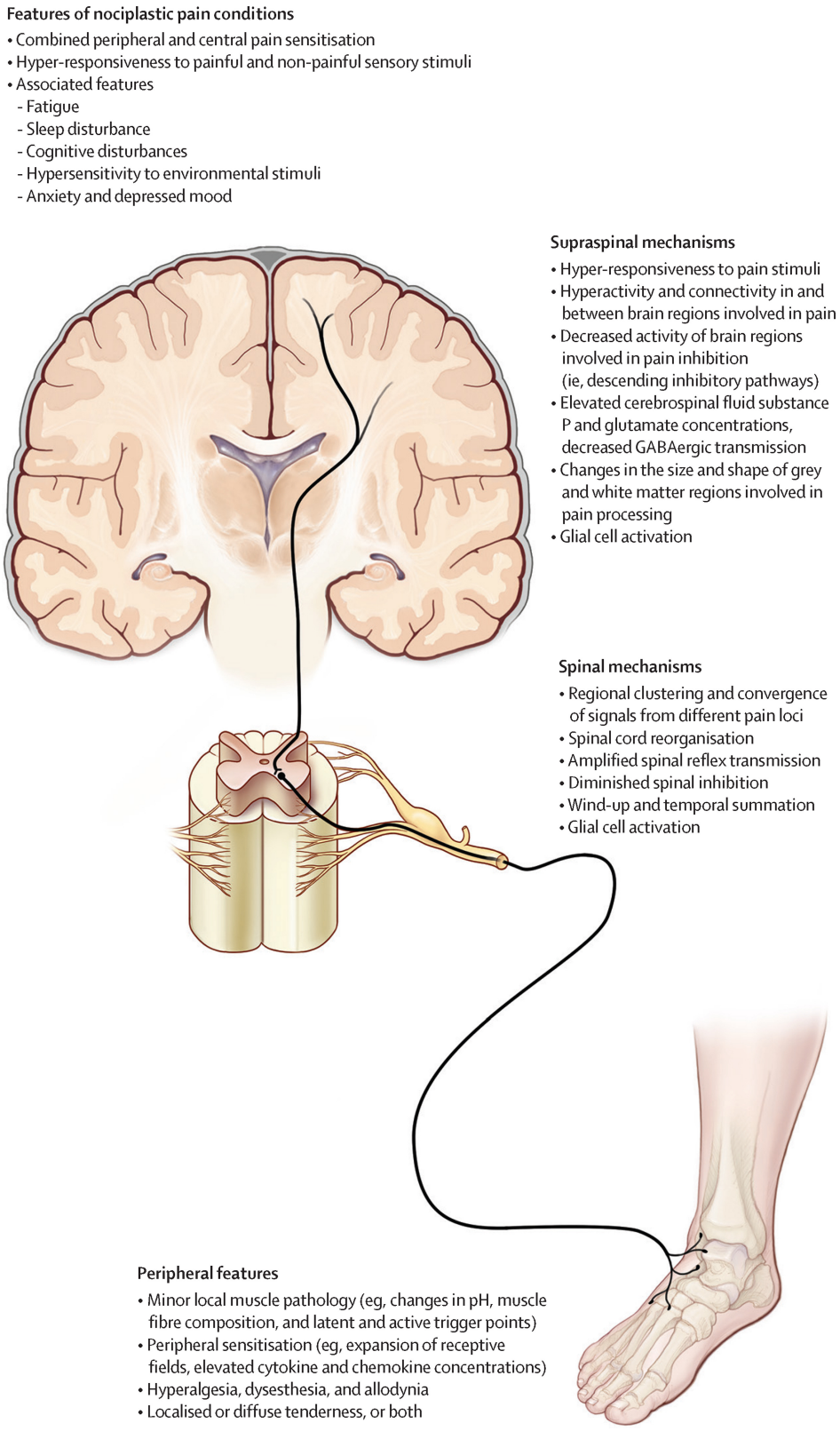

Figure 2.6: Features and mechanism of nociplastic pain. Reproduced from [32]

Central

Sometimes, as a result of high level of activity in the nociceptive afferents, the excitability of the neurons in the dorsal horn of the spinal cord increases greatly. This change is rapid, and because of this, activity levels in the nociceptive afferents that were previously sub-threshold is able to generate action potentials in the already excitable neurons in the dorsal horn, which leads to an increase in pain sensitivity. This effect can sometimes be generalised to low-threshold mechanoreceptors, in addition to the nociceptors, and hence, innocuous sensations (such as brushing against the skin lightly) are no longer perceived as innocuous, but as painful instead (allodynia, as defined earlier). Such sensitisation usually occurs immediately after a painful event but can outlast the original painful stimulus by several hours [13].

2.4 Assessment of Pain

Assessment of pain in patients suffering from pain and pain related disorders is important for the treatment of those disorders. However, in addition to that, conducting research with human subjects that are healthy and pain-free can help with improving our understanding of the physiological and psychological mechanism of pain perception, mediation and modulation. Such research can lead to increased knowledge about the nociceptive system, which is important in the further development of diagnosis and treatment methods.

In general, pain studies in humans consist of procedures that involve stimulating the nociceptive system (usually mildly painful stimuli) and measuring the nociceptive response to this stimulation. This response can be measured in terms of physiological measures as well as psychophysical ones. The main factors involved in pain research, and specifically in the research conducted in this study, will be described in this section.

2.4.1 Stimulation

One of the fundamental methods of studying pain mechanisms is to stimulate the nociceptive afferent nerve fibres in the skin. As stated before, the Aδ fibres and C fibres are associated with nociception, so these fibres need to be selectively stimulated in order to avoid interference from other sensory systems. Several methods can stimulate the Aδ fibres but also stimulate other fibres as well, so the stimulation method, the stimuli, etc need to be chosen such that only nociceptive fibres are activated. The stimulation method also needs to be quantifiable, reproducible for further research, and safe for the subject.

The stimulation can be of different types, such as mechanical, thermal, or electrical. which all have their own advantages and disadvantages. Mechanical stimulation is usually slow and cannot be controlled precisely, while the biophysical effects of ultrasound stimuli are not properly known. Thermal stimulation, for instance laser stimulation, is considered one of the best tools in the diagnosis of dysfunction of the nociceptive system. However, the slow heating of cutaneous tissue leads to asynchronous responses from the central and

peripheral neurons and depends on factors such as skin pigmentation and other individual skin properties [34]. Electrical stimulation, on the other hand, is easy to control (especially the stimulation timing) and the stimuli are highly quantifiable; parameters of the electric pulse can be controlled and varied with great accuracy. The control of the timing is of special importance if stronger stronger nociception is to be achieved by less strong stimuli using temporal summation (described in Section 2.2.3).

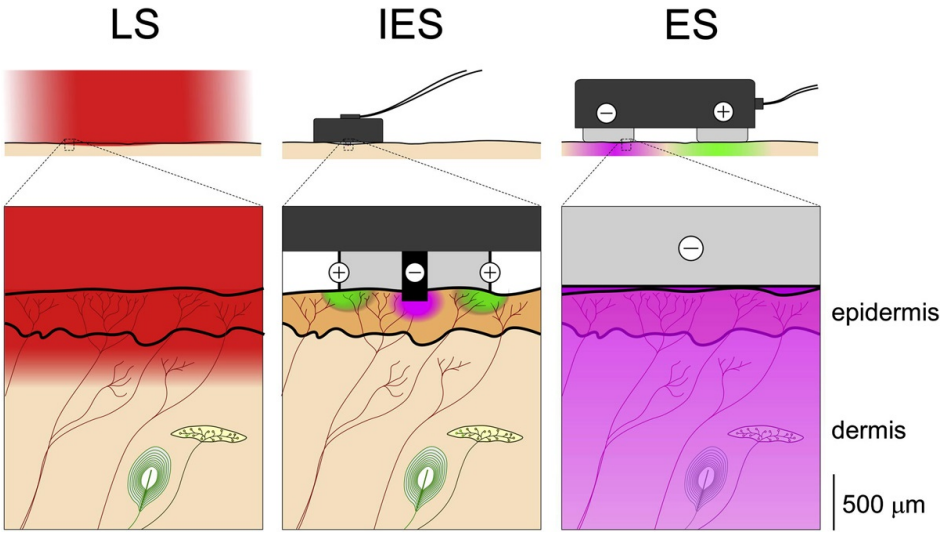

Figure 2.7: Schematic representation of laser stimulation (LS), intra-epidermal electrical stimulation (IES) and transcutaneous electrical stimulation (TES) of the skin. Left: the heat delivered during LS affects the area around the stimulation point, even entering the dermis. Centre: the electric current generated by IES is spatially restricted to the epidermis, so it is expected to selectively activate nociceptive-free nerve endings located in the epidermis. Right: the electric current generated by TES activates non-nociceptive $A\beta$ nerve fibres located deeper in the dermis. Reproduced from [35]

Intra-Epidermal Electrocutaneous Stimulation (IES):

The superficial Aδ fibres, which reside very close to the surface of the skin, can be selectively activated using a method called *intra-epidermal electrocutaneous stimulation*. In this method, an electrode with micro-needles is pressed against the skin gently, such that the needle tips penetrate the epidermis and are hence in proximity to the desired nerve endings [36]. A current passed between this electrode and a surface electrode placed nearby on the surface of the skin has been shown to preferentially activate Aδ fibres [35, 36].

Some advantages of the IES method are that no expensive equipment is required for the stimulation, and the stimulation can be applied to any part of the body. IES also generates a strictly time-locked response since the stimuli induce a single action potential in peripheral nerve fibres. However, a drawback of this type of stimulation is that the amount of current has to be limited if selective activation of Aδ fibres is desired, as a current of more than twice the nociceptive detection threshold has been observed to activate Aβ fibres as well [35]. This means the selective activation of Aδ fires can be achieved with this method as long as the current delivered with IES is restricted to less than twice the nociceptive detection

threshold (NDT).

Stimuli

A stimulus can be defined as an event that causes a functional reaction in an organ or tissue; in case of IES, the stimulus is the electrical signal (current) that is in the form of separate pulses or pulse trains, of constant or varying amplitude. Other parameters that can be controlled are the pulse width (PW), the inter-pulse interval (IPI) in case of stimuli with multiple pulses, the frequency, etc.

A factor such as frequency is important when using long trains that contain numerous stimuli occuring at a specific frequency. Such a stimulus is used to elicit tonic (sustained) response, and is used for frequency tagging, for instance, and other frequency analysis [37]. In other cases, a transient (or phasic) stimulus is used, and the transient response to separate stimuli is investigated, either independently or in relationship with responses to other stimuli. As mentioned before, the stimulus amplitude must remain less than twice the nociceptive detection threshold in order to achieve selective stimulation of nociceptive fibres.

A stimulus may contain one pulse, in which case it is aptly called a single pulse (SP) stimulus; stimuli containing multiple pulses can be double pulses (DP) or quadruple pulses (QP) and so on. When stimuli with multiple pulses are used, the IPI is adjusted to a small enough value that temporal summation may occur. In other words, the pulses have to be very close together in time, especially compared the time interval between each set of pulses that counts as a single stimulus. A study using various combinations of stimulus parameters showed that the detection thresholds tend to be lower and the slopes of the psychometric curve tend to be steeper with the addition of a second pulse [12]. Thus, it was shown that using double pulse stimuli increases the probability of detection of the stimuli. Similar results were seen in another study with multiple threshold tracking, which used double pulse stimuli with an IPI of 10 ms and 40 ms [11]. These studies also showed that tracking NDTs with double pulse stimuli was less prone to habituation or drifts of the threshold (Figure **??**.

Stimulation Methods

Several methods of applying such stimuli to the subject have been developed. For example, in the method of constant stimuli, the subject is stimulated with set pre-determined stimuli which the subject has to compare to a reference stimulus, and determine whether the stimulus is stronger or less strong than the reference [19]. A more adaptive method is the method of limit, wherein the stimulus amplitude is varied in ascending or descending steps, and the subject must report if the stimuli are stronger or weaker than the reference. Such a paradigm requires fewer repeated measures as it can be expected to converge to approximately the true value [19].

The procedure used for these kinds of stimulation is usually the go/no-go (GN) procedure. However, another one called the two-interval forced choice (2IFC) method also exists. These procedures will be expanded upon in a later section (see section 2.4.3).

2.4.2 Measures

The response of the subject to the stimulation of nociceptive nerve fibres can be measured in two main ways. One of them, called psychophysics, relates an event in the external physical world, in this case the stimulus, to the psychological domain, which is the perception of that stimulus. The other is the measurement of cortical activity induced by the stimulation, i.e., the temporal and spacial variations in local potentials. Some important concepts included in both types of measures will be described here.

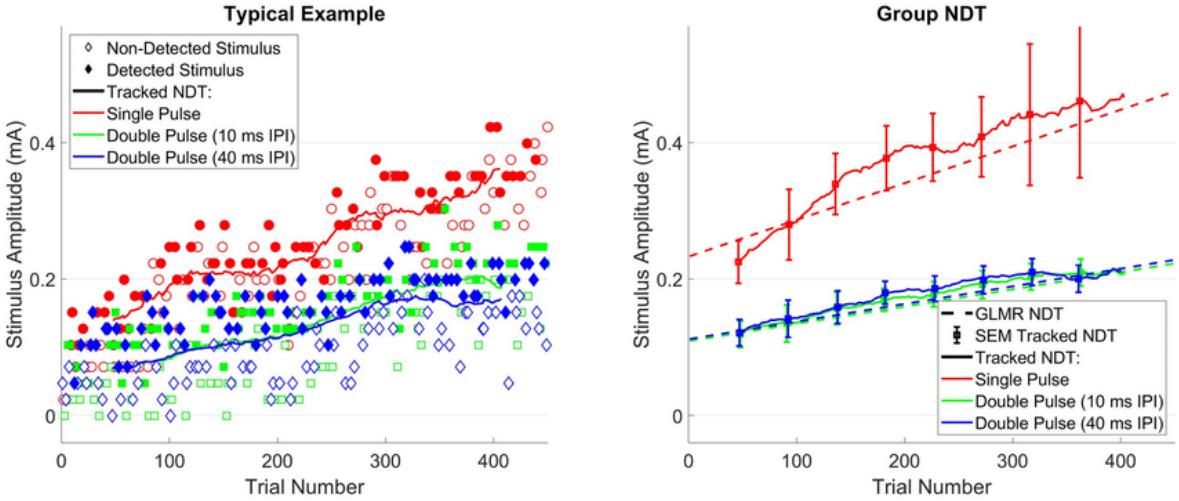

Figure 2.8: Left) a typical example of tracked nociceptive detection thresholds (NDTs), and (right) group level NDTs, for multiple thresholds being tracked. The bars in the group level figure indicates the standard error of the mean of tracked thresholds. For each NDT, it can be observed that the threshold increased over time due to habituation, but the threshold for DP stimuli was much lower for the SP stimuli. Reproduced from [11]

Psychometric Curve and NDT

According to the high-threshold theory, a stimulus is perceived when the accumulated sensory evidence exceeds a fixed internal criterion. As per this theory, whether the subject perceives the stimulus or not depends on a large number of neurons, each of which have a random probability of firing, which means the noise of sensory evidence can be assumed to be normally distributed [38]. However, psychophysical measurements show that the error distribution converges in its tails to a constant value instead of converging to zero. The left-sided tail occurs because the subject's mind may perceive a stimulus in the absence of an actual stimulus, due to background activity; the right-sided tail occurs because the subject's mind may fail to perceive a stimulus despite the the clear presence of one, due to background activity or distractions. These phenomena are referred to as *guessing* and *lapsing*, respectively.

The graph of the relationship between the amplitude of the electrical stimulus and the response of the subject expressed in terms of detection probability can be obtained using stimulus-response pairs, and is called the psychometric curve. By applying stimuli that have

a detection probability of 0.5, the psychometric curve for each stimulus (or stimulus set) can be made. This curve is used to estimate the average Nociceptive Detection Threshold (NDT) and its slope indicates the reliability of the detection, i.e., how accurately the subject was able to distinguish when a stimulus was present or absent; a higher slope indicates higher reliability.

For adaptive methods, an accurate and unbiased value for the NDT can be obtained, but the slope is inaccurate and negatively biased [19]. This negative bias exists because the adaptive methods are designed to converge to the correct threshold value from below, and can be avoided by taking a large number of measurements.

Electroencephalography (EEG)

The cortical activity related to nociceptive processing can be measured in terms of temporal and spacial variations using a variety of methods such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG), electroencephalography (EEG) and microneurography (MN). Out of these, EEG is more commonly used as it is relatively low-cost, is easy to use, and gives a good trade-off between temporal and spacial accuracy.

EEG measures the voltage fluctuations in the neurons of the brain due to ionic currents by recording the electrical activity on the scalp. This is done by placing a large number of electrodes over the whole scalp. The recorded signals represent spontaneous activity occurring in the brain over a certain period of time; this activity is generally not of any use in research. In EEG measurement, the signals of interest is the time-locked response to an stimulus, activity or thought, and is called an Evoked Potential (EP) or an Event Related Potential (ERP).

Evoked Potentials An *evoked potential* (EP) is a transient response of the cortex to applied stimuli and can, hence, be used to characterise cortical activation due to nociceptive stimuli in pain research. This is done by measuring phase-locked activity in the EEG, occurring after the stimulus application.

In general, relevant components in the EP are described using the polarity of peaks found in the EP, i.e., positive (P) and negative (N), and the order in which they appear (for instance, P1, P2 or N1, N2, N3). Figure 2.9 shows an example of typical EPs observed in pain research. The negative peak N1, seen in the figure, is thought to represent early sensory processing, while the negative peak N2 is known to be related to attention and stimulus awareness. Both of these components are thought to originate in the somatosensory cortex and hence measured on the lateral side of the head. The positive component P2 is also related to attention and arousal due to the stimulus, and is thought to originate in multiple areas of the brain, primarily the anterior cingulate cortex (ACC), and is hence measured at central locations on the scalp.

The EEG data is usually obtained via multi-stimuli experiments conducted on multiple different subjects, and contains a significant amount of background activity. The data is

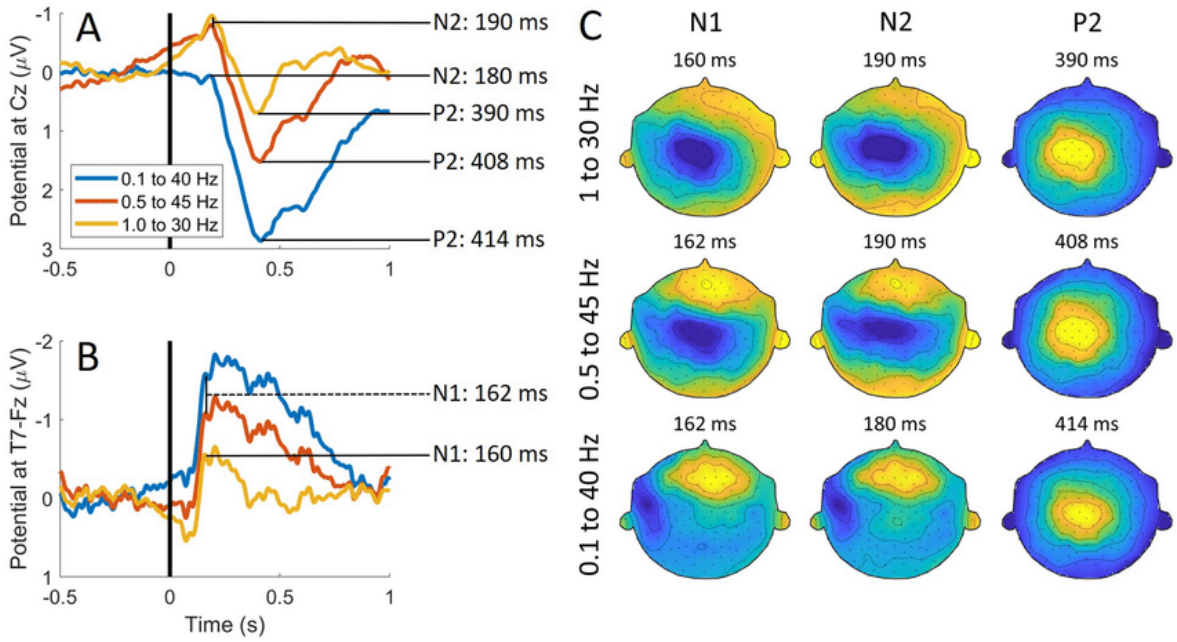

Figure 2.9: (Left) Example of a typical evoked potential (EP). A. EP at Cz, which is the central electrode, and B. EP at T-Fz derivation. The signals are band-passed filtered in three ways. Latencies at which peaks occur in each waveform are also marked. (Right) Evoked potential topographies at the latencies of N1, N2 and P2 (for each band pass filter) corresponding to the EPs shown to the left. Reproduced from [11]

pre-processed using band-pass filtering and removal of EOG (eye movement/blink) artefacts. Despite this, the low SNR (signal-to-noise ratio) of the measured signals leads to high trial-to-trial variability. To extract relevant information from these signals, various statistical techniques such as time-locked averaging and linear regression are used. Currently, time-locked averaging seems to be the golden standard in nociception research, and is used frequently [19], despite requiring a large number of trials to achieve accuracy and despite being influenced by habituation effects.

From the processed EEG data, other types of diagrams or figures, such as the topographical distribution of the signals on the scalp can also be obtained.

2.4.3 NDT-EP Method

As explained before, stimulus-response pairs obtained from stimulus detection experiments are used to obtain the psychometric curve, and the tracking of the thresholds can be used to observe the modulation of nociceptive processing. However, the underlying cause of the changing NDTs can be physiological or psychological; for instance, it could be due to the neuroplasticity of nociceptive processing or due to a change in a subjective detection criterion. The NDT-EP method combines both of these aspects such that the psychophysical detection thresholds and the evoked potentials are tracked simultaneously. In this method, EEG activity is measured for each of the detected as well as undetected stimuli, which gives the corresponding EPs for all of those stimuli.

The nociceptive detection threshold itself is determined by applying the adaptive staircase method mentioned before. The initial threshold is found by increasing the stimulus amplitude step-wise until the subject reports that they detected the stimulus. Subsequently, a vector of 5 different amplitudes with a step-size of 0.025 mA is initialised. One of these five amplitudes is randomly chosen to determine the amplitude of the next stimulus. If the stimulus is detected, all amplitudes in the vector are decreased by 0.025 mA, and if the stimulus is not detected, those amplitudes are increased by 0.025 mA.

The procedure used for this type of stimulation is usually the go/no-go (GN) procedure in which (1) an adaptive series of stimuli is presented, (2) the participant is asked to indicate when a stimulus was detected, and (3) the stimulus amplitude is increased or decreased depending on whether the stimulus was detected. However, it has been observed that the response of the subject during this method could reflect neural activity evoked by exceeding an internal ”response criterion”, rather the presence of sensory evidence. The response criterion may be sensory, perceptual or decision-based [39], and could be potentially avoided by using the two-interval forced choice (2IFC) method, in which the subjects have to indicate during which, out of two observations, a stimulus was applied [38, 40]. Non-zero EPs in response to undetected stimuli have also been seen when the GN method is used, which is another drawback of this method.

2.5 Summary of Relevant Research

2.5.1 Findings with NDT-EP Method

In one study, the changes in NDT that were related to stimulus parameters were successfully demonstrated [11]. Another study measured how the peripheral sensitisation caused by the application of capsaicin affected the NDTs [41]. Using Generalised Linear Mixed Models (GLMM), a model to evaluate the influence of individual stimulus parameters on the EPs was formed; this model was especially useful in the visible effects of habituation on the EP (that conventional averaging methods would not normally show) [42] as well as individual differences in the influence of different intensities and trials on the EPs [43]. It was seen that the EPs are modulated by all the stimulus parameters in healthy subjects [11, 44], while in patients with failed back surgery syndrome (FBSS), no modulation or less modulation by certain parameters was observed [45]. A possible explanation for the above-mentioned discrepancy is that the relationship between induced stimulation and pain perception is less clear in chronic pain patients compared to healthy subjects [46]. Another study with lower-back pain patients showed that habituation as seen in EPs was altered in these patients, and demonstrated that this method could be potentially very useful in identifying altered nociceptive processing in individuals with chronic pain [47].

2.5.2 Presence of Novelty Component in EP

Based on all this research, it can be said that the investigation of EP modulation would be highly interesting for the characterisation of the nociceptive system as well as for future diagnosis and management of chronic pain. However, currently, the EPs obtained in such studies are heavily affected by the stimulus detection itself, for example, due to task-related factors (also called novelty components) like attending to the stimulus [18, 48, 49], and is also affected by saliency [50]. Thus, the information obtained from EPs reflects mainly the brain processes consisting of, at least partially, the detection and reaction to salient stimuli, and not just the nociceptive-specific response. For the optimal evaluation of the extent to which the stimulus parameters are encoded in the EPs, it would be useful to reduce or possibly even completely remove these task/novelty-related components.

One study used pairs of identical stimuli but repeated them across blocks with either variable or constant inter-stimulus interval (ISI) and showed a reduction in EP that could potentially be due to lower novelty [51]. They found that the EP elicited by the second stimulus was suppressed when the ISI was constant, but not when the ISI was variable. In fact, when the ISI was constant, the second stimulus elicited EPs that were comparable to the ones elicited by the first. This study concluded that novelty or temporal predictability of the stimulus greatly influenced the saliency of the stimulus and thus affected the response to the stimuli, implying that the reduction in the ampltitude of the second EP resulted from predictability (or lack of novelty) of the stimulus.

Another recent study used repeated identical stimuli applied at a frequency of 1 Hz for 60 seconds [52]. The results showed that the EPs from the second stimulus show a sharp reduction in amplitude, while the subsequent stimuli showed a steady slow decay in amplitude. The authors concluded that the habituated response observed in the second EP in their study was not due to neural refractoriness or fatigue, but the obligatory component of the EP, with the novelty-related components largely removed, while the steady decrease in amplitude of EPs thereafter was likely due to the neural refractoriness or fatigue.

2.5.3 Other Relevant Research

A more recent (unpublished) study used pairs of stimuli, with the second stimulus in each pair having double the number of pulses, i.e., single pulse - double pulse being one pair, and double pulse - quadruple pulse being another pair [38]. This study used the first stimulus of both pairs, i.e., the single pulse in the first pair and the double pulse in the second, to track NDTs; once again, the pair that used the DP for the tracking showed similar results to the ones described above. An interesting observation was that when comparing the EPs elicited by the first stimuli of each pair or the second stimuli of each pair, both of the first stimuli resulted in similar EPs, and both of the the second stimuli also resulted in similar EPs. There was however, a drastic increase in the amplitude of the EPs elicited by the second stimuli of either pair compared to the first stimuli of those pairs.

2.6 Implications

To summarise some of the research mentioned throughout this chapter, the IES-5 electrode has been shown to selectively activate the nociceptive aδ fibres in multiple independent studies, as long as the current amplitude is kept below 2x the detection threshold. The NDT-EP method was developed to track the detection threshold throughout an experiment and obtain EEG data at the same time. Studies done using this method have shown that studying how stimulus parameters module evoked potentials in healthy subjects and chronic pain patients would help in characterising the nociceptive system better, which could in turn be useful for future diagnosis and treatment. However, it is also seen that the EPs measured in such studies are heavily affected by non-nociceptive cognitive components such as the novelty of the stimulus, which hinders the analysis of these EPs in terms of modulation due to stimulus parameters. Hence, it would be beneficial to remove such components from the measured EPs.

Some studies showed that using a second identical stimulus at a predictable interval could result in EPs that are free from the novelty component, as the predictability of the interval and stimulus strength removes the novelty of the stimulus itself. This could mean that EPs without the novelty component could be obtained by adding a second stimulus after the stimuli used for tracking thresholds in the NDT-EP method. However, in the NDT-EP method, all the applied stimuli are around the detection threshold, and thus are supposed to have a 50% detection probability ($p \sim 0.5$), so a second stimulus of the same amplitude is not guaranteed to be perceived by the subject, even if the first one was. To remove this uncertainty, we would have to make sure that the second stimulus has a much higher probability of detection ($p \gg 0.5$). This could be accomplished by applying a stimulus that has higher strength/intensity than the first, but still under 2x the detection threshold, e.g., by using double the number of pulses in the first one. On the other hand, the increased strength of the second stimulus would lead to higher salience, so the enhanced EP elicited by this stimulus would not be the EP elicited by the first stimulus minus the novelty component. A third stimulus that is identical to the second and at a predictable interval, however, would be expected to produce an EP that would not contain the novelty component but only the obligatory nociceptive component, and can be compared to the EP from the second stimulus. A fourth stimulus could be used to replicate the study by Mancini et al (2018) [52] showing a smaller decrease in amplitude due to habituation, compared to the decrease in amplitude due to possible novelty removal.

New Stimulus Protocol

Based on the above implications and the resulting train of thought, we hypothesise that it should be possible to evaluate the EP without novelty-related components using a new stimulation protocol during the NDT-EP method. In this protocol, after each stimulus with an amplitude determined by the NDT method, three stimuli at 1 Hz are provided. Three repeated stimuli have the same amplitude as the first one, but have higher strength; this is

implemented by using double pulse stimuli for the first stimulus, and quadruple pulse stimuli of equal amplitude for the three repeated stimuli. Table 4.1 shows the way this stimulus protocol would be applied:

Stimulus Nr.	NoP	Amplitude	Timing	Comment
1	2	determined by NDT	determined by NDT	Tracks threshold, EP contains novelty
2	4	equal to Stimulus 1	variable after detection of Stimulus 1	Higher salience than Stimulus 1, EP is enhanced, contains novelty
3	4	equal to Stimulus 1	exactly 1 s after Stimulus 2	Same salience as Stimulus 2, predictable, EP is reduced significantly, no novelty
4	4	equal to Stimulus 1	exactly 1 s after Stimulus 3	Same salience as Stimulus 3, predictable, EP is reduced slightly, habituation

Table 2.1: Proposed stimulation protocol, with the goal of obtaining EPs without the novelty component.

Chapter 3

Materials and Methods

3.1 Participants

In March 2022, ten healthy participants (n=10) between the ages of 18 and 60 years were recruited from amongst the students at the University of Twente, the Netherlands, through an ad placed on the student website of the university, via a page meant for listing studies that need volunteers [1]. The group consisted of a mix of genders (6 male and 4 female) and ages (Range = 19 - 27 years, M = 23.4, SD = 2.73). The main exclusion criteria for the recruitment were as follows-

- Diabetes Mellitus
- Pregnancy
- Implanted stimulation device
- History of chronic pain
- Complaints of pain before the start of or during the experiment

The participants were asked to avoid consumption of alcohol and recreational drugs, as well as excessive consumption of coffee 24 hours before the time of their experiment; sufficient sleep the night before the experiment was also recommended. Each participant was asked to fill out a form indicating whether they fell under one of the criteria listed above; they were also asked to indicate their weekly amount of alcohol consumption, sleep, exercise, and any medications they take. Another exclusion criterion was implemented after obtaining results, based on detection rate (see Section 3.5.1).

Ethical Considerations

The participants were provided with written information about the study and the experimental procedure, in advance. A signed consent form was obtained from each participant before

[1] www.canvas.utwente.nl, accessible only with student log-in

conducting their experiment. The study was performed in accordance with the Declaration of Helsinki (October 2013) and the Medical Research Involving Human Subjects Act (WMO) and Good Clinical Practice (GCP) guidelines. The research protocol was assessed and approved by the Natural Sciences and Engineering Sciences Ethics Committee of the University of Twente [2].

3.2 Stimulus

Intra-epidermal electrocutaneous stimulation was achieved using sterilised IES-5 electrodes; this electrode (shown in Figure 3.1) consists of an array of five micro-needles that penetrate 0.2 mm into the *stratum corneum* (outermost layer) of the skin and has been shown to selectively activate the superficial (Aδ) nociceptive skin fibres [35]. The AmbuStim stimulation device (NociTRACK, University of Twente, Enschede, NL) was used to deliver the stimuli to the right arm of each participant, approximately 10 cm from the *medial epicondyle*. The instructions to this stimulation device were sent via Bluetooth from a personal computer, using a custom programme written in LabView 2013 (National Instruments, Texas, USA).

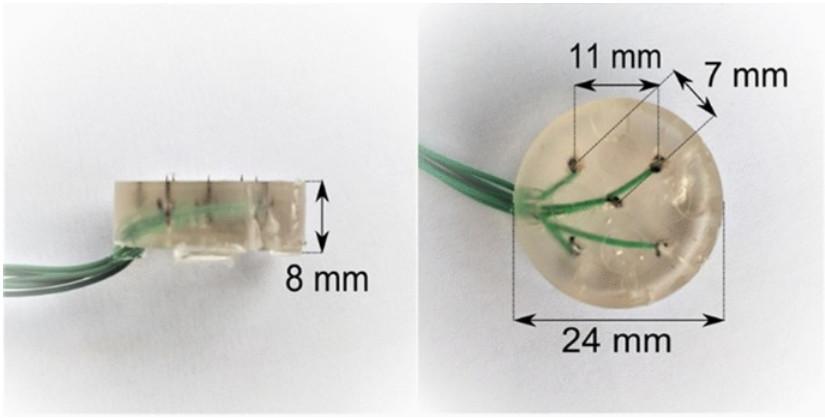

Figure 3.1: (Left) Side view, and (right) top view, of an IES-5 electrode, along with dimensions.

The stimuli themselves each consisted of a set of four stimuli. The first of each set was a double pulse with an amplitude that was set using the random staircase method used in NDT studies [53]; this amplitude is around the nociceptive detection threshold so as to ensure a $p = 0.5$ chance of detection.

If the stimulus was detected, as indicated by the subject themselves, then three more stimuli were sent. Each of these stimuli were quadruple pulses of the same amplitude as the earlier double pulse, such that there was $p \geq 0.5$ chance of detection so as to ensure that the repeated stimuli were perceived. The choice of doubling the number of pulses (NoP) to increase stimulus strength (via temporal summation, see section 2.2.3) instead of doubling the amplitude of the stimuli itself was made in order to keep the stimulus nociceptive selective, as it is known to not be selective at twice the detection threshold [35]. The time interval between the double pulse and the first quadruple pulse was variable (approximately 3-4

[2] Research protocol number: 2022.123

seconds), due to the variability of the Bluetooth communication speed, but the inter-stimulus intervals between the quadruple pulse stimuli were set to exactly one second. Figure 3.2 illustrates what the stimuli may look like.

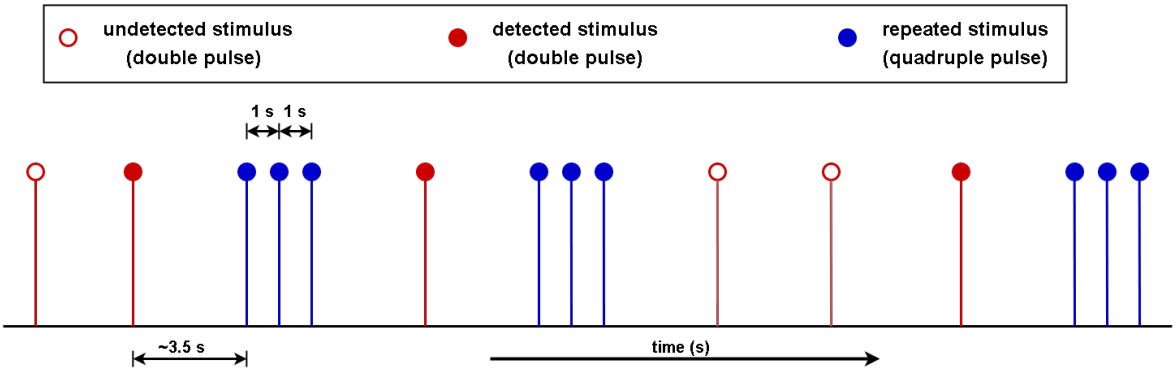

Figure 3.2: An example of how a series a stimuli in the newly-designed protocol may look like. If a DP stimulus is detected, it is followed by a set of three equal QP stimuli separated by 1 s each.

As per the random staircase method [53], the amplitude of the double pulse stimulus (first stimulus in each set) in the subsequent set of stimuli was based on whether the earlier one was detected. A set of five equidistant amplitudes was defined around the initial detection threshold using a step size of 0.025 mA, and the amplitude of the next double pulse stimulus was selected from this set. If the subject detected the stimulus, the amplitudes in the predefined set were increased by 0.025 mA, and decreased by 0.025 mA if the stimulus was not detected. A stimulus was considered to be detected if the subject indicated this (by releasing the button) within once second after the stimulus was applied. A total of one hundred detected stimuli were delivered, i.e., each experiment ended when the subject had detected a total of one hundred stimuli. Each of the stimuli was made up of pulses with the following characteristics:

- Pulse shape: Square pulse
- Pulse width (PW): 210 μs
- Inter-pulse interval (IPI): 10 ms

3.3 Measures

3.3.1 Nociceptive Detection Threshold

The double pulse stimuli were used for tracking the nociceptive detection threshold, based on the subjects response to each of these stimuli. As explained earlier, upon receiving the stimulus, the subject indicated if the stimulus was detected or not, and these responses were used to determine the amplitude of the double pulse stimulus in the next set of stimuli, using the adaptive staircase method. These double pulse stimulus amplitudes were used to track their nociceptive detection threshold. (The quadruple pulse stimuli were not used for

threshold detection as they had a much higher chance of detection ($\geq$0.5), by design, and were hence not indicative of the detection threshold itself.

3.3.2 Electroencephalography (EEG)

The electrical activity occurring in the brain during the course of the experiment was recorded at a sampling rate of 1024 Hz via EEG using a REFA amplifier (TMSi B.V., Oldenzaal, the Netherlands) and a standard ANT Neuro Waveguard EEG cap; only 32 of the Ag/AgCl electrode channels were used, as those were deemed enough due to the exploratory nature of this study. The distribution and location of the electrodes over the cap was according to the standard International 10-20 system [54]. (See Appendix A for the detailed diagram of this EEG cap configuration.) The placement of the cap on the scalp was such that the Cz electrode was in the centre when measured between the two mastoids and between the nasion and inion. A ground electrode for the EEG place placed in the middle of the forehead just above the supraorbital ridge. The scalp-electrode impedance is verified to be lower than 5kΩ. The EEG data was recorded on a dedicated computer using the Polybench Designer (version 1.30.0, TMSi, Oldenzaal, NL) software. The subjects were told to avoid eye movement as well as muscle movement and contraction in order to minimise artefacts in the recorded data.

3.4 Procedure

Before the start of the experiments, general data about the subjects, such as age, sleeping and exercise habits, drinking and/or drug habits, history of illness and/or injury, etc. was collected for potential future analysis. The setup for the experiment and the EEG cap electrode configuration can be found in Appendix sdsf. The experiments themselves took between 1.5 and 2 hours each, and were carried out as described below:

Familiarisation

After the subject is seated in their chair and the EEG cap and stimulator and correspoinding cables are all attached, they are instructed to press a button on the stimulator. A series of stimuli having increasing amplitudes with a step size of 0.025 mA is applied, starting from zero. The subject is asked to keep the button pressed until they are familiar with what the stimulus should feel like. The subject is asked to release the button once they are familiar with the sensation of the stimulus.

Initial Threshold Determination

Once the subject is familiar with the stimulus sensation, they are asked to press the button on the stimulator once again, and this time release it the first instant they perceive the stimulus. An ascending ramp of stimuli is once again sent to the stimulator. The amplitude at which

the subject perceives the stimulus the first time and releases the button, is the subject's initial nociceptive detection threshold (NDT).

Repeated Stimulus Protocol

After the initial NDT is determined, the main part of the experiment, using the repeated stimulation protocol described earlier, begins. This part of the experiment can take roughly an hour to an hour and a half, so the subject is given instructions to ensure the experiment goes well. The subject is asked to relax their muscles and to not move or clench any muscles during the experiment. They are also asked to gaze at a picture posted on the wall in front of them and to avoid moving their eyes; they are asked to blink as they normally would.

Once the subject is ready, they are asked to press the button on the stimulator, and the experiment begins. Starting at the detection threshold determined earlier, a series of double-pulse and quadruple-pulse stimuli are sent to the stimulation device as per the adaptive staircase method described earlier, whereby the amplitude increases or decreases based on the subject's response (refer to section 3.2). When the subject indicates that they have detected a stimulus, the three more quadruple pulse stimuli are sent. The subject is asked to wait for some (6-7) seconds before pressing the button again, as the three quadruple pulses will be sent during this period.. The experiment goes on until one hundred such detected stimuli sets have been recorded.

3.5 Data Analysis

3.5.1 Nociceptive Detection Thresholds (NDT)

The individual NDT values were determined using the psychometric function, and a graph of the NDT values throughout the experiment was plotted using Generalised Linear Methods (GLM) for each subject separately with the use of Matlab (version R2020b, The MAthworks, Inc., Massachusetts, US). The shapes of these plots and the detection rate of the double pulse stimuli were used to exclude subjects whose recorded data showed unusual behaviour. Specifically, a detection rate of at least 0.4 (40%) was used as an additional exclusion criterion at this stage. Additionally, the graph of the tracked NDTs can also be used to exclude subjects that had a sharp and continuous rise in NDT amplitudes, as this is an indication of substantial habituation.

Boxplots of the NDT as well the slope of the graph formed by measurements over the course of the experiment were also made. These were used to find the medians of the NDT amplitude and slope, which can be used for comparison with earlier studies. These values can themselves give insight into the reliability of the measurements, and by comparing them to the values found in earlier studies, we can investigate how the change in the stimulus protocol affects the NDT measurements.

3.5.2 Processing of EEG Data

The EEG data was processed in Matlab (version R2020b, The MAthworks, Inc., Massachusetts, US) using a software toolbox FieldTrip (Radboud University, Nijmegen, NL), which is specialised for EEG and MEG signal processing. The trials were segmented into epochs using a window ranging between 0.2 s pre-stimulus and 1.0 s post-stimulus. The first fifteen epochs were omitted as these do not provide a reliable estimate for the detection threshold. The data was filtered using a 2nd order high pass filter with a cut-off frequency of 0.1 Hz and a 6th order low pass filter with a cut-off frequency of 40 Hz. After this, the data was baseline corrected and then manually cleaned in two steps. First, independent component analysis (ICA) was applied, and components with eye blink artefacts and/or muscular activity and EMG artefacts were removed. After this, the trials (and/or channels) that were outliers were removed from the data based on the variance of the measured data in the trials and channels.

3.5.3 Evoked Potentials (EP)

The EPs obtained by using the epochs are grand averaged over all ten subjects, and then analysed in the following ways. For the butterfly plot, an average of the electrode potentials related to all stimuli was taken, while for the topography and EPs themselves, the response to the undetected stimulus, the detected stimulus, and the three stimuli after the first detected one, were averaged and analysed separately.

Butterfly Plot

An overview of the grand averaged measurements from each of the 32 measured channels (electrodes) was displayed in a butterfly plot. This figure also showed the GFP (global field potential); the peaks in the GFP were used to find the average latencies at which the EPs have peaks. The electrode potentials can also show which area of the scalp has more activity.

Brain Topography

After finding the relevant latencies using the butterfly plot, scalp topographies at the determined latencies for the four different stimuli in each trial, and the response to the undetected stimulus, were all plotted. These topographies were used to compare the areas of the brain activated at those latencies by each stimulus and to compare the strength of the activation.

Evoked Potentials (EPs)

Based on the areas of the brain that were found to be activated the most at the specified latencies, EP derivations were chosen. For each of the chosen derivations (T7-F4: contralateral, T8-F3: ipsilateral, and Cz-M1M2: central), the EPs in response to each of the detected stimuli and in response to the undetected stimuli were plotted and compared to each other.

Chapter 4

Results

The results of the experiments include the neurophysiological and psychophysical response to the stimuli that are undetected, and the the stimulus that is detected as well the three stimuli that follow the first detected one. For the sake simplicity, these stimuli will be henceforth referred to as undetected, detected$_1$, detected$_2$, detected$_3$ and detected$_4$.

4.1 NDT

The individual thresholds and the slops of the NDT graphs for each of the subjects as well as each of their detection rates are given in Table 4.1. It shows that all the subjects except Subject 08 have detection rates above 0.4; subject 08 has a detection rate of $\sim$0.36, and hence falls within the exclusion criterion of having the detection rate below 0.4. It can be seen that the NDT calculated for this subject is also unusually high (1.936) compared to the other subjects, for whom the values are well below 1.0. Due to this, the data for Subject 08 is excluded from further analysis in this study.

The NDT graphs for all eleven subjects is shown in Figure 4.2 (full size versions can be found in B). The graphs for subjects 1-7 and 9-11 do not show unusual or remarkable behaviour or shape, compared to earlier studies, as expected, corroborated by the values seen in Table 4.1. The individual graph for Subject 08, on the other hand, shows a sharp increase in the NDT, and reaches a maximum value (almost 2.5 mA) that is much higher than the rest of the graphs, each of which remains fairly stable or increases very little compared to that of Subject 08. This gives additional support for the exclusion of Subject 08 from the study.

For the rest of the ten subjects that can be included in the study, the box-plots for their NDTs and slopes are shown in Figure 4.1. The median of the ten NDTs is 0.35 mA while the median of the slopes is 17.75 mA^{-}, with four out of the ten measurements falling outside the middle 50%.

Subject Nr.	NDT	Slope	Detection Rate
Subject 01	0.405	17.75	0.472
Subject 02	0.150	43.45	0.487
Subject 03	0.539	13.96	0.440
Subject 04	0.782	06.23	0.423
Subject 05	0.229	39.90	0.488
Subject 06	0.459	07.97	0.441
Subject 07	0.384	08.96	0.442
Subject 08	1.936	01.44	0.359
Subject 09	0.306	16.79	0.469
Subject 10	0.185	27.97	0.500
Subject 11	0.314	19.36	0.478

Table 4.1: For each subject, the nociceptive detection threshold calculated from the NDT-EP experiments, the slope of the NDT graph obtained during the experiments, and the rate at which each subject detected the applied stimuli. Notably, the detection rate for Subject 08 is lower than that permitted by our exclusion criterion (0.5), and the NDT for this subject is almost significantly higher than the NDTs of the rest of the subjects.

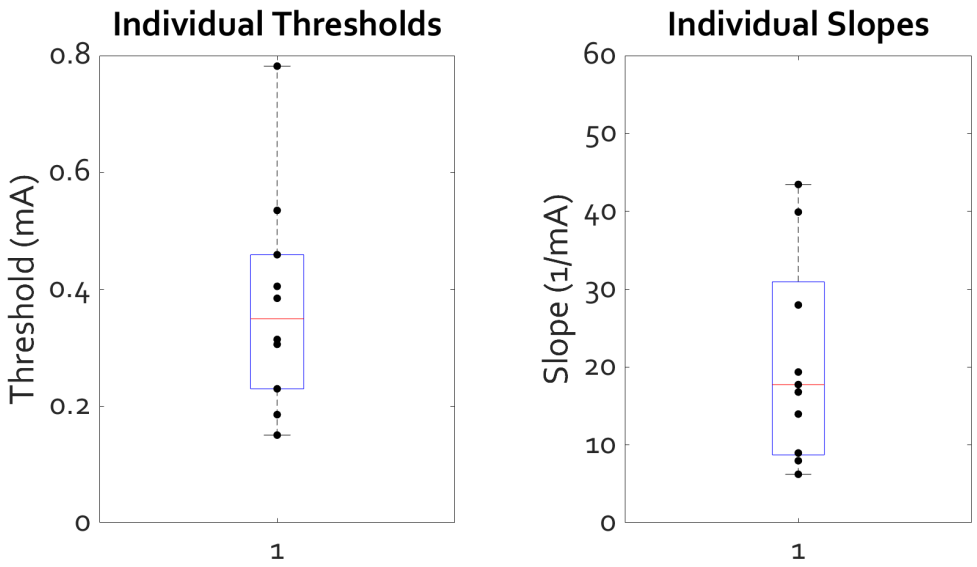

Figure 4.1: Boxplots of the average NDTs (left) and slopes (right) of the measurements for all ten subjects. The median of the NDTs is $\sim$0.35 and the median of the slopes is $\sim$17.75. Four of the ten measurements lie outside the middle 50% of values.

4.2 Electroencephalography

4.2.1 Butterfly Plot of All Channels

The Butterfly plot of the grand averaged EPs of each of the 32 channels used in the measurements, over all 10 subjects, within the chosen window of 0.2 s pre-stim and 1 s post-

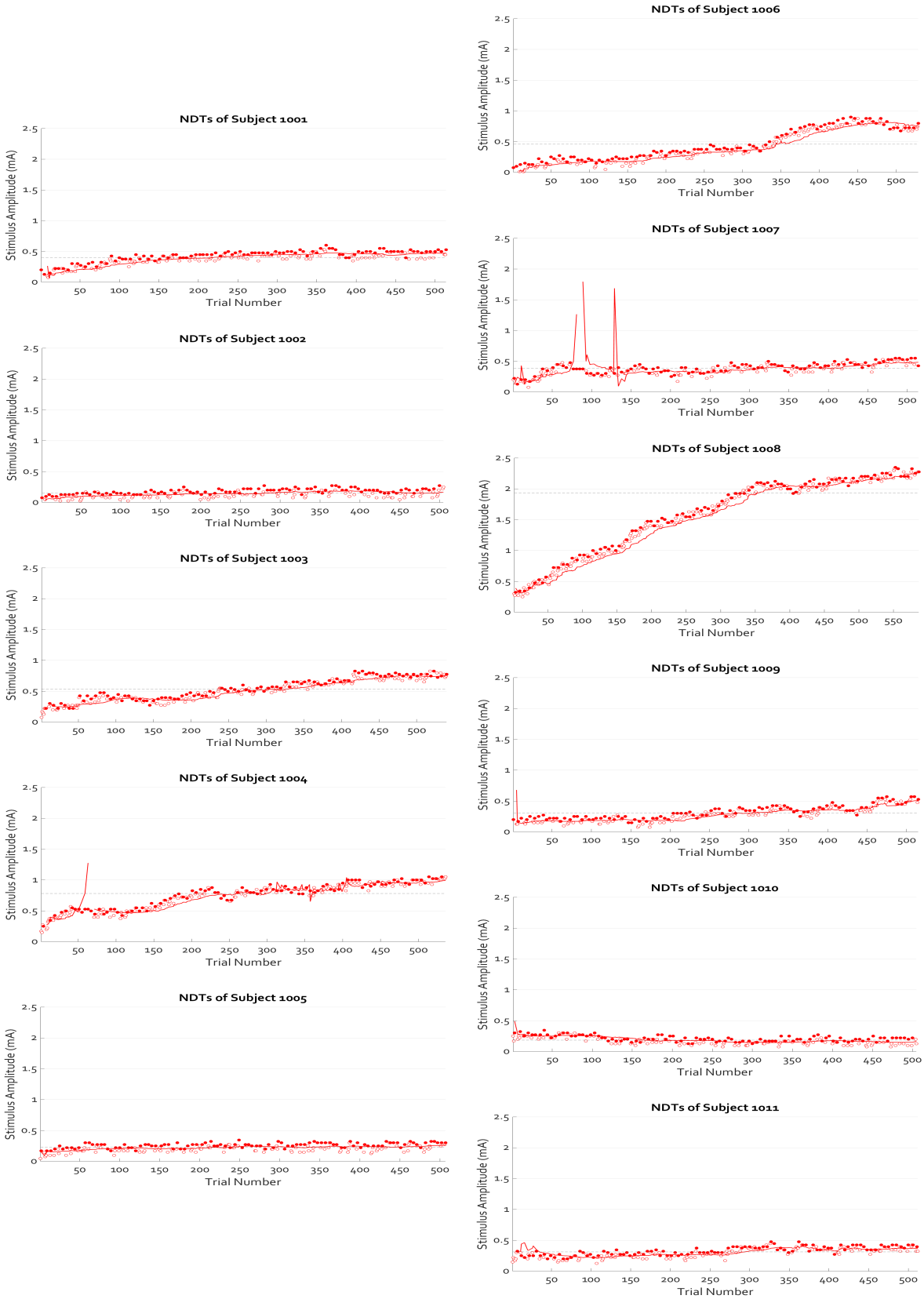

Figure 4.2: Tracked NDTs for each of the eleven subjects measured during this study. Full size found in Appendix ASAD. The thresholds remain fairly stable for most subjects, or increase a little over the trials for some subjects, compared to subject 08, whose tracked NDTs increase drastically over time.

stim, is shown in Figure 4.3. The figure also shows the global field power (GFP) over all the measurements. The latencies for the topographies and the EPs are chosen based on this plot. The figure shows the GFP has a large peak of around 2.2 μV at t=345 ms, and a smaller peak of about 1 μV at approximately t=165 ms. Hence, relevant EP components corresponding to the applied stimuli are expected to be found approximately at these time instances.

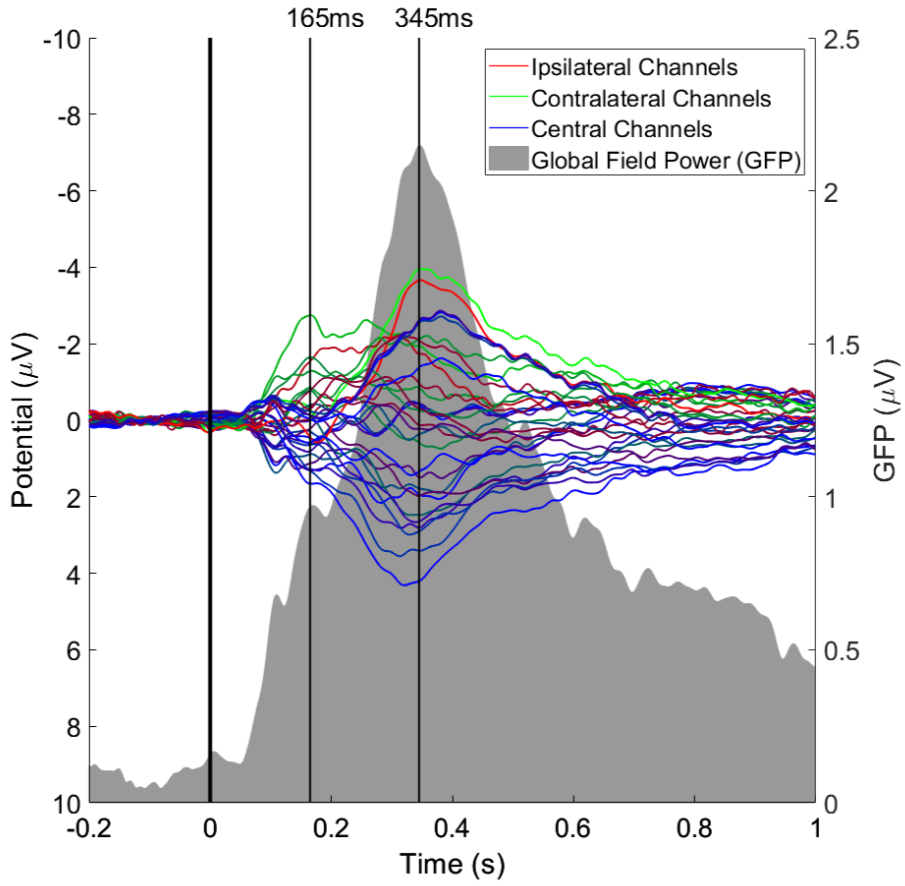

Figure 4.3: Butterfly plot of the grand average potential and global field power (GFP) of the 32 EEG channels in response to IES. A negative contralateral peak is seen at 165ms, while a positive central peak is seen at 345 ms, coinciding with the maximum GFP.

The electrode derivation with the highest potential ($\sim$4.3 μV) at t=345 ms is the central derivation (Cz-M1M2) while the electrode derivation with the largest peak (appr. -2.7 μV) at t=165 ms is the contralateral derivation (T7-F4). Grand averaged EPs are hence computed for both undetected and detected stimuli over the components of the two derivations, T7-F4 and Cz-M1M2, at latencies of t=165ms and t=345ms, respectively. For comparison, the EPs of the ipsilateral derivation (T8-F3) at a latency of t=165ms are also computed. Note that the contralateral and ipsilateral electrodes also show comparably large peak amplitudes of about -4.3 μV and -3.6 μV, respectively, at t=345 ms.

4.2.2 Grand Averaged Scalp Topographies

The average group topographies are plotted at the two peak latencies found in the earlier section, namely t=165ms and t=345ms. Figure 4.4 shows the scalp topographies at these latencies for the undetected stimuli, and the detected double pulse stimulus as well as each of the three repeated quadruple pulse stimuli that follow the first detected one.

The top row of images in Figure 4.4 shows the topographies at the latency of t=165ms. A negative contralateral component can be clearly seen in the area where the T7 electrode is located, along with a smaller negative contralateral component around the electrode T8, and a small positive central component around Cz too. The amplitude for the contralateral negative component increases going from detected$_1$ to detected$_2$, and then decreases for each of the consecutive detected$_3$ and detected$_4$ stimuli. There is also a weaker ipsilateral component that shows a similar trend. There is a positive central component present for the detected$_1$ and detected$_2$ stimuli, but the detected$_3$ and detected$_4$ stimuli seem to show frontal activity instead.

The bottom row of images in Figure 4.4 shows the topographies at the latency of t=345 ms. A strong positive central component is visible, centred around the area where the Cz electrode is located, along with a polar opposite negative component around the circumference of the scalp. The amplitude of this central activity increases going from detected$_1$ to detected$_2$, and then decreases for each of the consecutive detected$_3$ and detected$_4$ stimuli, similar in trend to the contralateral/ipsilateral activity seen at t=165 ms. There is no noticeable ipsilateral or contralateral activity in these topographies; however, for all of the positive central activity, there is some negative component around the circumference of the scalp.

The above components are mainly present (for both t=165 ms and t=345 ms) for the detected and consecutive stimuli. At both of these latencies, the undetected stimulus shows very little topographical activity; however, it is noteworthy that the undetected stimuli do show some activity, even if it's very weak. In general, for either of the latencies, the components in the scalp topography seem to show the following trend: the first and second detected stimuli show strong components in the scalp topography; the components for the second detected stimulus are stronger than the ones for the first detected stimulus; the third stimulus shows less strong activity and the fourth one shows even less activity. In other words, the strength of the components reduces for each of the consecutive two stimuli, namely, the third and fourth detected stimuli.

4.2.3 Evoked Potentials

Central Derivation (Cz-M1M2)

Figure 4.5 shows the grand averaged EPs for the undetected as well as the four detected stimuli at the central derivation, Cz-M1M2. All the detected stimuli show obvious positive peaks around t=345ms. The amplitude of the EP elicited from first detected stimulus is around 11 μV, while the EP corresponding to the second detected stimulus is much higher, at around 14.5 μV. Thereafter, the amplitudes of the EPs reduce, with detected$_3$ eliciting an

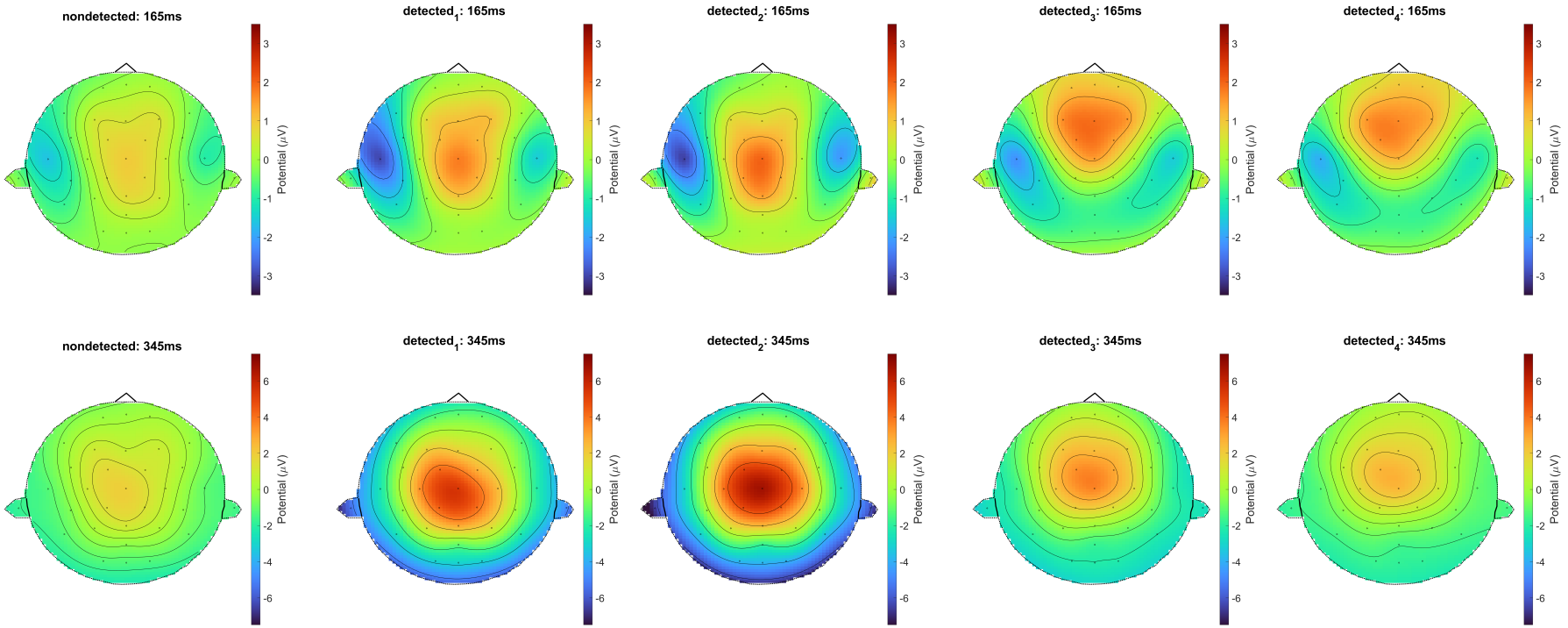

Figure 4.4: Group everaged topographies of all ten subjects at latencies of t=165 ms and t=345 ms after each applied stimulus. Top row: At t-165 ms, there is strong contralateral activity (max of -3.5 μV for detected$_2$) for all detected stimuli, as well as central activity (max of $\sim$2 μV for detected$_2$). A small ipsilateral component (max of -2 μV for detected$_2$) is also present. The central component seems to move towards the front for subsequent stimuli. Bottom row: At t-345 ms, there is strong central activity for all detected stimuli, for instance, with a max of $\sim$7.5 μV for detected$_2$.

EP of $\sim$6.5 μV and detected$_4$ eliciting and EP of $\sim$4 μV, both values being significantly lower the the first two.

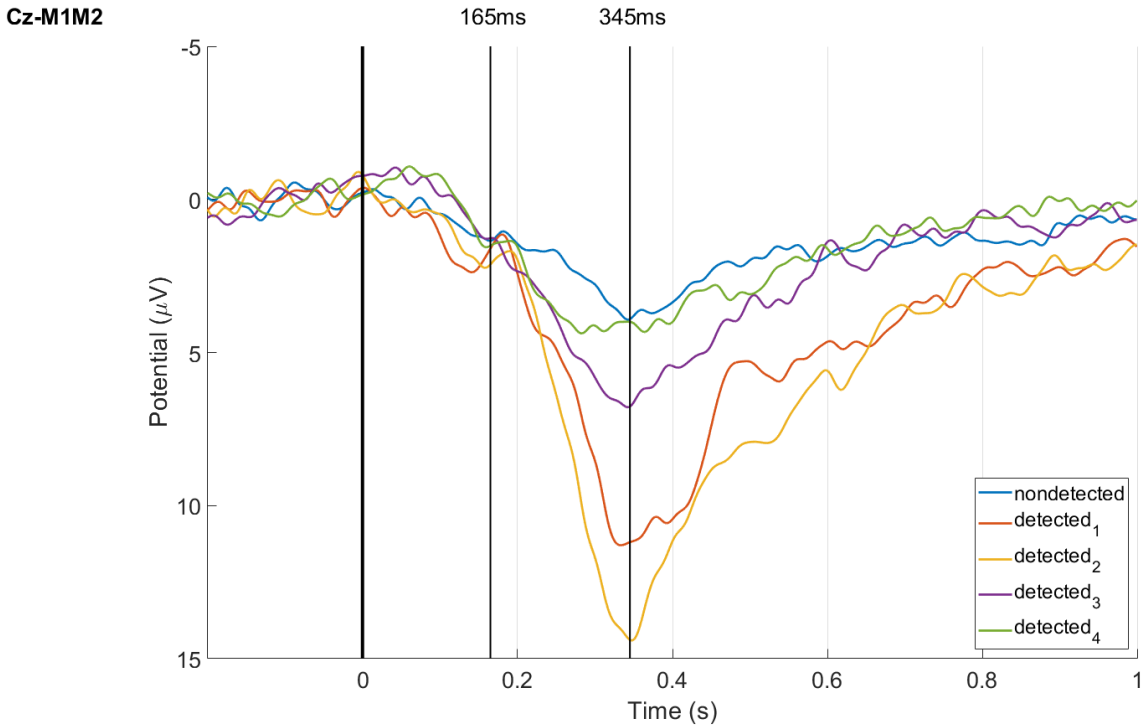

Figure 4.5: Group averaged EPs for the undetected stimulus and for the detected$_1$, detected$_2$, detected$_3$, detected$_4$ stimuli for the central derivation Cz-M1M2. All four show peaks at t=345 ms, with detected$_2$ being the highest, followed by detected$_1$, detected$_3$ and detected$_4$ in decreasing order of amplitudes.

Interestingly, the undetected stimulus seems to have elicited an EP as well. The detected$_4$ stimulus and the undetected stimulus both have an approximately similar shape overall and also alo have similar (and non-zero) amplitudes at the chosen latency of t=345 ms. Smaller positive peaks are also observed at t=165ms corresponding to the detected$_1$ and detected$_2$ stimuli.

Ipsilateral (T8-F3) and Contralateral (T7-F4) Derivations

Figure 4.6 shows the grand averaged EPs for the undetected as well as the four detected stimuli at the contralateral and the ipsilateral derivations, namely, T7-F4 and T8-F3, respectively. EPs seem to be elicited on both sides of the scalp.

For the contralateral derivation (top figure), all four detected stimuli shows clear peaks at t=165 ms, in the range of -3.5 to -5 μV. The amplitudes of the detected$_2$ EP is significantly higher than that of detected$_1$, after which detected$_3$ has a lower amplitude and detected$_4$ has an even lower one. The EPs of detected$_1$, detected$_3$ and detected$_4$ show no peaks at this latency, while detected$_2$ shows a prominently high peak of about -5.5 μV at t=345 s.

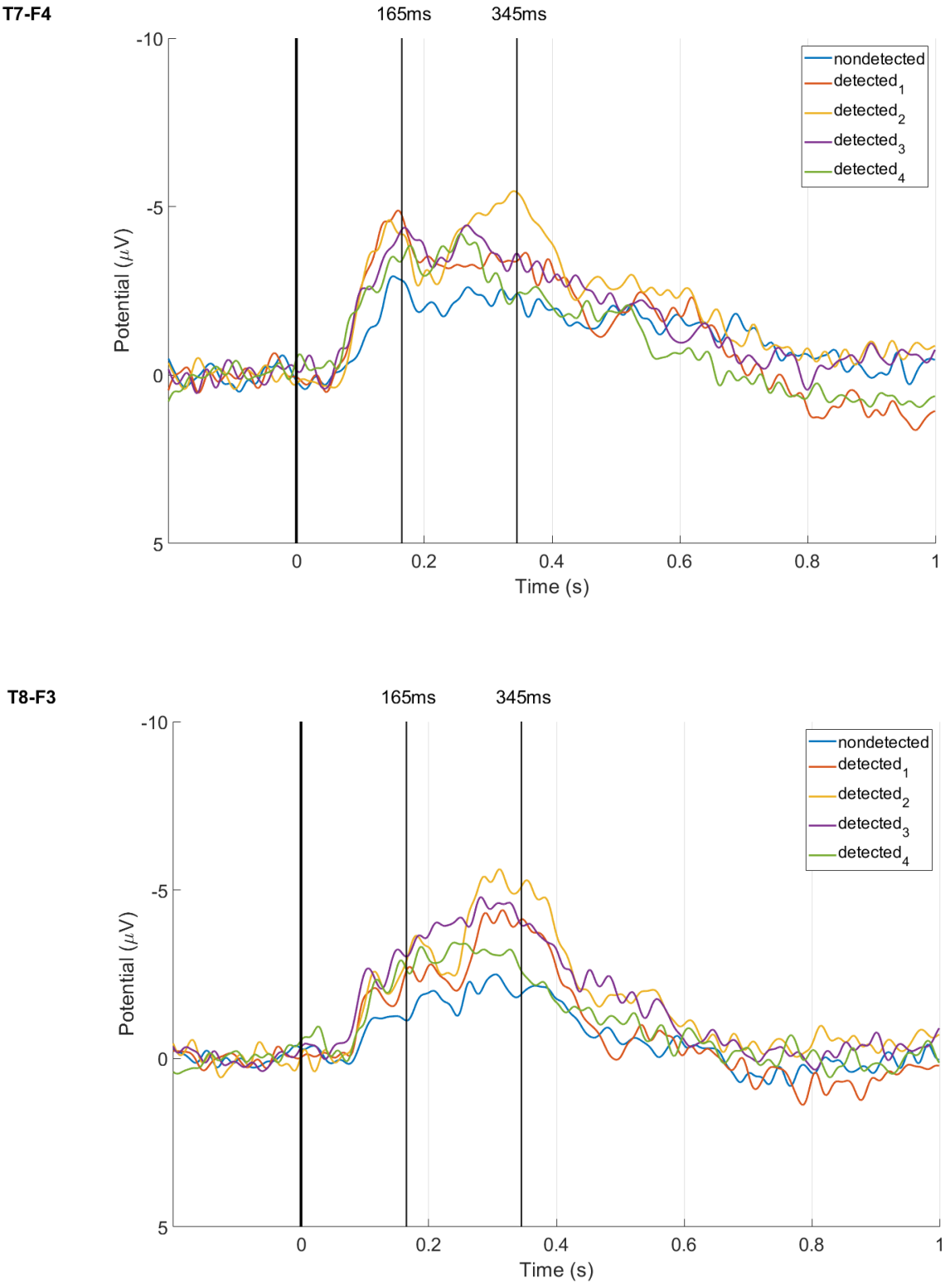

Figure 4.6: Group averaged EPs for the undetected stimulus and for the detected_1, detected_2, detected_3, detected_4 stimuli for the contralateral derivation (top) and ipsilateral derivation (bottom). Top: Peaks at t=165 ms show prominent peaks, with detected_2 being the highest, followed by detected_1, detected_3 and detected_4 in decreasing order of amplitudes. Bottom: Similar trend in peaks is seen at t=345 ms.

For the ipsilateral derivation (bottom figure), EPs show no peaks at t=165 ms for any detected stimuli, but show peaks at the latency of t=345 ms. At this latency, the amplitudes of all EPs are in the range of -4 to -5.5 μV and show a similar trend as the other derivations, with the amplitude increasing from detected$_1$ to detected$_2$ and thereby decreasing progressively for detected$_3$ and detected$_4$.

Once again, for both derivations at both chosen latencies, the undetected stimulus elicits a non-zero EP, similar to the central derivation. For the contralateral (T7-F4) derivation, the undetected EP shows a clear peak of around -3 μVat t=165 ms; however, there are no clear peaks at t=345 ms for this derivation. For the ipsilateral (T8-F3) derivation, there are no clear peaks for the undetected stimulus.

Chapter 5

Discussion

5.1 NDTs

The individual NDTs of each of the subjects were used to decide whether the results of that particular experiment could be included in the analysis. Ten out of the eleven datasets showed results that could be included in the analysis. These subjects showed a high detection rate, detecting on average 41.4% of the applied stimuli. As this is above the detection rate chosen as the exclusion criterion (40%), the data of these ten subjects was used for the further analysis discussed here.

For all ten subjects, the shapes of the tracked NDT graphs seem to show no unusual activity, with the tracked detection threshold staying fairly stable or increasing slightly; this is likely due to habituation. The box plots made subsequently for the NDTs of these ten subjects showed that a median NDT value of 0.35 mA (average 0.375 mA), which differ from values found in earlier studies. In earlier studies with healthy subjects that used NDT tracking using double pulse stimuli found average NDTs in the range of 0.15 to 0.25 mA [11, 12, 44]. In one study, where where pairs of double pulses and quadruple pulses were used during the NDT-EP experiments, the average NDTs were found to lie in the vicinity of 0.2 mA [38]. Thus, the NDTs found in this study were higher than the ones found in earlier studies.

The reason for this could be the habituation caused by the type of stimuli chosen in this study. Double pulses and quadruple pulses were used as stimuli here (rather than single pulses and double pulses), with every detected double pulse followed by three repeated quadruple pulses. This choice was made because past studies done with the NDT method demonstrated that the detection rates for double pulse stimuli were higher and the detection thresholds were lower than for single pulse stimuli, possibly resulting from temporal summation or short-term plasticity [12, 44, 55] or peripheral mechanisms like sub-threshold/supra-threshold excitabilities [44, 56]. The values of average NDT and slope from past studies with single pulses did indeed show higher thresholds (0.3to 0.5 mA), which is as per expectations, but the values seen in this study are higher than previous studies using double pulses for the threshold tracking. The exclusion of single pulse stimuli and the repeated stimulation with quadruple stimuli which are more intense, may be resulting in more habituation.

It would be interesting to perform similar experiments with single and doubles pulses, or by using pairs of single-double and double=quadruple pulses simultaneously, similar to the pair probing done in [38]. Comparing results in such experiments might help in evaluating more accurately the specific effects of using the double-quadruple stimulus types on the NDTs.

Interestingly, this study found a median slope of 17.75 mA^{-} (average of 23.23 mA^{-}), which is higher than the slopes found in past studies (average of 15 to 22 $.A^{-}$) [11, 12, 44]. This may indicate that the results from this study are fairly reliable, as higher slopes are associated with better reliability of results.

For the data that met the exclusion criterion based on detection rate, the detection rate was seen to be $\sim$0.36, which was lower than the other subjects, all of whom had detection rates above 0.4. The tracked NDTs for subject 08 increased rapidly to a very high value ($\sim$2.4 mA), compared to the other subjects, for whom the increase is slower and not a lot, as the maximum amplitude reached is around or often less than 1 mA. The slope of this data was 1.44 mA^{-}, which was drastically low, further showing that this dataset would not give reliable results.

5.2 Topographical Distribution and Evoked Potentials

The top view topography in Figure 4.4 shows the activity elicited by the applied stimuli at latencies of t=165 ms and t=345 ms. The stimuli elicit a negative contralateral activity at t=165 ms, which has been seen in the topographies obtained in earlier studies [11, 38]. However, in this study, an ipsilateral component of a smaller amplitude is also present, which has not been seen in most studies. It is interesting to note that it is specifically the study conducted by S. Wijers ([38] that shows such bilateral activity. As that study also uses a stimulus followed by a stimulus that has double the number of pulses as the stimulus, i.e., double pulse after single or quadruple pulse after double pulse, it shares this similarity with this study, which also uses a double pulse followed by quadruple pulses; the difference is that there are multiple quadruple pulses in the repeated stimulus protocol used in this study. Based on this similarity, it is possible that the use of this type of stimulus pair causes an ipsilateral component. However, the reason for this is not known. On the other hand, at t=345 ms, a strong positive central component is seen, which seems to match the topographies observed in earlier studies [11, 38].

The EPs obtained in this study are in agreement with the topography. For example, for $detected_2$, there is a central component in the topography of around 7 μV, which would lie in the area where the Cz electrode is present, along with a component at the circumference (where M1 and M2 electrode are located, behind the ears) of the scalp with an amplitude of also around -7 μV. Thus, the topography shows a potential difference of around 15 μV for $detected_2$, which is also seen in the EP shown in Figure 4.5. Also similar to the topography, the contralateral T7-F4 derivation shows a sharp peak at t=165 ms, but the ipsilateral derivation T8-F3 has a lower amplitude around the t=165 ms mark.

At t=345 ms, the strong positive central component along with the strong negative component around the circumference leads to higher amplitude of the EPs of both of the above derivations. The high amplitude central component seen in the topographies is also seen in the EPs of the derivation Cz-M1M2; all detected EPs shows noticeable peaks at this latency. The central derivation has a small peak at the t=165 ms, which probably corresponds to the central activity seen at t=165 ms in the topographies.

Each stimuli set contained four separate stimuli, as has been explained before. Comparisons of the EPs and scalp potentials caused by the different stimuli have several interesting features, which are discussed in the next section.

The third and fourth stimuli also cause similar distribution but with smaller amplitudes. The EPs elicited by the stimuli agree with the topographical distribution. The EP at contralateral derivation shows a sharp negative peak at a latency of 165 ms, while the central derivation shows both N165 and P345 peaks.

5.2.1 Effect of Repeated Stimulus Protocol

The repeated stimulus protocol was designed with the purpose of possibly removing the undesirable novelty component from the EPs elicited by the applied stimuli. To this end, it was expected that the EP of the first stimulus detected$_1$ would include the novelty component, and the EP of the second stimulus detected$_2$ would also have the novelty component present due to the variable time window between the detected$_1$ and detected$_2$ stimuli. The EP of detected$_2$ would also be expected to have higher saliency than detected$_1$. A subsequent stimulus would have the same saliency as detected$_2$ if that stimulus is repeated, but would perhaps not have the novelty component if the repeated stimulus were applied the same exact (and hence, predictable) time interval [51, 52]. The study intended to see if this can be achieved with the designed stimulation protocol.

The EPs of the central derivation seen in Figure 4.5 show that detected$_1$ elicits an EP with a sharp peak, and detected$_2$ elicits an EP that has a peak of higher amplitude than that of detected$_1$. This is, as expected, due the higher saliency caused by the increase in the strength of the stimulus (from double pulse to quadruple) and the variability of the interval between them, as explained earlier. The next two stimuli, detected$_3$ and detected$_4$, show peaks of much smaller amplitude in the EPs, compared to the first two stimuli. The scalp potentials seen in the topographies show a similar trend. It is possible that this is the result of the removal of the novelty component. If the novelty component were removed, we would expect the third and fourth EPs to have lower amplitudes, with only the nociceptive component present. One recent study showed that when pairs of stimuli were applied at varying time intervals but with exactly the same time interval between the two stimuli, the EP elicited by the second one was of smaller amplitude, containing only the obligatory nociceptive response [51]; this is similar to what is seen in the present study where the EP from the third stimulus (second of the repeated set) has a lower amplitude. However, it is also likely that this trend may simply be due to habituation, as the same stimulus is applied multiple times. In an earlier study with stimulation at 60 Hz, i.e., with intervals of 1 second, it

is seen that this predictable repetition leads to a sharp reduction in the second EP (possibly because the novelty component is absent), and thereafter, a slow decay of EP amplitude, which is speculated to be because of habituation (neural refractoriness) [52]. Similar to that study, in the current study, the reduction from the second EP to the third EP is sharp, and the reduction from the third to the fourth EP is much less. It would be interesting to add two (or more) stimuli to the current four-stimulus protocol used in this study, and see whether the EPs generated by these stimuli also only decrease very slowly after the second repeated stimulus (third in overall protocol), as seen in the 60 Hz repeated stimulus study.

5.2.2 Response to Undetected Stimuli

In the EPs, especially prominently at the central derivation, it was observed that the undetected stimulus has an EP of discernible amplitude, although it should have been more or less zero amplitude, as these stimuli were not detected by the subjects during the experiments. The topographies also show some central activity at t=345 ms and some central as well as bilateral activity at t=165 ms. The amplitude of the scalp potentials for the detected stimulus is very small, but non-zero. The EPs of the undetected stimulus, on the other hand, are of significant amplitude. At t=345 ms, the central derivation shows an undetected EP with an amplitude that is almost equal to the detected$_4$ EP (about 4 μV). For the contralateral derivation, a sharp peak of around 3μV, while the ipsilateral derivation shows increased activity of around 1.5 μV at t=345 ms. The EPs and scalp potentials elicited by the undetected stimuli have lower amplitudes compared to the detected stimuli, but they are not zero or close to zero. Their presence itself is unusual but could be because of lapsing. Lapsing is said to have happened when the subjects did not press the button despite detection, either because they were not sure if they perceived the stimulus, or because they were not attentive enough to press the button within the allowed time after the stimulation happened (1 s). Another explanation for this unexpected activity could be the distinction between nociception and pain. It is possible that nociception occurred, leading to the measured signal, but the subjects' internal response criterion (see section 3.2) was not exceeded, so no 'painful' sensation was experienced. Past research has shown that this internal criterion affects the measurements when a go/no-go (GN) type of stimulation method is used; those results also showed a significant non-zero response to undetected stimuli when the GN method was used (see appendix C) [40]. The same study showed that using a different stimulation method which was described in Section 2.4.3, known as the two-interval forced choice (2IFC) method, eliminated this problem; the evoked response for undetected stimuli was absent in the results [40].

5.2.3 Frontal Component in Topography

The topographies of the response to the third and fourth stimuli show interesting behaviour at the t=165 ms mark. At this latency, there is a weak central component present for the first two stimuli, but for the third and fourth stimuli, a frontal component is seen. The reason for

this is not known, and further research is needed to verify and study this interesting result. It is possible that the difference between nociception and perception of pain, as explained before, could result in differences in the areas of the brain activated during either process. This could be tested, once again, by replacing the go/no-go stimulation method in this study with the two-interval forced choice (2IFC) method, which is thought to not suffer from the drawback of depending on an internal criterion of pain perception [40]. Besides that, the button-releasing action during the experiments relates to the detection of the first stimulus, and thus confirms the detection of the first stimulus only. While it is safe to say that the second stimulus would also definitely be perceived (the increase of NoP was chosen in order to specifically ensure a much higher than 50% chance of detection), it is unknown whether the third and fourth stimuli were detected at all. A possible way to verify whether these stimuli were detected could be to make a modification to the study in some way, such that the subject can indicate whether the subsequent stimuli were perceived, or can indicate how many stimuli they perceived, which would also give an indication of whether or not the third, fourth and any further stimuli were, in fact, perceived by the subject.

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

The objective of this study was to design a new stimulation protocol that might be useful in removing the novelty component from nociceptive EPs. To that end, a repetitive stimulation protocol was designed, consisting of sets of stimuli, each containing a double-pulse stimulus, followed by three quadruple-pulse stimuli with an inter-pulse interval of exactly one second. The pulses in all four stimuli had the same amplitude, and the interval between the double-pulse and the first quadruple-pulse was variable.

The results of the experiments conducted using this repetitive stimulation protocol achieved the expected reduction in the amplitudes for the EPs of the third and fourth stimuli. Such a reduction was earlier predicted to be the result if the protocol were successful in the removal of the novelty component. This means it is possible that the novelty component was indeed removed from the EPs.

Thus, the present study shows that the repetitive stimulation protocol proposed in this thesis shows promise in achieving the goal of obtaining nociceptive EPs that do not have the novelty component in them. However, more research is needed to verify if the results obtained here are indeed due to the removal of the novelty component or due to some other factor. For further analysis, the stimulation protocol itself could be optimised, for instance, in several ways, some fo which are described in the following section.

6.2 Recommendations

Based on the results of this study, and the implications of those results, the following modifications could be made for future studies conducted along this line of research:

- In order to check the effect of habituation on the decrease in amplitudes of the third and fourth stimuli further experiments could be conducted with four or five quadruple stimuli (instead of three, as in this study), to see how the trend of gradual amplitude decrease continues.

- The experiments could be conducted with a two-interval forced choice procedure rather than the go/no-go procedure used in the current study, in order to remove the influence of the response criterion on the resulting EPs, including the EP of the undetected stimulus.
- To ascertain whether all the stimuli in each set were detected, a way for the subjects to indicate whether they perceived each of the stimuli, or to indicate how many stimuli they perceived each time, could be added to the experiment procedure.

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Appendix A

10-20 System for EEG

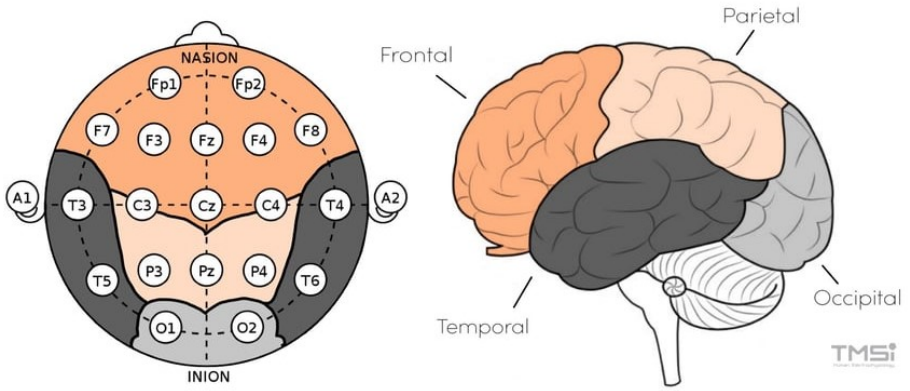


Figure A.1: (Left) Electrode layout of the 10-20 system, and (Right) corresponding brain regions (right).

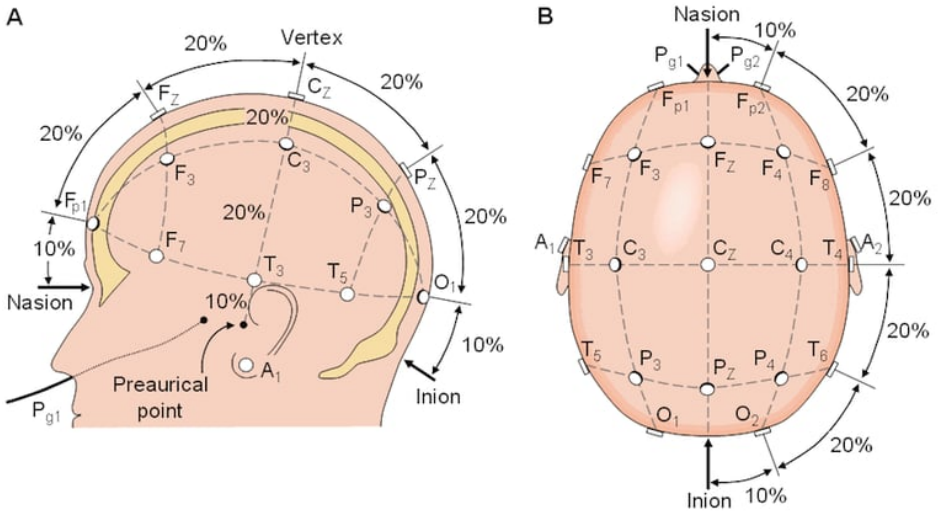
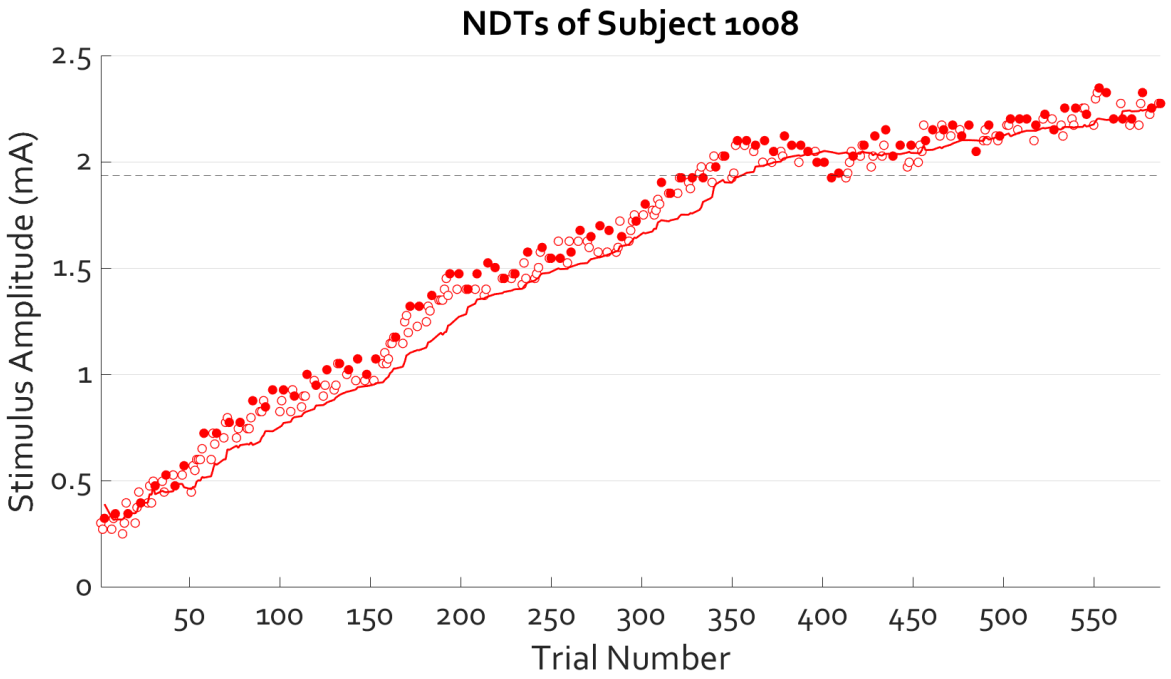


Figure A.2: The 10-20 system with front-back (nasion to inion) 10% and 20% electrode distances.

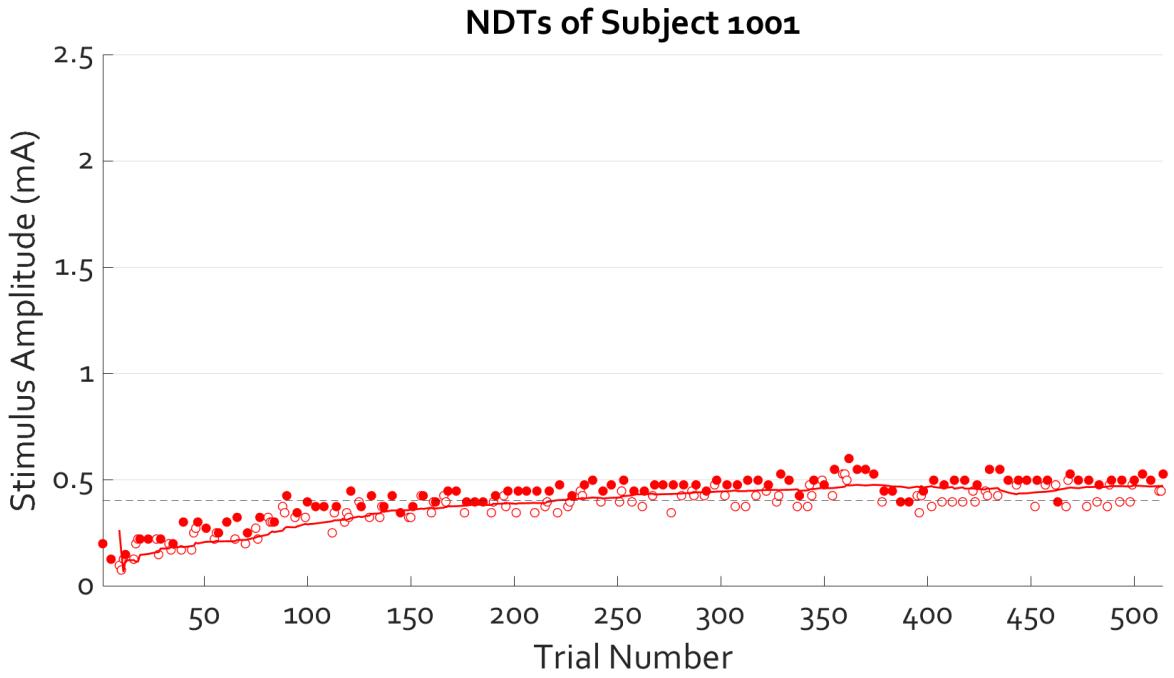
Images taken from TMSi website. For more info, visit https://info.tmsi.com/blog/the-10-20-system-for-eeg

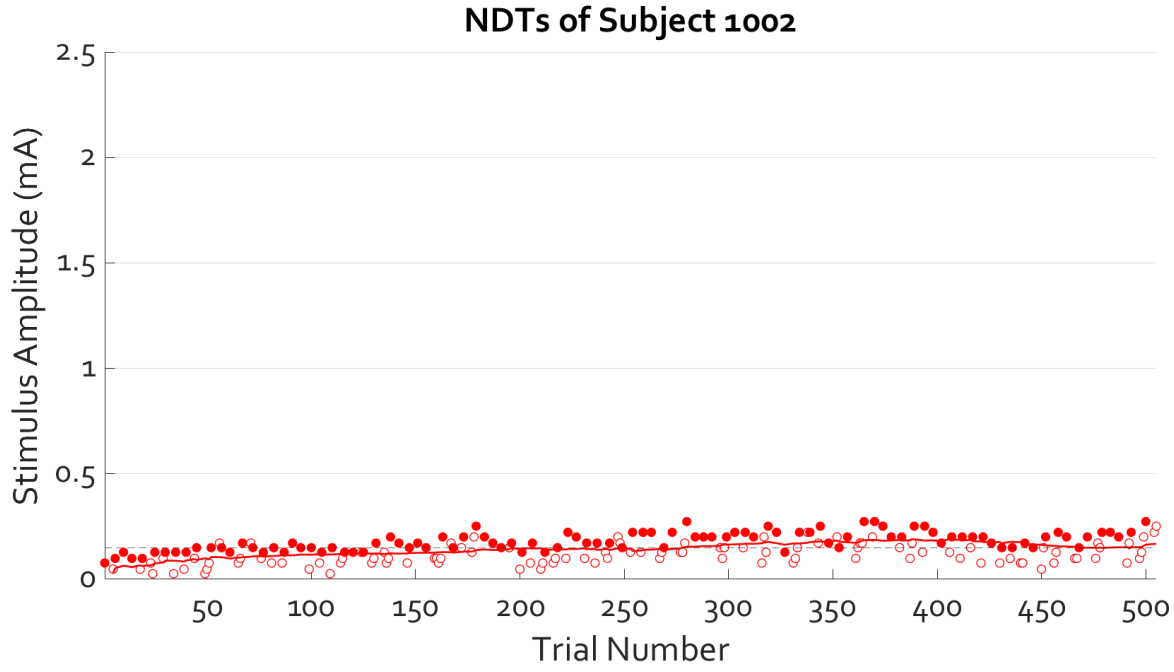
Appendix B

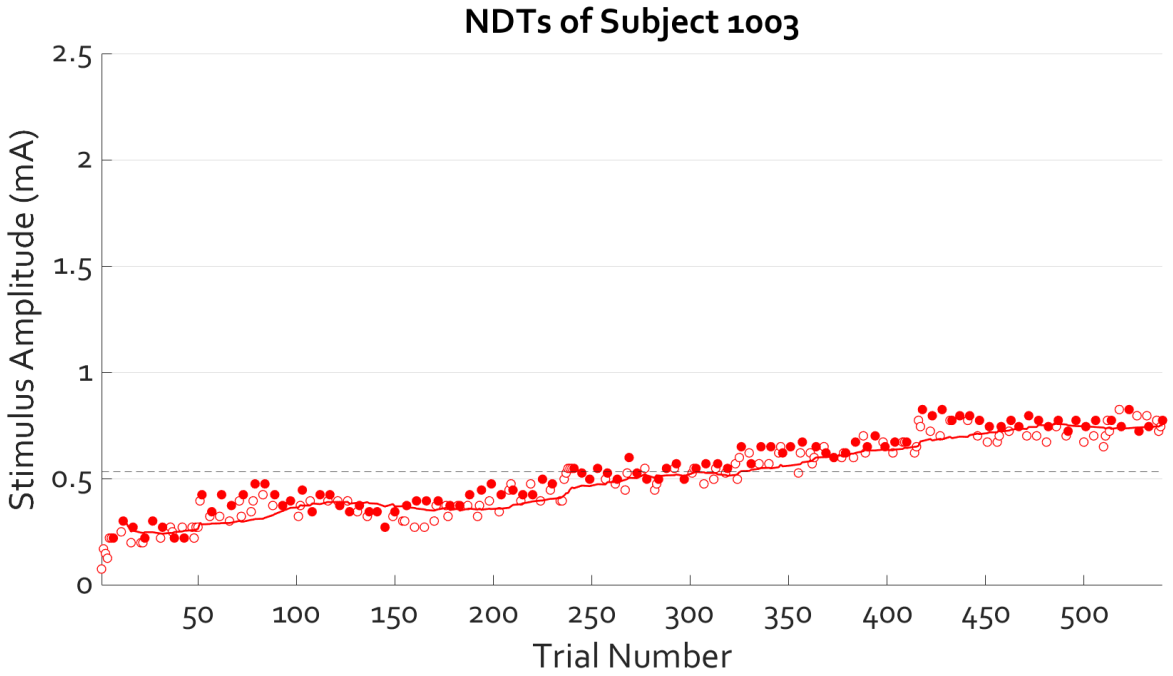
Individual NDTs of Each Subject

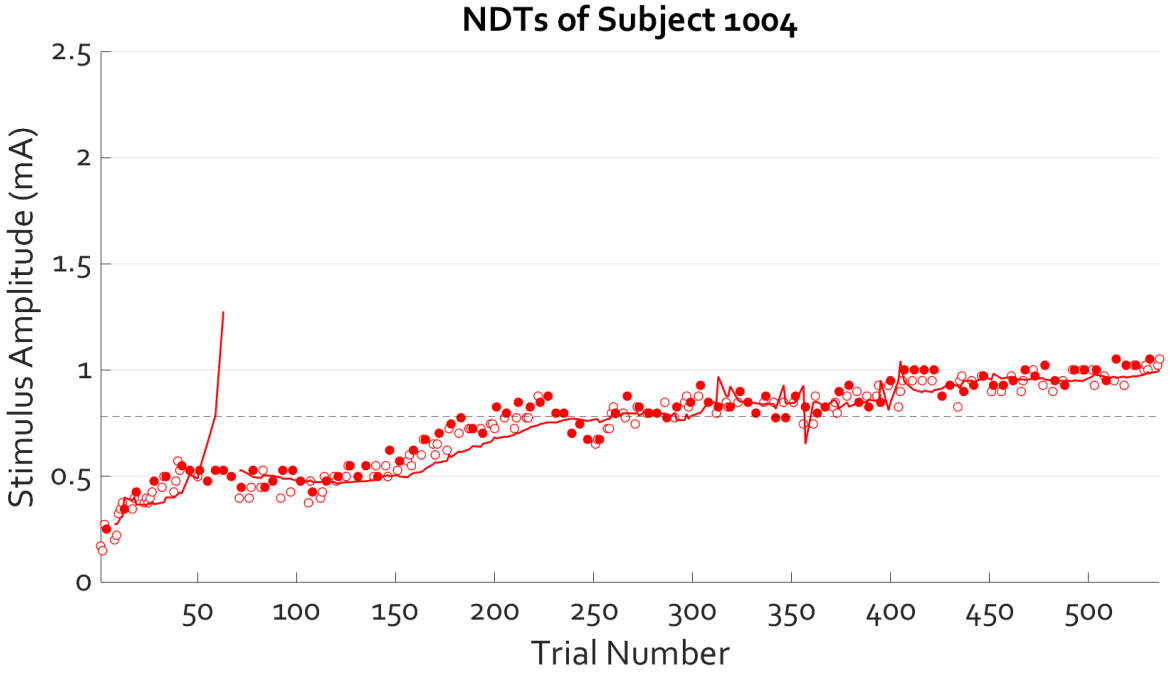


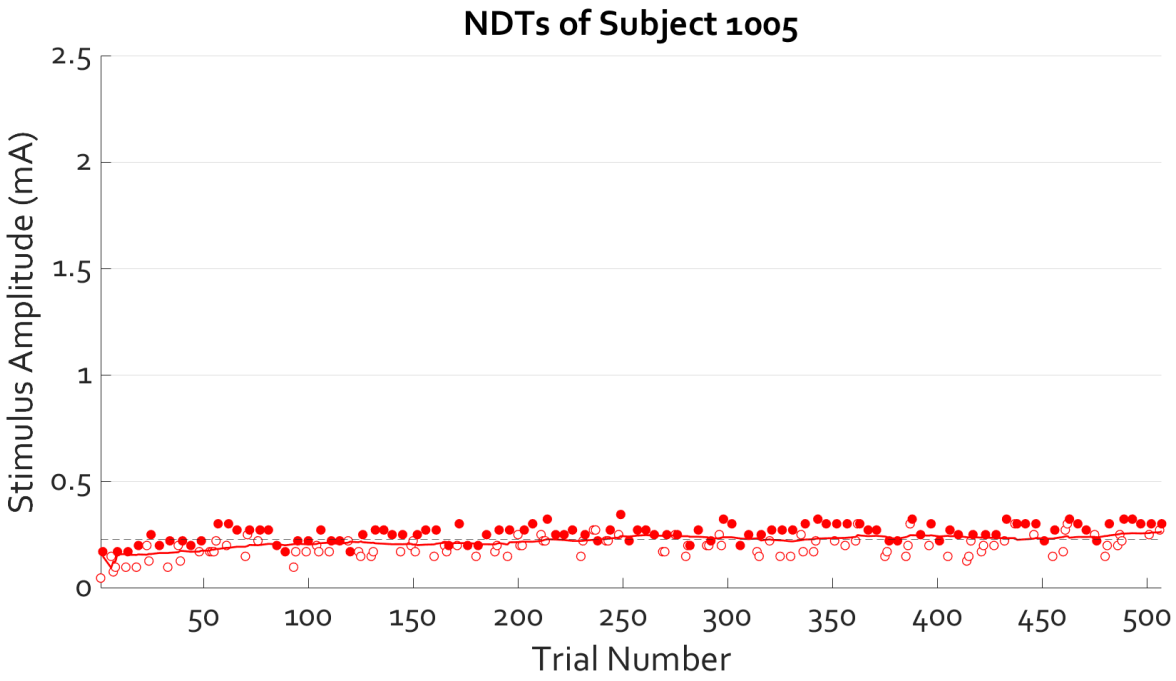
The tracked NDT for subject 08 increases sharply, displaying a high amount of habituation, and reaches a high value at the end. The NDTs of the remainder of the subjects (seen next), do not show as much habituation, and do not reach a high amplitude by the end of the experiments.

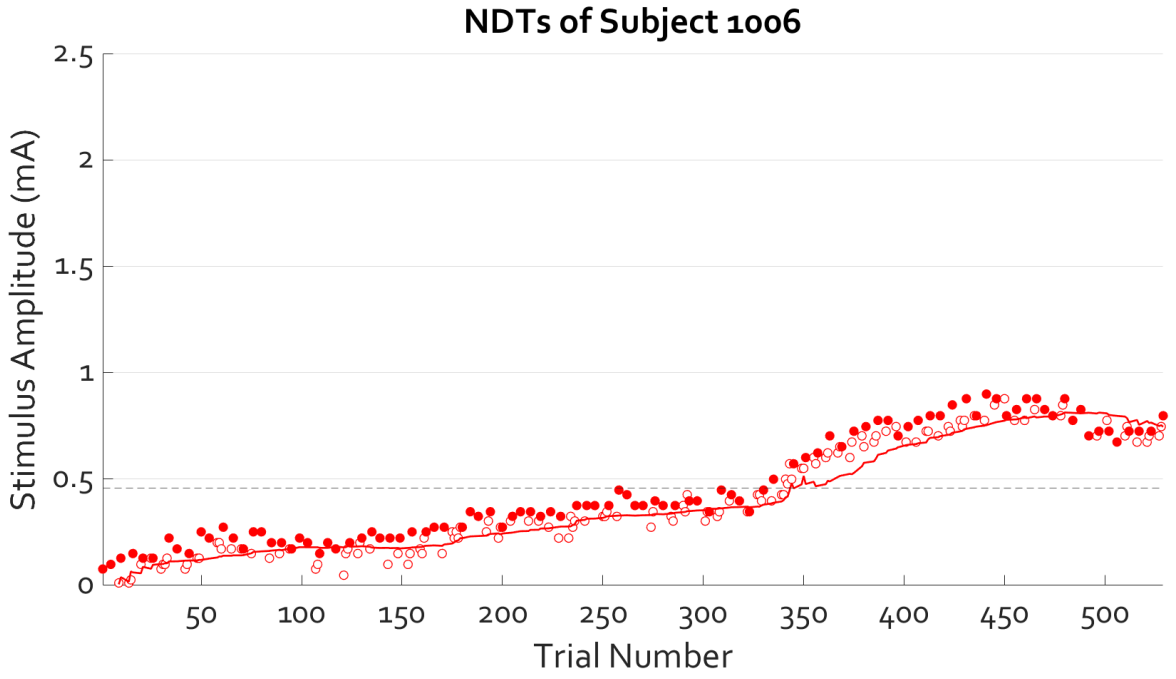

NDTs of Subject 1001
Stimulus Amplitude (mA)
2.5
2
1.5
1
0.5
0
50
100
150
200
250
300
350
400
450
500
Trial Number

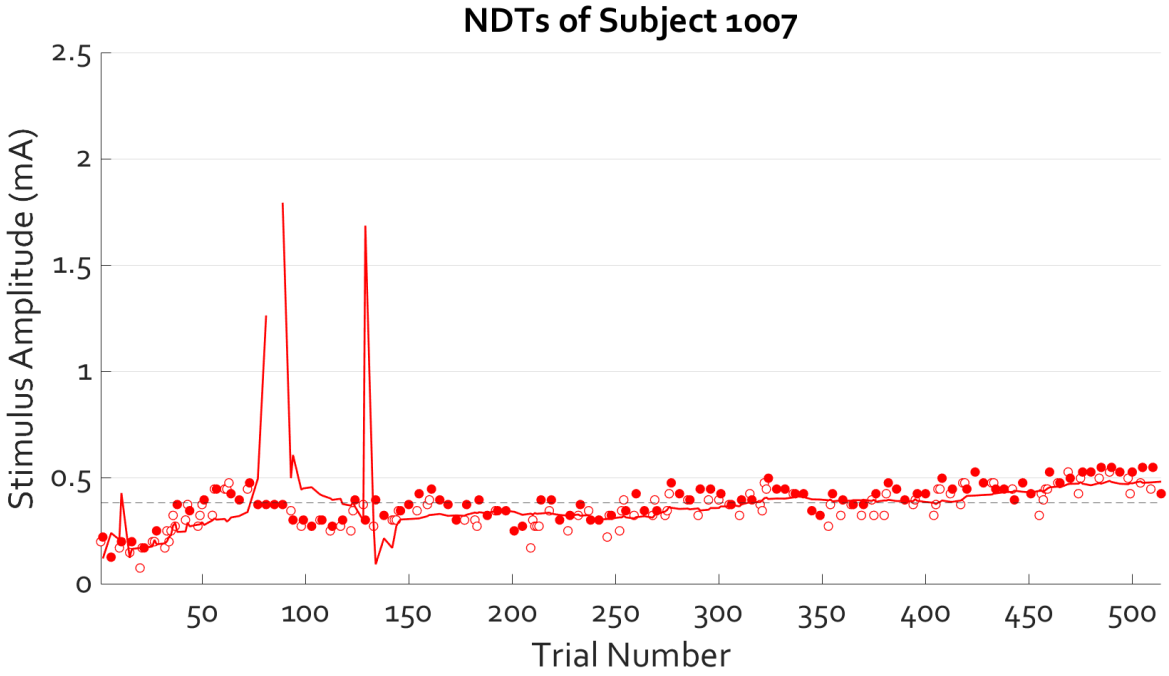

NDTs of Subject 1002
Stimulus Amplitude (mA)
2.5
2
1.5
1
0.5
0
50
100
150
200
250
300
350
400
450
500
Trial Number

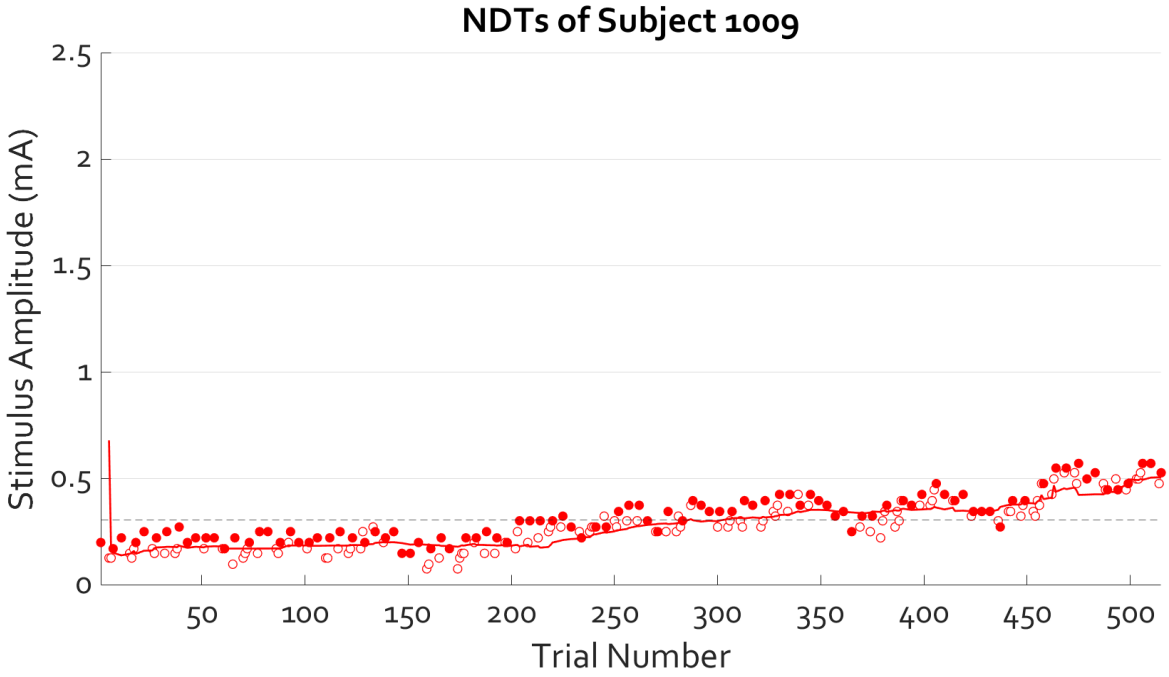

NDTs of Subject 1003
Stimulus Amplitude (mA)
2.5
2
1.5
1
0.5
0
50
100
150
200
250
300
350
400
450
500
Trial Number

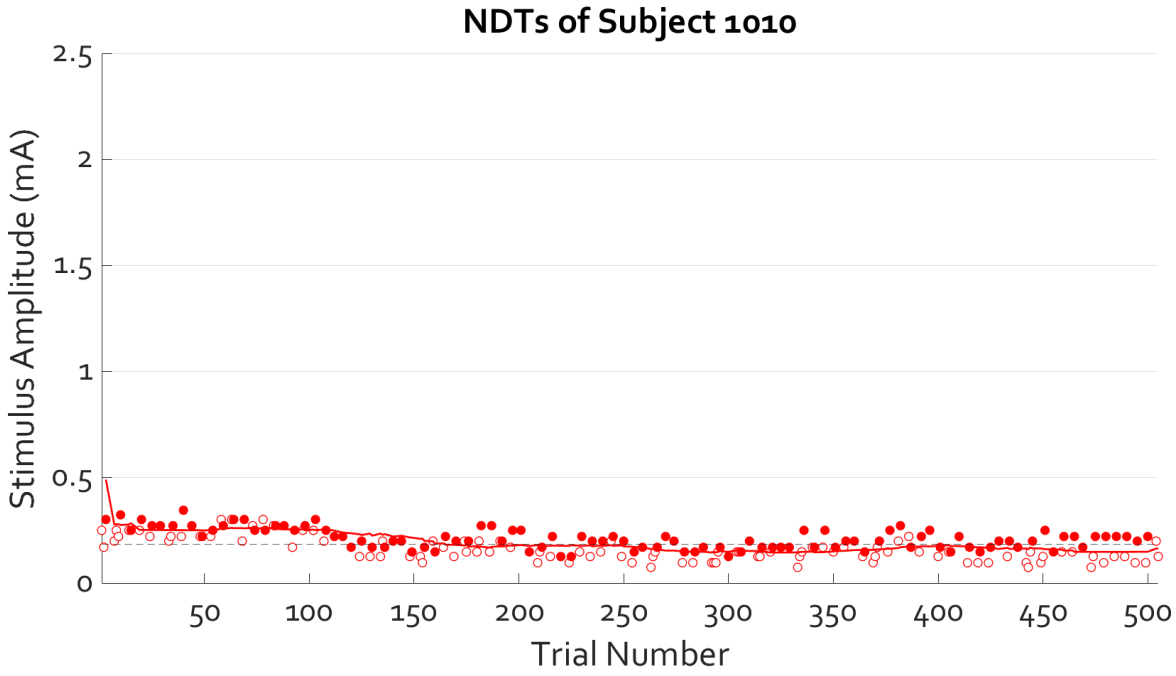

NDTs of Subject 1004
Stimulus Amplitude (mA)
2.5
2
1.5
1
0.5
0
50
100
150
200
250
300
350
400
450
500
Trial Number

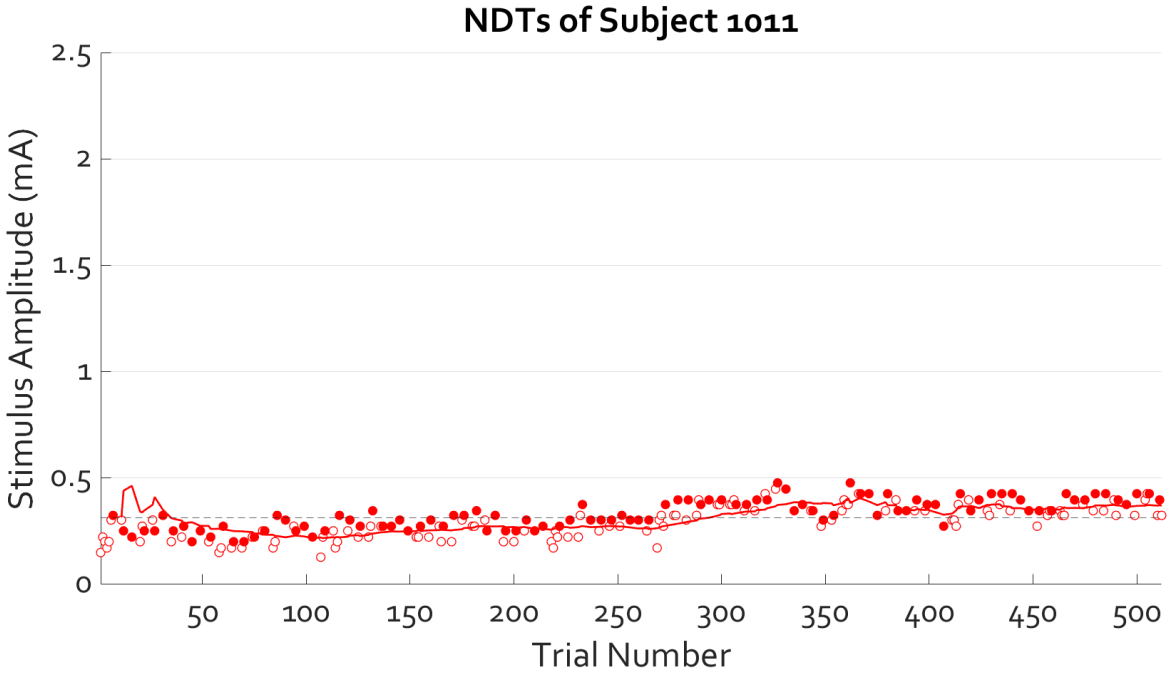

NDTs of Subject 1005
Stimulus Amplitude (mA)
2.5
2
1.5
1
0.5
0
50
100
150
200
250
300
350
400
450
500
Trial Number


NDTs of Subject 1006
Stimulus Amplitude (mA)
2.5
2
1.5
1
0.5
0
50
100
150
200
250
300
350
400
450
500
Trial Number


NDTs of Subject 1007
Stimulus Amplitude (mA)
2.5
2
1.5
1
0.5
0
50
100
150
200
250
300
350
400
450
500
Trial Number


NDTs of Subject 1009
Stimulus Amplitude (mA)
2.5
2
1.5
1
0.5
0
50
100
150
200
250
300
350
400
450
500
Trial Number


NDTs of Subject 1010
Stimulus Amplitude (mA)
2.5
2
1.5
1
0.5
0
50
100
150
200
250
300
350
400
450
500
Trial Number


NDTs of Subject 1011
Stimulus Amplitude (mA)
2.5
2
1.5
1
0.5
0
50
100
150
200
250
300
350
400
450
500
Trial Number

Appendix C

Undetected EP for Gn vs 2IFC

The figure below shows the grand averaged evoked potentials at the Cz electrode for the go/no-go (GN) method and the two-interval forced choice (2IFC) method. The figures to the right are obtained using DP stimuli, which is of relevance to this thesis. It can be seen that the evoked potentials were significantly larger than baseline for detected as well as non-detected stimuli, when GN procedure was used, as opposed to when 2IFC was used.

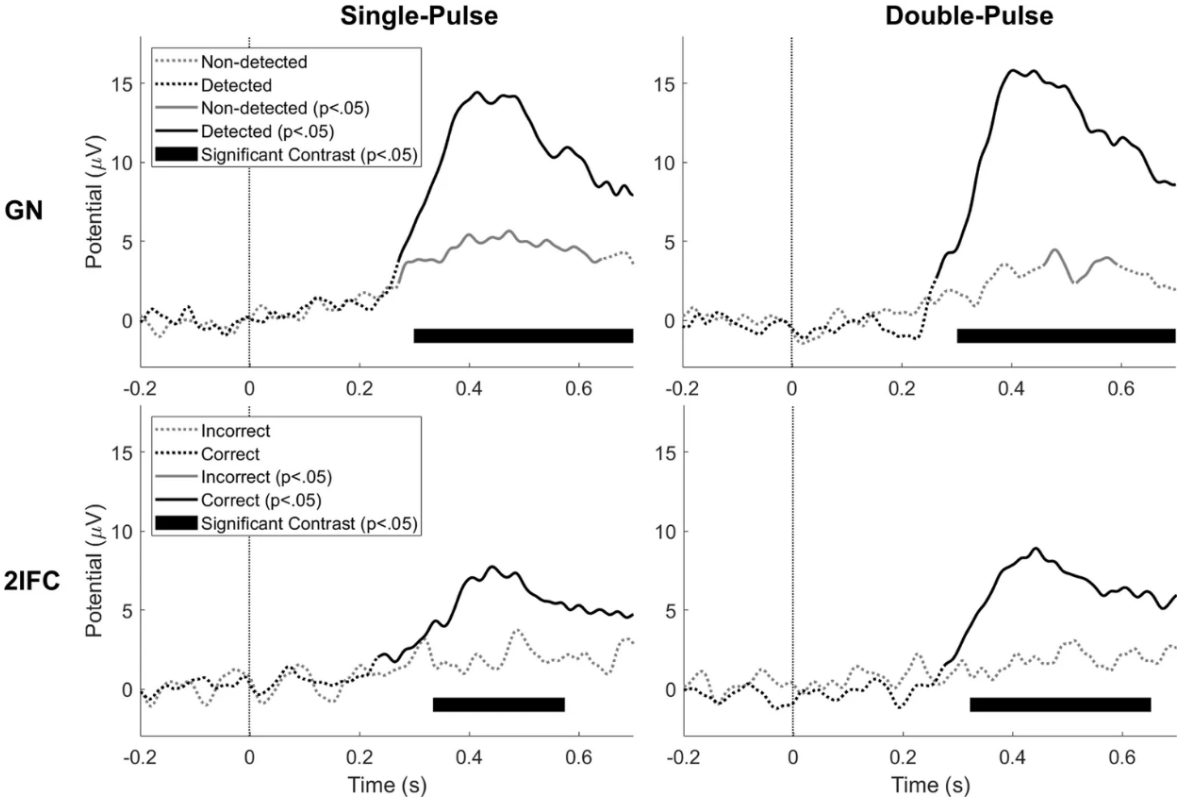


Figure C.1: (Grand averaged evoked potentials at the Cz electrode. (Left) single pulse, and (right) double pulse. (Top) Go/No-go method, and (bottom) two-interval forced choice method.