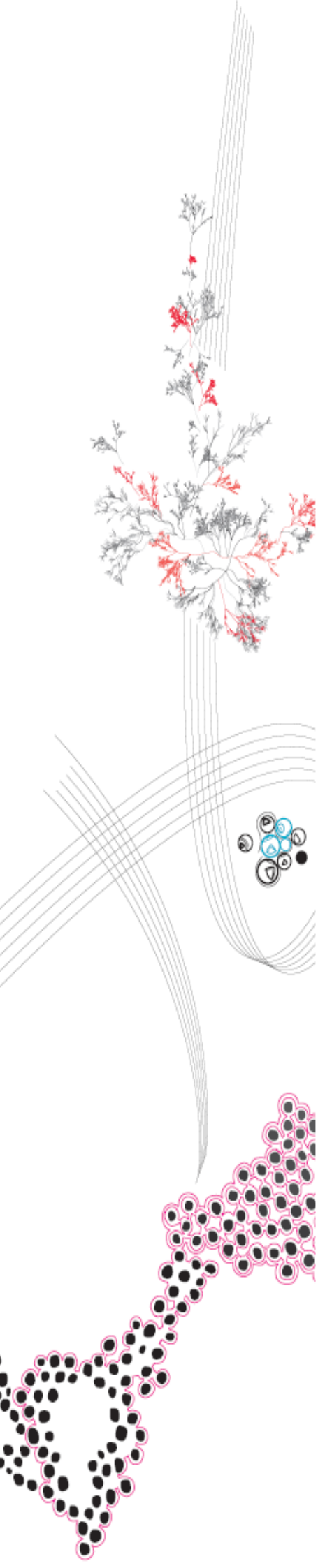

UNIVERSITY OF TWENTE.

Faculty of Electrical Engineering, Mathematics & Computer Science
Biomedical Signals and Systems

Measurement of Steady-State Evoked Potentials During Transcutaneous Electrical Stimulation: A Pilot Study

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Preface

I would like to thank the members of my supervising committee, namely, B. van den Berg MSc, Dr.ir. J.R. Buitenweg and Prof.dr.ir. R.N.J. Veldhuis, for their support and assistance during this study, and for providing me with the opportunity to undertake this interesting and illuminating study. Most importantly, I would like to thank the subjects who volunteered to participate in this study, for taking the time to undergo the long experiments.

I would like to dedicate this study to the myriad of people who silently suffer from invisible illnesses such as chronic pain, chronic fatigue, and several other neurological and mental disorder, and who are looking to the scientific community to find definitive diagnostic and treatment methods.

Summary

Chronic pain disorders affect a large of number of people all across the world. It manifests as pain lasting over the course of months or years, and is known to adversely affect the lives of those suffering from it. As of yet, there is no effective treatment for chronic pain, as its exact mechanism is unknown. However, it is believed that this disorder may be the result of abnormalities in the nervous system. For this reason, research into the characterisation of the human nervous system, especially the sensory system, is crucial. A part of this research is understanding the mechanosensory system, which is associated with the perception of touch.

In this study, sustained electrical stimulation of long duration applied to arm of the subjects is used to evoke a steady-state response in the subjects' brain in the form of steady-state evoked potentials. By recording and measuring the EEG data from the subject during the stimulation, *frequency tagging* is used to identify a signal associated with the stimulation in the brain activity, as such a response is 'tagged' by the stimulation frequency. Two different types of stimuli were used in the experiments, and specific descriptions of the stimulus perception, such as intensity and area, reported by the subjects were also noted. After the collected EEG data was processed in several stages, it was analysed to check if the frequency-tagged brain response is discernible, and to explore the influence of certain experimental parameters on the outcome of the experiment.

The results showed the clear presence of the tagged brain response in all of the subjects. Some results were seen to have a noticeable stimulus artefact in them, but even after the exclusion of these specific results, the expected output component remained observable in the average of the remainder of the results. However, the reliability of these results can be considered questionable. The lack of efficacious methods for the removal of noise and other undesirable signals in the data is found to be a serious impediment to this research, so new and better methods are needed. Additionally, it was found through this study that the area over which the subject feels the stimulus directly influences the amplitude of the measured output. This inference can be used to ensure better results in experiments and needs more investigation. Another important finding is that the parameters set for the stimulation signal have a marked effect on the results that are observed. This opens up a wide range of

possibilities for future research.

Contents

List of acronyms

BSS	Biomedical Signals and Systems
CNS	central nervous cystem
CT	computerised tomography
ECG	electrocardiogram
EEG	electroencephalogram
EMG	electromyogram
EOG	electrooculogram
EP	evoked potential
ETP	electronic potential
FFT	Fast Fourier Transform
ICA	independent component analysis
IES	intra-epidermal electrocutaneous stimulation
IPI	inter-pulse interval
LTMR	low-threshold mechanoreceptor
MRI	magnetic resonance imaging
PNS	peripheral nervous system
PW	pulse width
SNR	signal-to-niose ratio
SSEP	steady state evoked potential
TES	transcutaneous electrical stimulation

TMSi Twente Medical Systems internation B. V.

Chapter 1

Introduction

1.1 Context

Chronic pain is a ubiquitous medical condition with diverse physiological and psychological mechanisms. Defined roughly as pain lasting for an unusually long duration and without conceivable cause, it is a debilitating condition that afflicts many people. A meta-analysis study conducted in several Western countries found that the prevalence of chronic pain varied with a median 15% of patients [1]. Another study found that an estimated 20.27% of the overall population reported experiencing chronic pain, with a majority reporting daily, and moderate to severe pain [2]. This affliction has been shown to cause problems in the lives of the individuals, adversely affecting their quality of life. It also leads to several psychological disturbances and results in unsatisfactory social interactions and problem in family and relationships [3]. As a whole, it poses a significant burden on society, taking away from the labour force, for instance [4].

Yet, treatment for this condition has proven to be insufficient, with most traditional treatments being largely ineffective [5]. This may be due to various reasons, some of which are yet unknown, but an underlying cause of such pain seems to be abnormal sensitisation of the nervous system (see Appendix A). For this reason, the plasticity of the human nervous system in both patients and healthy humans needs to be studied.

For such research purposes, a setup with an electrical or thermal stimulus to the nerve fibres of the hand or another body part, and a device that acquires the EEG (electroencephalogram) of the subject to measure the fluctuations in brain activity brought about by the stimulus, is often used [6], [7]. Recently, a new method developed at the BSS (Biomedical Signals and Systems) group of the University of Twente was shown to be suitable for measuring the changes in EEG brought about by phasic electrical stimuli [4]. In this method, linear mixed models were used in combination with evoked potentials to objectively observe and study stimulus-related brain activ-

ity at the subject-level and group-level simultaneously. Due to this, and due to the fact that this method was shown to reduce the influence of parameter changes on the variation of evoked potentials, it can be considered a suitable method for such research.

The next step is to use steady-state stimulation to invoke sensation that corresponds to tonic pain, similar to the pain seen in chronic pain disorders. Sustained sensory stimulation has been found to to evoke a steady-state response, called steady state evoked potentials (SSEPs), thought to be due to the entrainment or resonance of a population of neurons that respond to the stimulus at the frequency of the stimulation [8]. As SSEPs do not die out as long as the stimulation is sustained and have a high signal-to-noise ratio [9], tonic stimulation is suitable for recording the cortical response to sensation, and thereby for studying the properties of the somatosensory system, specifically in the frequency domain.

1.2 Problem Statement

The aim of this thesis is to show that a sequence of frequency-modulated stimuli can be used to invoke a steady-state response in the brain, that can be measured and analysed using steady state evoked potentials in the EEG . It is important to determine whether this method can be used to measure activity corresponding to solely nociceptive (pain-related) stimulation or if the changes in brain activity correspond to both nociceptive and tactile (touch-related) stimulation. A separate study has measured the EEG response to nociceptive stimulation, which needs to be compared to the response to tactile stimulation to examine if the brain response is nociceptive specific. Hence, the primary objective of this thesis to measure the EEG variations caused by tactile tonic stimulation and to investigate some factors that may affect the acquisition of quantifiable and reliable results. A secondary objective is to analyse the obtained measurements to characterise properties of the somatosensory system by analysing it as an input-output system with the stimulation as input and the EEG as output, and to investigate the obstacles to such analysis.

1.3 Strategy and Organisation

Chapter 2 describes the neurophysiological basis required to conduct this study; this is followed by a description of the equivalence of the human nervous system and a black-box electrical system. The stimulation and analysis methods expounded in these chapters are employed suitably to conduct the experiments for this study, as explained in Chapter 3. The results obtained from the experiments are noted

in Chapter 4; a number of inferences can be drawn from these results, and the significance of the results and their inferences is discussed thoroughly in Chapter 5. The conclusions drawn from the discussion and the implications of this study are stated in Chapter 6, along with recommendations for further research.

Chapter 2

Background

2.1 Human Nervous System

This section describes some background information about the human nervous system and how it functions. The overall structure and the various relevant components of the nervous system, as well as the mechanisms of neural signals are explained.

2.1.1 Structure and Components of the Nervous System

The human nervous system is responsible for receiving innumerable bits of information from the world and from the body itself and integrating these to determine the response of the body to that information. The central nervous system is made up of billions of basic functional units called neurons, through which signals enter the body as synapses and travel uni-directionally to the brain [10].

Neurons do not function in isolation but in complex ensembles called neural circuits, which can be classified in a broad way based on their function or based on anatomy. Anatomically, the nervous system is divided into the central nervous system (CNS) which includes the brain and the spinal cord, and the peripheral nervous system (PNS) which consists of the sensory neurons that connect the sensory receptors inside or on the surface of the body. Functionally, the nervous system consists of the sensory systems which acquire and process information from the external environment, and the motor system which responds to this information by generating behaviour, like movement [11]. The sensory system will be described in more detail in Section 2.2.

The nerve cells themselves can be of two types: the afferent neurons, which carry information from the periphery (e.g. body surface) to the brain or spinal cord, and the efferent neurons, which carry information away from the brain or spinal cord [11]. Additionally, the nerve fibres in the body can be classified based on the type of signals that they transmit and based on their diameter sizes; $A\beta$, $A\delta$, and C

fibres are some examples. The relevant fibres will be described in Sections 2.2.2 and 2.2.3.

2.1.2 Neural Signalling

The neurons in the body encode, transfer and store the information they acquire by means of different types of electrical signals, the generation of which is based on the flow of ions across the plasma membranes of the neurons [10]. Usually, a negative potential of about $-70mV$, called the resting membrane potential, is generated across the neurons due to the difference in the concentration of $Na+$ and $K+$ ions dissolved in the intra-cellular and extra-cellular fluids. Excitatory stimulation of the nerve cells usually takes the form of electronic potentials (ETPs).

Receptor potentials occur due to the activation of sensory neurons such as the ones responsible for mechanical disturbances in the skin (touch). Synaptic potentials occur from the activation of synapses, and allow transmission of information from one neuron to another. Information is carried through the nervous system in a self-generating wave of electrical activity called an action potential, which is an all-or-nothing change in potential across the cell membrane, like a spike, in response to stimuli. This is responsible for the long-range transmission of information in the nervous system [10].

The synapses mentioned above are functional contacts between the numerous neurons residing in our nervous system. These can be either of two types, chemical or electrical, depending on their transmission mechanism. Chemical synapses communicate via the secretion of neurotransmitters while electrical synapses communicate via the flow of current through specialised fluid channels called gap junctions [10], [12]. Note that the rate of transmission of signals depends inversely on the diameter of the nerve fibres that participate in the transmission.

2.2 Somatosensory System

This section describes the somatosensory system which is responsible for the sensations of pain, touch, temperature and so on. The mechanism of touch will be explained in depth as it is most relevant to this thesis.

2.2.1 Sensation and Sensory Processing

Sensation can be considered the ability to encode and perceive information generated by external or internal stimuli. This occurs when afferent nerve cells called

receptors transmit signals related to mechanical force, light or sound waves, odorant molecules or ingested chemicals to the brain. Neurons in the body are capable of gauging what the signal represents, how strong it is, and what is the location of the stimulus [10]. Activity in the nervous system due to these stimuli is initiated by sensory receptors such as the visual receptors in the eyes, auditory receptors in the ears, tactile receptors on the surface of the body and so on. Usually, such sensory experience engenders immediate reaction from the brain, and the sensation is stored in memory long-term to generate fast bodily reactions in the future [12].

The component of the nervous system that transmits sensory information from receptors is called the somatosensory system, shown in Figure 2.1.a. The information enters the CNS through the peripheral nerves and is conducted immediately to multiple areas such as the spinal cord, the medulla, the cerebellum, the thalamus and areas of the cerebral cortex.

Numerous types of receptors can be found in the human body, each having a different and specific function corresponding to a type of stimulus or sensation. In a broad classification, the receptors can be:

1. Mechanoreceptors: detect mechanical compression or stretching of receptors or adjacent tissue
2. Thermoreceptors: detect changes in temperature, cold or warmth
3. Nociceptors: detect physical or chemical damage to tissue
4. Electromagnetic Receptors: detect light on the retina of the eye
5. Chemoreceptors: detect taste In the mouth, smell in the nose, oxygen level in arterial blood, amongst other chemical factors from the body.

Of these, the mechanoreceptors related to tactile sensation and the nociceptors (related to pain) are of importance to this report and will be discussed in following sections.

In the brain, the sensory signals terminate in the primary and secondary somatosensory cortex, as shown in Figure 2.1.b, after which several brain areas involved in cognitive processing of the stimulus are activated. Various regions of these cortices are activated based on the location of the incoming signal Figure 2.1.c.

2.2.2 Mechanosensory System (Touch)

The subsystem of the somatosensory system that is involved in the detection of mechanical stimuli such as light touch, pressure and vibration is called the mechanosensory system [10]. This system helps us identity the shape and texture of objects,

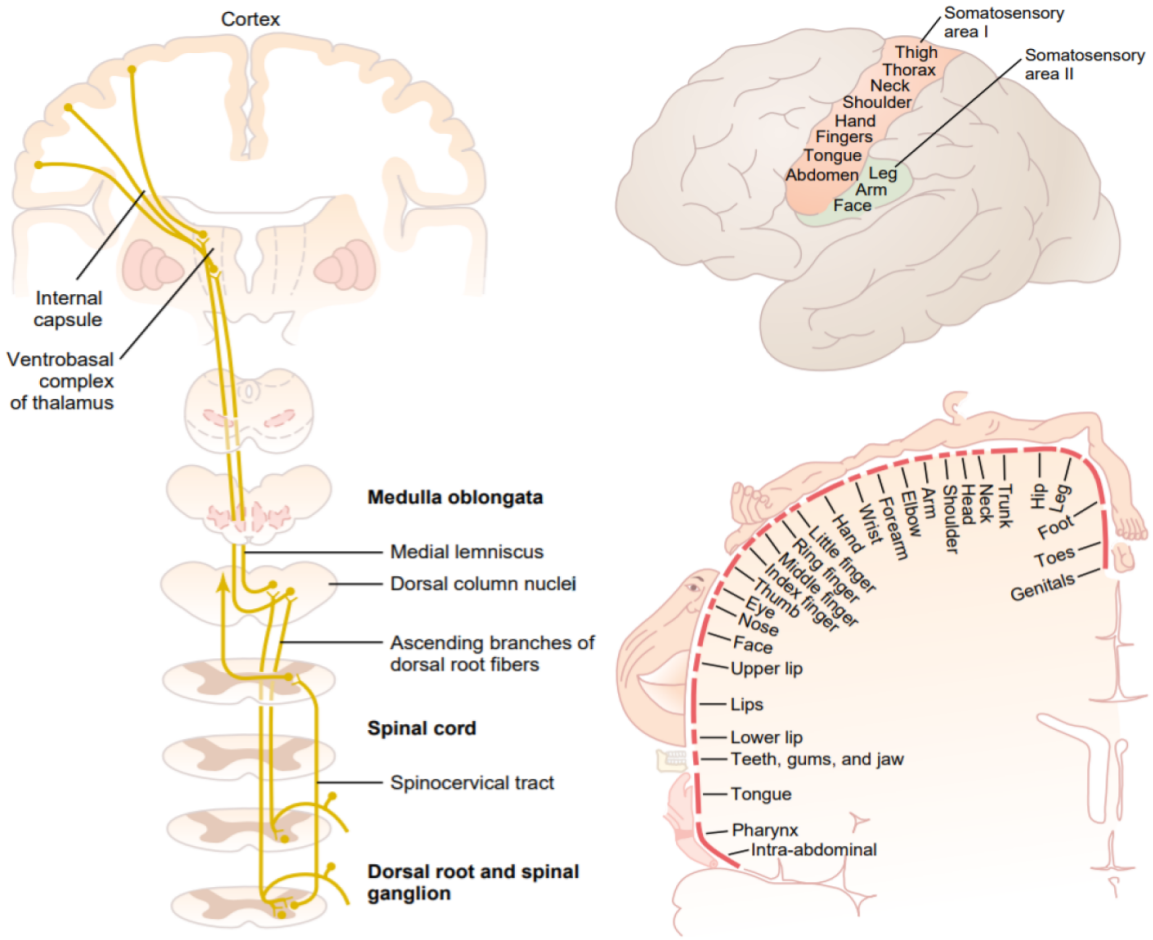

Figure 2.1: (a) The dorsal columnmedial lemniscal pathway for transmitting critical types of tactile signals (left), (b) Two somatosensory cortical areas, somatosensory areas I and II (upper right), (c) Representation of the different areas of the body in somatosensory area I of the cortex. Reproduced from [12]

assists in moving through space, and facilitates social behaviours like hugging and shaking hands. This system is most relevant for this thesis.

The cutaneous mechanoreceptors that mediate innocuous touch are one of the so-called low-threshold mechanoreceptors (LTMRs) [13]–[15]. These receptors are innervated by the $A\beta$ nerve fibres found deeper within the skin, which leads to the perception of tactile sensation. These fibres have a low threshold of activation and require only a weak electrical stimulus to produce an action potential. They have a larger diameter of about $6 - 12\mu m$ and have higher conduction velocities of about $40 - 80m/s$ [10], [12], [14].

The sensory pathway for transmitting sensory signals into the CNS is depicted in Figure 2.1.a. As shown, the sensory information enters the spinal cord through the dorsal roots of the spinal nerves, and are then carried rapidly and with special fidelity to the brain via the dorsal column-medial lemniscal system. This system carries the sensory signals in the dorsal (posterior) column of the spinal cord, as implied by its name. After this, via the medial lemniscus, the signals cross over to the opposite side in the medulla oblongata and pass upward through the brain stem to the thalamus [12].

2.2.3 Nociceptive System (Pain)

Pain is a protective mechanism of the human body and is activated when there is tissue damage, causing the individual to react and thereby remove the source of the pain (to prevent further damage) [12]. It is also important clinically as pain is the most common reason for people to consult a doctor, leading to the diagnosis of any number of maladies [16]. The subsystem of the somatosensory system that is involved in the perception of pain, also called nociception, is aptly called the nociceptive system.

The dedicated high-threshold receptors that detect painful stimuli are called nociceptors, which are free nerve endings found in skin and other tissue [14]. The $A\delta$ fibres are mechanical and thermal nociceptors that respond to strong mechanical stimuli, whereas the C fibres are polymodal fibres that respond to noxious mechanical, thermal or chemical stimuli. The $A\delta$ fibres are about $3\mu m$ in diameter and have a conduction velocity of about $2m/s$, while the C fibres are $0.5 - 2\mu m$ in diameter and conduct at a speed of about $20m/s$ [10], [12].

The transmission of pain-related signals to the brain activates feedback circuits that can inhibit the effects of the painful sensory input at the spinal cord, brainstem, or cortical level [16]. There are two sensory pathways for nociceptive signals depending on whether there is fast-sharp pain or slow-chronic pain. The stimulation of the brain-areas involved in pain perception have a strong arousal effect on the ner-

vous activity throughout the brain, which explains why people in pain are strongly aroused or are unable to sleep [12].

2.3 Black Box Approach

The earlier section described the human nervous system, including the somatosensory system, has been described to have a complex pathway, starting from nerve fibres that acquire information from the physical world to the relevant cortical areas in the brain that process this information . In an attempt to understand and describe the changes that occur in a physical system over time due to an external stimulus, it can be treated as a black-box input-output system. Thus, a mathematical representation for this system (its internal working), can be found by providing, using a controlled input (stimulus) and by measuring the output (brain response). This requires the use of a variety of stimuli and different types of analyses.

The effects of tonic stimuli on the nervous system can be studied using a method known as frequency tagging. In this method, by stimulating somatosensory receptors with stimuli of certain frequencies, it can be determined and verified if somatosensory processing cortical areas contain response signals at these frequencies as well, which would indicate that a nervous pathway exists [17]. These frequency-tagged stimuli elicit SSEPs which show up as peaks at the same frequency as the stimulus, in the frequency spectrum of the brain responses [18]. Frequency tagging has been done successfully in a number of studies related to the characterisation of visual neural pathways [8], [19], [20], tactile neural pathways [20]–[22], as well as nociceptive pathways [9], [22].

2.3.1 Somatosensory Stimulation

To study the mechanisms of touch or pain, selective afferent nerve fibres in the skin can be stimulated. The stimuli can be of different types, such as mechanical, thermal, or electrical. which all have their own advantages and disadvantages.

Mechanical stimulators that are used conventionally are slow and cannot be controlled precisely, while the biophysical effects of ultrasound stimuli are not properly known. For thermal stimuli, 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 [23]. Electrical stimuli are easy to control (especially the stimulation timing) and are highly quantifiable; they are hence used often in the stimulation of afferent nerve fibres. Parameters of the electric pulse can be controlled and varied, which can be used to

characterise the system. These parameters include not only the amplitude and frequency, but also the type of modulation.

Transient vs Tonic Stimulation

The stimulus itself can be of two types, namely, transient or continuous. Transient stimuli consist of short duration stimuli like, for instance, single or double pulses, with long inter-stimulus intervals. This allows the underlying physiological system to recover after each stimulus and facilitates the observation of a detailed course of post-stimulus processing [24]. The effect of transient stimuli on brain activity has been studied numerous times [4], [9].

Continuous sensory stimulation on the other hand contains, as the name suggests, uses stimuli of longer duration, obtained by the periodic modulation of a long-lasting stream of sensory input such as a pulse train [22]. It has a number of advantages over transient stimuli as it allows the continuous investigation of neural processing by eliciting SSEPs [24], which are elaborated in Section 2.3.2. Such a stimulus also imitates natural pain or touch related stimuli more closely, as any such sensation is likely tonic. Studies have identified the SSEPs related to the periodic stimulation of nociceptive afferent neurons thereby introducing a new opportunity for studying the cortical processing related to pain perception [9].

In general, when real systems need to be characterised, the choice of excitation signal is important as it affects the output of the system, and some signals may be more suitable than others for the modelling of a specific system. The signals may also require optimisation and compromise to be of use in the characterisation of the system. Some types of stimulus signals are considered next.

Square Wave Modulated Pulse Train

Tonic stimuli resulting in SSEPs are repeating patterns with certain repetition frequencies. The purpose of using this type of stimulus is to verify that the stimulation frequency is also evoked in the somatosensory processing area of the brain, which would imply that a nervous pathway exists [17]. Studies have shown that it is possible to acquire SSEPs using trains of constant-current pulses with periods of no stimulation in between. A major study also showed a difference in the cortical activity related to nociceptive and non-nociceptive responses elicited by continuous stimulation [22].

The stimulus used in these experiments is always a train of equal fixed-amplitude pulses modulated by a square wave of a much lower frequency such as the one shown in Figure 2.2. With this type of stimulus, there are a number of ways in which the stimulus signal can be varied for further analysis of the nervous pathways.

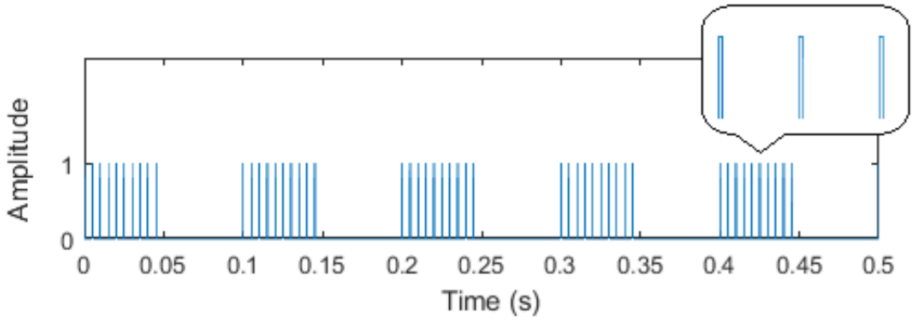

Figure 2.2: Square wave modulated pulse train. Reproduced from [17]

Apart from the frequency of the modulating wave, its duty cycle can be varied which changes the intensity of the signal; most studies mentioned earlier used a 50% duty cycle wave [25]. Additionally, the frequency of the constituent pulse train can be varied too. The pulse-width (PW) and the inter-pulse interval (IPI) in case of multiple pulses can also be controlled [26].

Stimulation Methods

The electrical stimulation described above can be done using two different methods, dictated by the type of electrodes and/or the strength of the stimulus that is used. These methods are described below (illustrated in Figure 2.3):

Intra-Epidermal Electrocutaneous Stimulation (IES): This method is used to selectively activate the nociceptive $A\delta$ fibres based on the fact that these fibres are found in the superficial portion of the skin, i.e., not very deep from the surface [27]. The method does not require expensive equipment and can be applied to any part of the body [4]. A low current must be used, as it has been shown that a stimulus of more than $2.5mA$ also activates non-nociceptive $A\beta$ fibres. However, as this thesis does not concern itself with the nociceptive fibres, this method cannot be used.

Transcutaneous Electrical Stimulation (TES): In this method, the $A\beta$ fibres that are located deeper in the skin, and which are involved with non-nociceptive or tactile sensation only, are activated. This is because the current delivered by this method using round skin electrodes penetrates deeper into the skin. While this current activates both nociceptive and non-nociceptive fibres, as the non-nociceptive $A\beta$ fibres have a lower activation threshold, they are activated preferentially [27]–[29]. This can be ensured using a controlled value of the stimulus current. In this case, studies have found that threshold for perception is about $1 - 2mA$ [30]. As

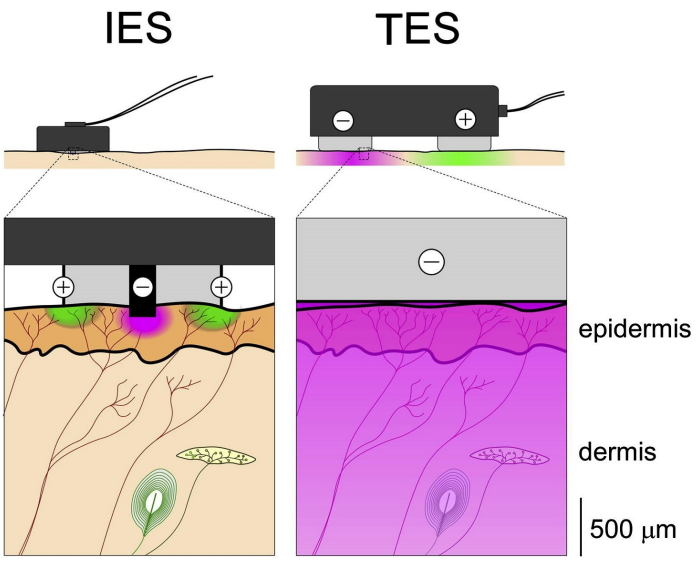

Figure 2.3: Schematic representation of intra-epidermal electrical stimulation (IES) and transcutaneous electrical stimulation (TES) of the skin. Left: the electric current generated by IES is spatially restricted to the epidermis, it is expected to selectively activate nociceptive-free nerve endings located in the epidermis. Right: the electric current generated by TES activates deeper-located non-nociceptive $A\beta$ nerve fibres preferentially. Adapted from [27]

this method is useful for activating the non-nociceptive nerve fibres preferentially, it is the method that is of importance for this thesis.

2.3.2 Analysis of Cortical Response

Brain activity can be recorded using various methods such as Computerised Tomography (CT), Magnetic Resonance Imaging (MRI), functional MRI, Magnetoencephalography, magnetic source imaging, etc. These methods require the use of heavy and specialised machinery and are, thus, beyond the scope of this study.

Another method for investigating cortical response is via scalp recordings, which is known as Electroencephalography (EEG). This method involves the application of a large set of electrodes to the scalp; these electrodes can be used to measure electrical activity (current), corresponding to fluctuations caused by synaptic connections in deeper layers of the cortex. The main advantage of EEG is its simplicity and high temporal resolution, which makes it suitable for the investigation of cortical activity, even though it has the serious limitation of poor spatial resolution [10].

The EEG response of the CNS to specific sensory stimulation can be measured in the form of sensory evoked potentials (EP), which can be visual, auditory or so-

matosensory, based on which sensory system is stimulated [11]. A transient EP is measured by giving the sensory system a kick, which is a short stimulus, and measuring the voltage against time [31]. On the other hand, an SSEP is similar to the steady state response of a resonance circuit, and occurs in response to repetitive sensory stimulation [32]. Additionally, the power of the SSEP is concentrate at the stimulated frequency and its harmonics, whereas the power of the non-cerebral artefacts such as eye blinks and muscle movements is spread over a wide range of frequencies. This leads to a relatively low SNR as the contribution of the non-stimulus-related signals is low at the specific frequency of the SSEP [9]. Also, SSEPs can be elicited by selective stimulation of afferent fibres and can be fully described by amplitude and phase, which makes them suitable for studying the cortical response of pain and touch in humans. Another important advantage is that it is possible to obtain reliable results in short periods of time.

Undesirable Components in EEG Data

While the advantages of EEG mentioned in the earlier section make it suitable for clinical research, EEG does suffer from certain drawbacks, such as a relatively low SNR and a variety of noise sources affecting it. Scalps potentials are low in amplitude, ranging from $20 - 100\mu V$ [33], which means the signals under investigation are likely to be buried in noise, which is simply brain activity unrelated to the stimulus or event being studied, and some other interfering electrical activity. The sources of noise could be electrical, magnetic or irradiated, or they could be biological, or introduced by the instruments involved in measurement. Some major sources of noise are discussed here:

Stimulus Artefacts The most common and problematic type of artefact is the one which is time-locked and phase-locked with the stimulus, known as the stimulus artefact. Such an artefact is made up of electrical or magnetic signals that pass directly from the stimulator to the recording electronic devices due to electromagnetic coupling [33]. The presence of this artefact may be misleading as the recorded data will not necessarily reflect the desired signal, i.e., the cortical response evoked due to the stimulus, or it may be a mixture of the response and stimulus artefact.

Response Artefacts Artefacts that are not necessarily time-locked with the stimulus are the cognition-locked artefacts, which may be due to voluntarily (consciously) or involuntarily (subconsciously) occurring responses from the subject. These include (a) eye movements (EOG: electrooculogram), which are especially significant when the eyes are closed, (b) eye blink artefacts, which cannot be pre-

vented and are dependent on blink rate; they are also associated with eye rotation [33]. A third type of artefact is associated with the *readiness potential*, which is some involuntary response to the act of pressing the button or a response to the stimulus, as well as other muscle movement (EMG: electromyogram) [33].

Other Sources of Noise Another important source of noise is the skin potential artefact which depends on the conductance of the skin. This conductance is, in turn, influence by the thickness of the skin, the number of sweat glands and hair follicles, and the degree of skin hydration [34], all of which defer from one subject to another. Other common sources of noise are the high frequency white noise or high frequency noise from the amplifier or from spontaneously generated signals from random brain activity. Alpha waves from background brain activity are usually expected to be present in the data. Coupling with mains AC power lines in the experiment room also introduces noise of $50Hz$ in the data.

Elimination of Noise Sources

Some sources of noise are unavoidable, but others can be removed completely or at least mitigated. Amplifier and other recording instrument noise is low due to the internal electronics that are meant to reduce noise. However, other sources of electromagnetic noise such as cellphones and unnecessary equipment can be removed from the vicinity of the experimental setup during the experiment. Insulating the experimental room in a Faraday cage can serve as an effective but costly measure. The AC power line signal can be ignored as its fixed frequency is known [35].

While ECG activity is unavoidable, asking the subject to relax and sit in a comfortable position before starting the experiment and instructing them to avoid large muscle movements or speech can reduce EMG noise. Blinks are an involuntary unavoidable activity, but eye saccades, which are rapid involuntary eye movements can be minimised by giving the subject a fixed point to focus on. Noise introduced due to insulating properties of the skin can be reduced by using as abrasive cream and scratching the skin area where electrodes are to be placed [35].

EEG Data Processing Methods

The EEG data obtained through the experiment can be investigated by analysing its temporal as well as spectral properties. However, as mentioned above, despite precautions taken to alleviate the occurrence of noisy signals, some unavoidable noise will still be present in the data. Thus, in order to ensure that the analysis of the required cortical response is not hindered by such noise, some pre-processing

of the data needs to be done. There are several methods used for different types of noise, some of which are described below:

Signal Averaging The best and simplest way to improve the reliability of the results is to average the signal recorded over repeated occasions. Assuming that the noise is random in relation to the events of interest, it will cancel itself out upon averaging, leaving behind a stable EEG response to the event of interest. However, it should be noted that only noise that is random and symmetric will be cancelled out [35]. Other specific types of noise can be rejected or removed using other methods before averaging and analysis of the signals, as explained next.

Rejection of Noisy Data Some channels of the EEG cap may record a high incidence of noise, or some trials may have more noise due to movement or other activities occurring during those trials. Abnormal trends and outliers in the minimum and maximum and kurtosis over all trials and all channels can be used to reject bad trials or channels to remove the trials or channels that have too much noise. However, care must be taken as some desirable information is also often rejected during this process [35].

Rejection of EOG Artefacts Nuisance signals can be fully and accurately removed by using blind source separation (BSS), which is the separation of source signals from a set of mixed signals without knowledge of the individual sources or the mixing process. For instance, independent component analysis (ICA), which is a special case of BSS, uses various measures of statistical independence to decompose the measured signal into a number of original source signals. Visually recognisable noise such as blink, eye movement and muscle movement artefacts can be removed by removing the source signal in which such artefacts are observed [35].

Filtering An easy way to remove noise from the raw data is by filtering it out. High pass filtering can be used to remove noise due to sweating and drifts in electrode impedance; a cut-off frequency of $0.01Hz$ is recommended. High frequency noise can be removed using a low-pass filter, while specific frequencies such as the $50Hz$ one can be removed using a notch filter, if required. However, due to the existence of roll-off and ripples, filtering can also lead to loss of data and must be limited [35].

Inspection of Stimulus Artefact

The presence of stimulus artefacts is a major problem that can affect the reliability of the results of the experiment, as explained before. To ensure that the measured data represents only the cortical response to the stimulus, such an artefact should be prevented, if possible. If present, it needs to be detected so that it can removed in future experiments.

Glitches (voltage spikes) present in the stimulus definitely lead to a stimulus artefact and must, hence, be removed by adapting and improving the internal electronics of the stimulator. When such a stimulator is used, it is still necessary to check if the stimulus artefacts have been prevented for sure. For this, time-course graphs of the recorded signal and of the stimulus input can be compared to see if sharp spikes corresponding to time-locked events are present in the recorded data. Furthermore, cross-correlation can also be used to check the similarity between these two signals.

Chapter 3

Method

3.1 Participants

The participants consisted of healthy students ($N = 10$) at the University of Twente, the Netherlands, with a mix of gender (7 male and 3 female) as well as age ($M = 23.2, SD = 2.62$). The participants were contacted via email and given further instruction once they accepted the request to participate in the research. They reported no history of neurological or chronic pain disorders, or other significant health issues. They were instructed to avoid use of alcohol and narcotics, and the overconsumption of coffee for at least 24 hours before the experiment; sufficient sleep was recommended before the experiment. Each participant was given complete details of the experimental procedure and was familiarised to the stimulus at the onset of the experiment. Written consent forms were obtained from all participants.

3.2 Stimulus

Somatosensory stimulation of the non-nociceptive $A\beta$ fibres was achieved through Transcutaneous Electrical Stimulation (TES), which is expected to preferentially activate $A\beta$ fibres (see Section 2.3.1). Using a NociTRACK AmbuStim stimulator ($compliance\, voltage = 90V$), the stimuli were delivered to the right arm of the participants, approximately over the radial nerve, at about $10cm$ distance from the medial epicondyle using standard disposable ECG electrode.

The detection threshold of the subject is the lowest amplitude of stimulus current at which the subject perceives a stimulus. During the experiment, a current that is twice this threshold was used for the nerve stimulation. The stimulus was, in each case, a steady sequence of pulses ($PW = 0.5ms, IPI = 10ms$) occurring periodically. The chosen frequency ($43Hz$) is expected to show a relatively high amplitude of SSEPs as compared to other tested frequencies [22]. As per earlier

studies, stimuli with these parameters are expected to preferentially stimulate non-nociceptive $A\beta$ fibres. Two different variations of the electrical stimulus sequences were used in each experiment, which are described below:

1. The first stimulus sequence consisted of single pulses that occur at a frequency of $43Hz$, which means the time between each pulse is $23.25ms$.

2. The second stimulus sequence consisted of double pulses that occur at a frequency of $43Hz$, which means the time between the first pulse in one pair of pulses and the first pulse in the next pair is $23.25ms$.

3.3 Procedure

The experiments were conducted in a silent lab containing all the required equipment in it. The subjects were seated in an arm-chair in a self-decided comfortable position, with a photograph affixed on the wall in front of them. They were asked to keep their eyes focused on this photograph to avoid saccade artefacts.

The two stimuli were applied to the right arm in separate blocks. The experiment consisted of four blocks, two each for the single-pulse and the double-pulse stimulus. The order of the two blocks was randomised across the subjects by applying each of the two stimuli in a 1212 scheme for 5 subjects and a 2121 scheme for the other 5 subjects. Each block consisted of 20 trains (trials) of pulses with $10s$ trains of pulses alternating with $10s$ periods of no stimulus; the latter serve as the control condition for the experiment. Each train of pulses within the 20 trials consisted of either the first or the second stimulus, obtained by applying a boxcar function with a periodicity of $43Hz$.

Each block was separated by a period of about 15 minutes during which certain questions were asked, the instructions were reiterated, and the new detection thresholds of the subject were measured (as the threshold changes after continuous stimulation due to habitation). The entire experiment took about 1.5 hours in addition to preparation of about 1.5 hours.

The procedure and stimuli are represented in the Figure 3.1. A complete step-by-step description of the experimental protocol can be found in Appendix B.

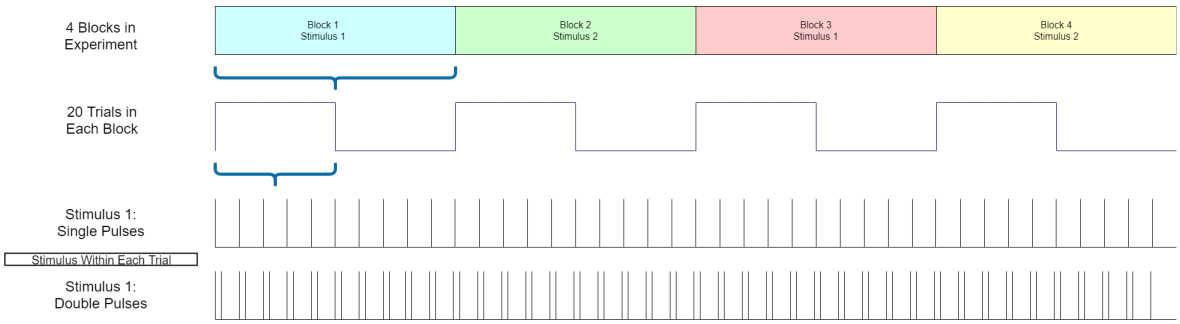

Figure 3.1: (From top to bottom) (a) Each experiment consists of 4 blocks, (b) Each block consists of 20 trials, (c) Stimulus 1: Single pulses, (d) Stimulus 2: double pulses

3.4 Measures

3.4.1 Behavioural Measures

After each of the four blocks, the subjects were asked a set of questions and the answers were noted for future reference. First, the subjects were asked to describe the sensation they felt during stimulation and then they were asked to rate the intensity of the sensation. The rating was a self-evident four-choice scale, namely, neutral, mildly uncomfortable, very uncomfortable and painful. They were also asked if the sensation was localised to a small area under or around the electrode or if it was felt all over their arm. The exact questions asked are noted in Appendix C.

3.4.2 Electrophysiological Measures

To obtain the EEG data during experimentation, a standard EEG cap with 128 Ag/AgCl electrodes was placed on the scalp of the subjects. Electro-gel was used to keep the impedance of the electrodes below $5k\Omega$. The signals were amplified by connecting the EEG cap to a 128-channel Refa amplifier, with the ground connected to the centre of the subjects forehead. The subject was asked to keep their eyes fixed on a picture attached directly in front of them to reduce the occurrence of EOG artefacts. They were also instructed to avoid any muscle movement during the experiment so as to prevent the occurrence of muscle movement artefacts.

3.5 Data Analysis

The EEG data was measured and recorded using the programme PolyBench (TMSi, the Netherlands). The pre-processing and processing steps required for data anal-

ysis were done using Matlab (Version: 2017b) (Mathworks, USA) and the FieldTrip (Radboud University, the Netherlands) toolbox.

In the pre-processing, the runICA function is used to decompose of the recorded mixed signals into latent source signals. After this step, the separate components can be viewed; the ones that are found to contain EOG artefacts are selected for rejection . After this, the variance of all the channels and all the trials are plotted in a graph. Channels and/or trials that have high variance (outliers) are selected for rejection; these would be excluded from all further analysis. The workflow of this pre-processing step and also, the subsequent processing steps, is illustrated in Figure 3.2. Examples of EOG artefacts and high variance channels/trials are shown in Figure 3.3 and Figure 3.4 respectively.

Prior to looking at the average, two channels, Cz and Fpz, are selected for the analysis of the signals and spectra obtained for the EEG measurements. Cz is located at the approximate centre of the head and EEG cap, and is an appropriate choice as it is the common reference electrode for all the other channels. Literature shows that for a $43Hz$ signal the frontal area of the head gives a higher output [22]. The presence of stimulus artefacts is also most visible in the channels at the front. Hence, Fpz, which is the first electrode at the very front of the EEG cap, is chosen as the second electrode.

3.5.1 Presence of Stimulus Artefacts

The presence of stimulus artefacts in the EEG data renders the data unreliable, as explained in Section 2.3.2. Hence, the data must be examined specifically for detecting this artefact, if present . For this, the averaged waveform after EOG artefact removal is viewed in the time domain, for each subject. The input (stimulus) signal is also plotted, so that the two waveforms can be compared visually to detect the presence of stimulus artefact, which would present itself as sharp peaks in the output waveform time-locked with the stimulus events. If stimulus artefacts are detected, the subjects that show these artefacts are excluded for the data analysis.

3.5.2 Frequency-Domain Analysis of EEG Data

The frequency spectrum of the EEG data is viewed in two ways using FFT (Fast Fourier Transform) in Matlab. The artefact-free EEG epochs are averaged in order to attenuate the contribution of components that are not phase-locked with the stimulus. This gives the mean signal over all the trials for each channel included in the cleaned data; the spectrum of this mean is considered for the spectral analysis. Besides this, taking the spectrum of each of the 40 trials of either of the two stimuli and

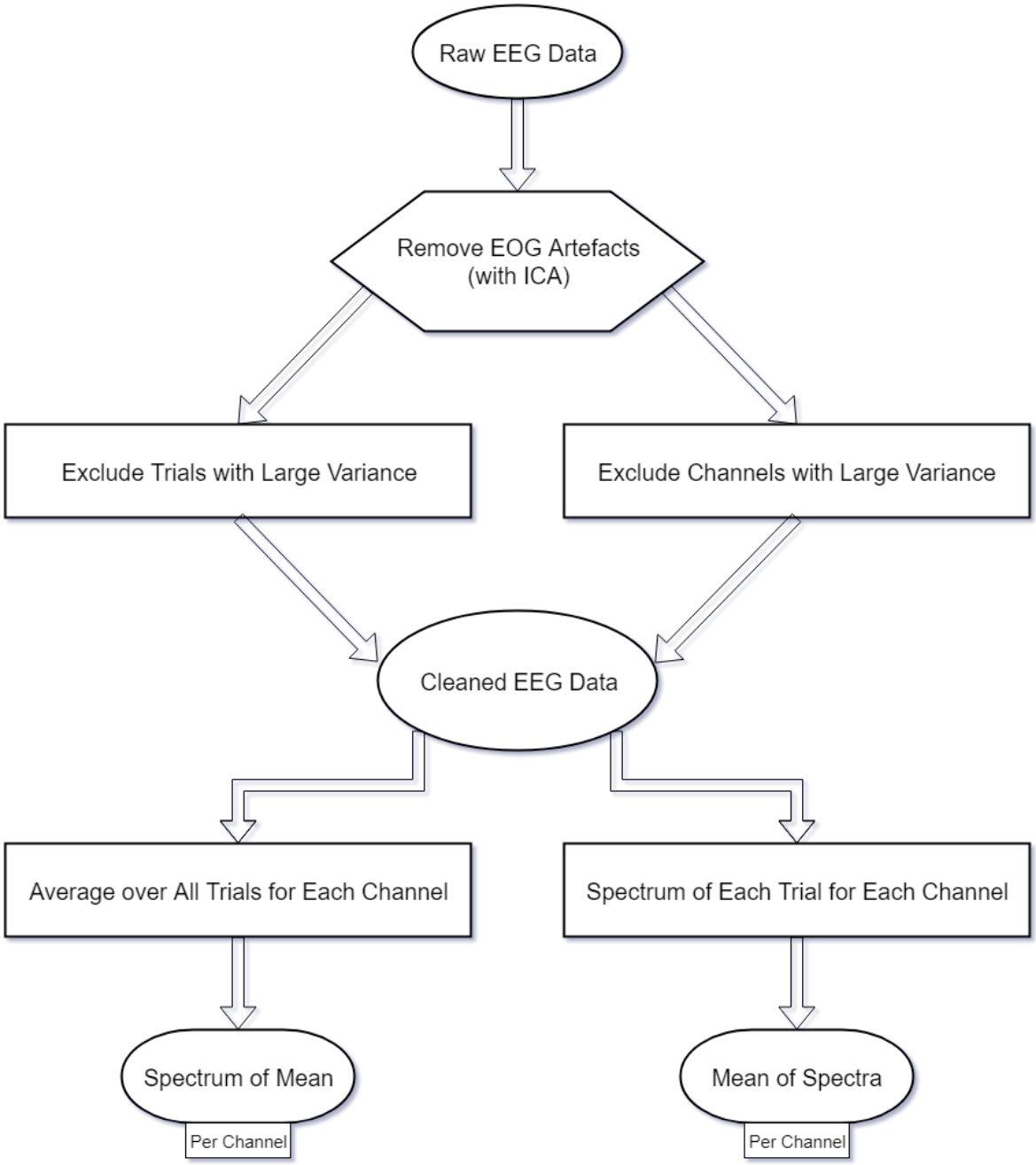

Figure 3.2: Workflow of the pre-processing and processing steps applied to the recorded EEG data of each subject until the required (spectral) results are obtained.

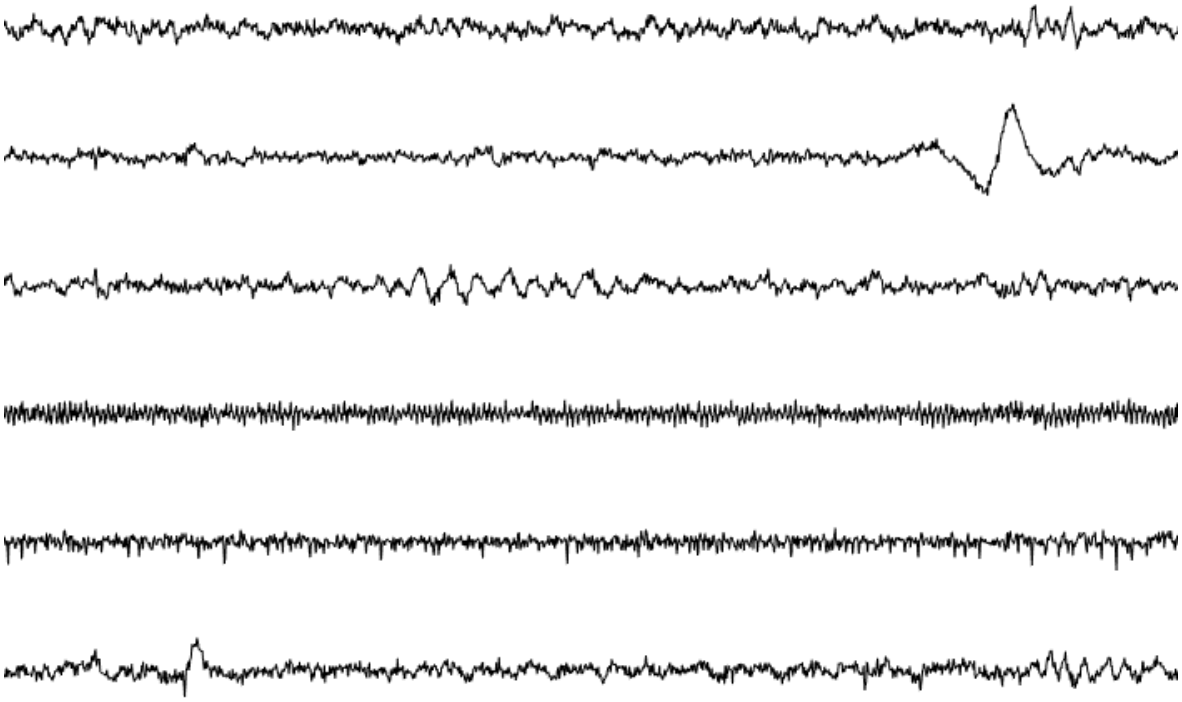

Figure 3.3: EEG data components (ICA): Second component from the top shows EOG artefact.

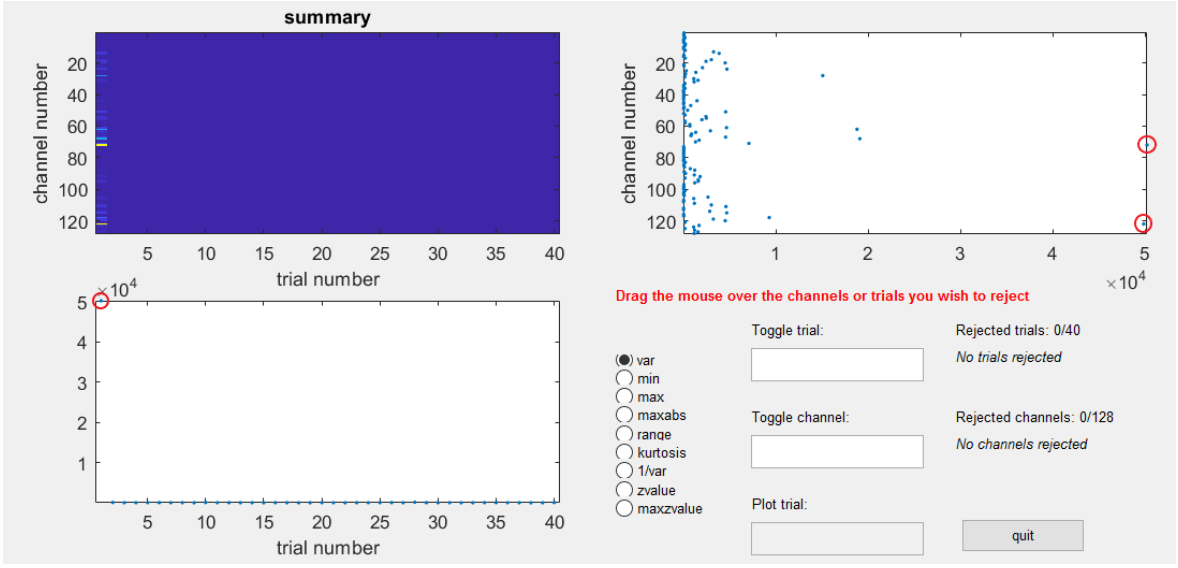

Figure 3.4: Window used for rejection of high variance trials and/or channels. Left bottom: One trial shows high variance. Right top: Two channels show high variance

averaging these spectra gives the mean of all the trial spectra for each channel.

The two frequency spectra obtained for each subject can be further averaged over all the subjects to obtain a single global spectrum (of either type) that can be used for detection of the desired frequency component. In general, at least the first 4 harmonics of the desired component are viewed so that the first 2 odd and the first 2 even harmonics can be compared. The harmonics can be used as a rudimentary indication of the linearity; if the shape of the output resembles the shape of the input precisely, the system can be considered to be linear.

Effect of Variation in Number of Pulses

The above method, used to obtain the averaged frequency spectrum all subjects, is applied to the EEG data elicited by both stimuli separately. As the stimuli differ in the number of pulses, these two spectra can be compared to investigate the effect of varying the number of pulses on the SSEPs elicited during the experiment.

Effect of Variation in Area of Perception

The spectra for the set of patients who felt a localised sensation and the set that felt the sensation all over their arm (both self-reported) can be averaged separately. The two spectra obtained from the averaging can be compared to investigate the effect of sensation area, and thereby, of the location of the electrodes on the arm, on the elicited SSEPs.

Chapter 4

Results

In this study, 10 subjects were given two different types stimuli separately to their forearm. Their cortical response to this stimulation was recorded as EEG data, which was then processed to obtain results. After removal of random noise and signals unrelated to stimulus events, the EEG data obtained during the experiments was stored separately for all the channels for each of the subjects. This data was analysed to see if the stimulated frequency can be 'tagged' in the cortical response.

For the two stimuli (single-pulse and double-pulse) (see Section 3.2), the frequency was intended to be $43Hz$ each. However, due to an error in the stimulation software, Stimulus 1 (single-pulse) has a frequency of $42.38Hz$ and Stimulus 2 (double-pulse) has a frequency of $45Hz$.

Unfortunately, the data for 2 out of the 10 subjects participating in this study could not be used. This is because the stimuli for these two subjects had a different frequency than the stimuli used for the other eight subjects, also due to a problem with the stimulation software. Hence, the results presented in this section include the data of only these eight subjects.

In each case, the signals or spectra for separate subjects are shown in grey. Also, the label 'input' is used for the signal from the stimulator; the labels 'output' and 'control' are used for the EEG data measured during the experimental and control conditions, respectively.

4.1 Subject Response

The answers given by the subjects to the questions (refer to Appendix C) asked after each block of the experiment indicated that Stimulus 2 was felt more strongly by all subjects compared to Stimulus 1. However, neither of the stimuli could be categorised as painful, although they caused discomfort proportional to the amplitude of the stimulation current. Out of the 7 subjects used in further analysis, 4 of the

subject stated that they felt the stimulus only in a small area around the electrodes attached to their arms, while 3 of the subjects reported to have felt the stimulus over their whole forearm.

4.2 Visual Detection of Stimulus Artefact

Although eye and muscle movement artefacts are removed from the data during pre-processing, the processed results may still contain stimulus artefacts. These artefacts are detected by comparing the input (stimulus) signal and the output (EEG response) signal.

4.2.1 EEG Signal

The input and output signals (and their averages) for stimulus 1 are shown in Figure 4.1. It can be seen that the presence of an artefact for one subject (shown in red) is clearly visible in channel Fpz but not in channel Cz. In both cases, the average of all subjects (shown in green) does not exhibit the presence of the artefact. Figure 4.2 shows the input and output signals for stimulus 2. For this stimulus too, the artefact for one subject (same subject, also shown in red) is distinctly visible in channel Fpz but not in Cz, and the average over all subjects (shown in green) does not show the presence of the artefact. It is interesting to note that the stimulus artefact manifests as sharper and more distinct peaks for Stimulus 1 than for Stimulus 2.

The results hereafter are obtained with the exclusion of the subject that shows the clear presence of stimulus artefact. The spectral analysis is done with only the data for the remaining 7 subjects included.

4.2.2 Spectrum After Artefact Removal

Figure 4.3 shows the spectra for Stimulus 1 for the two chosen channels, while Figure 4.4 shows the spectra for Stimulus 2. The mean of the spectra of each trials as well the spectrum of the mean signal over all the trials is shown. The average over all subjects is also given.

For Stimulus 1, the stimulation frequency component of $43.38Hz$ is visible in all cases, i.e., for both channels and for both types of output spectra, but is not visible in the control situation. For Cz, only the fundamental frequency component is visible in the mean of the spectra, while the second harmonic is visible in addition to the fundamental frequency in the spectrum of the mean signals. For Fpz, the first three harmonics are visible in the mean of the spectra, but the spectrum of the

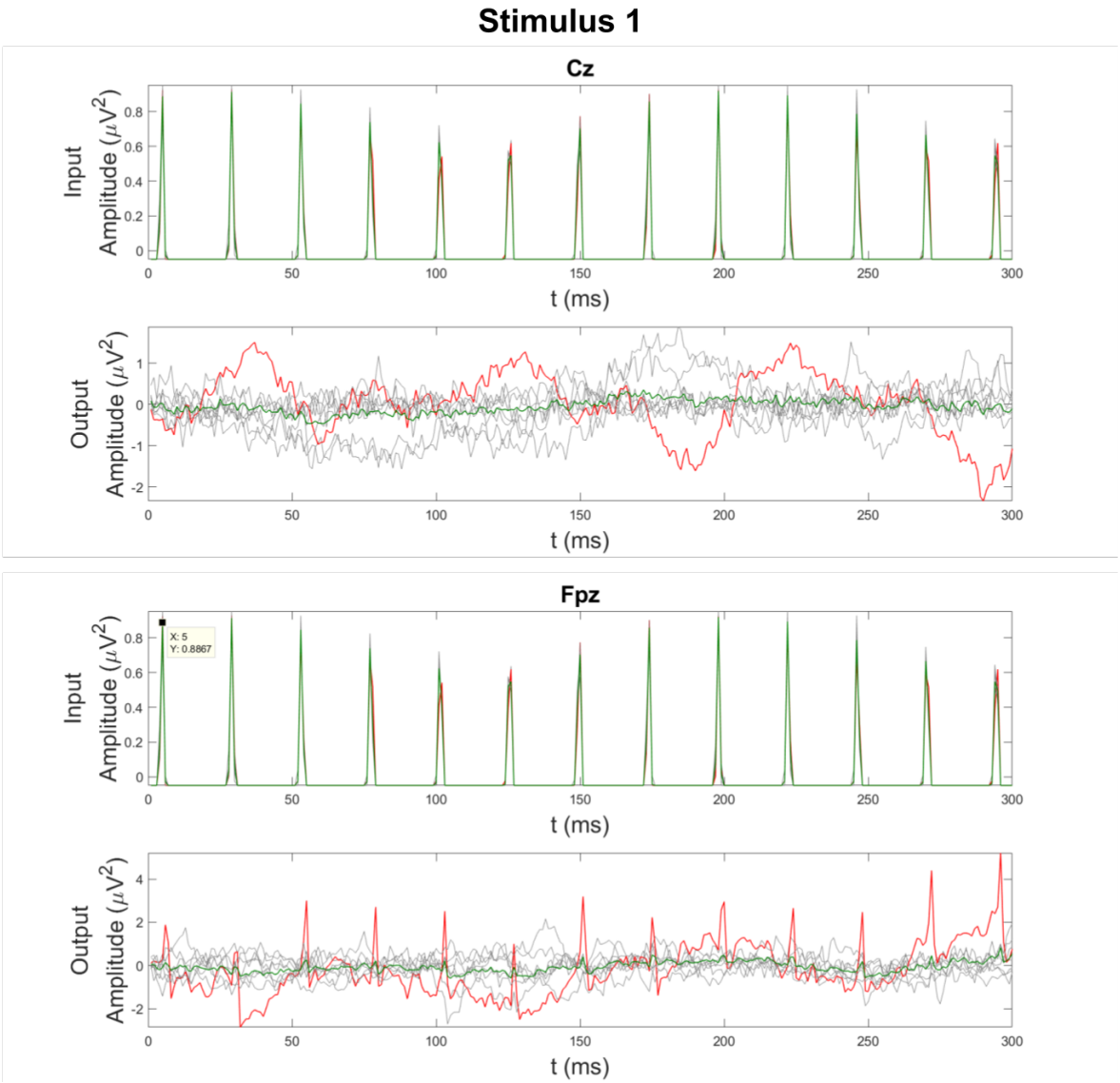

Figure 4.1: Average signal over all trials using Stimulus 1 for channel Cz (top) and Fpz (bottom). In green: average over all subjects, in red: subject showing presence of stimulus artefact, in grey: signals for rest of the subject (without artefact)

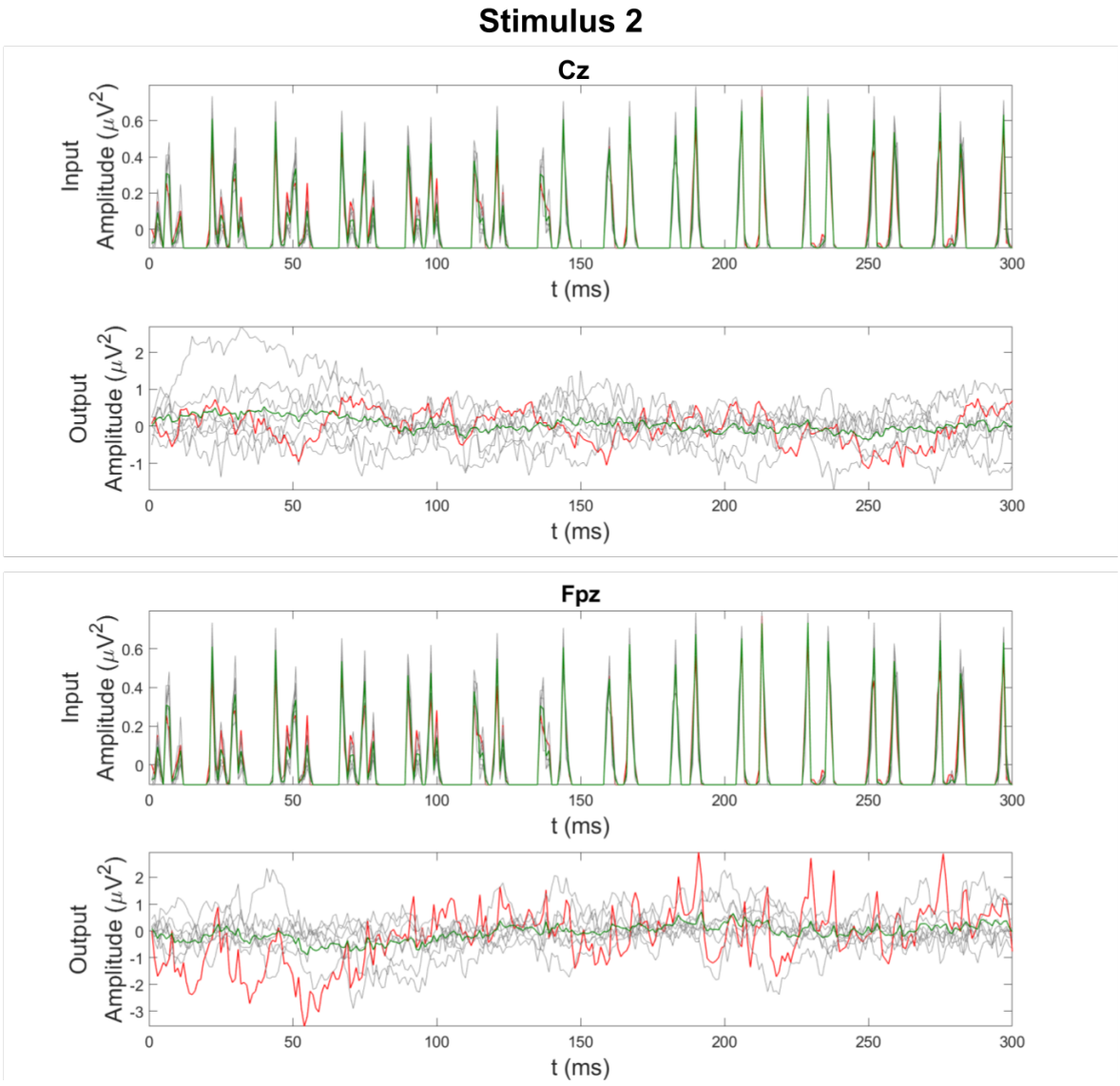

Figure 4.2: Average signal over all trials using Stimulus 2 for channel Cz (top) and Fpz (bottom). In green: average over all subjects, in red: subject showing presence of stimulus artefact, in grey: signals for rest of the subject (without artefact)

mean distinctly shows the first seven harmonics. Note that these seven harmonics in the output spectrum are linearly scaled with the seven harmonics seen in the input spectrum.

In the case of Stimulus 2 as well, the $45Hz$ stimulation frequency component of is clearly visible in the output spectra, but not in the control situation. In this case, the mean of the spectra shows the harmonics $f, 2f, 3f$ for Cz and only f for Fpz, but the spectrum of the mean signals shows the harmonics $f, 3f, 4f, 6f$ for Cz and $f, 3f, 6f$ (clearly) for Fpz. Furthermore, it should be noted that for Stimulus 2, in all cases, the spectrum of the input signal itself presents distinct side-lobes for all visible harmonics.

For both stimuli, the amplitudes of these components are higher in Fpz than in Cz. Furthermore, it can be seen that the spectrum of the mean signal (mean taken over all seven subjects) shows less noise (backgrounf brain activity) and a more distinct output component (with higher amplitude) at the required frequencies. As this trend is observed for all four combinations of stimuli and channels, the results henceforth will use the spectrum of the mean signal for further analysis. Additionally, the harmonics for both stimuli will be investigated further in Section 4.4.

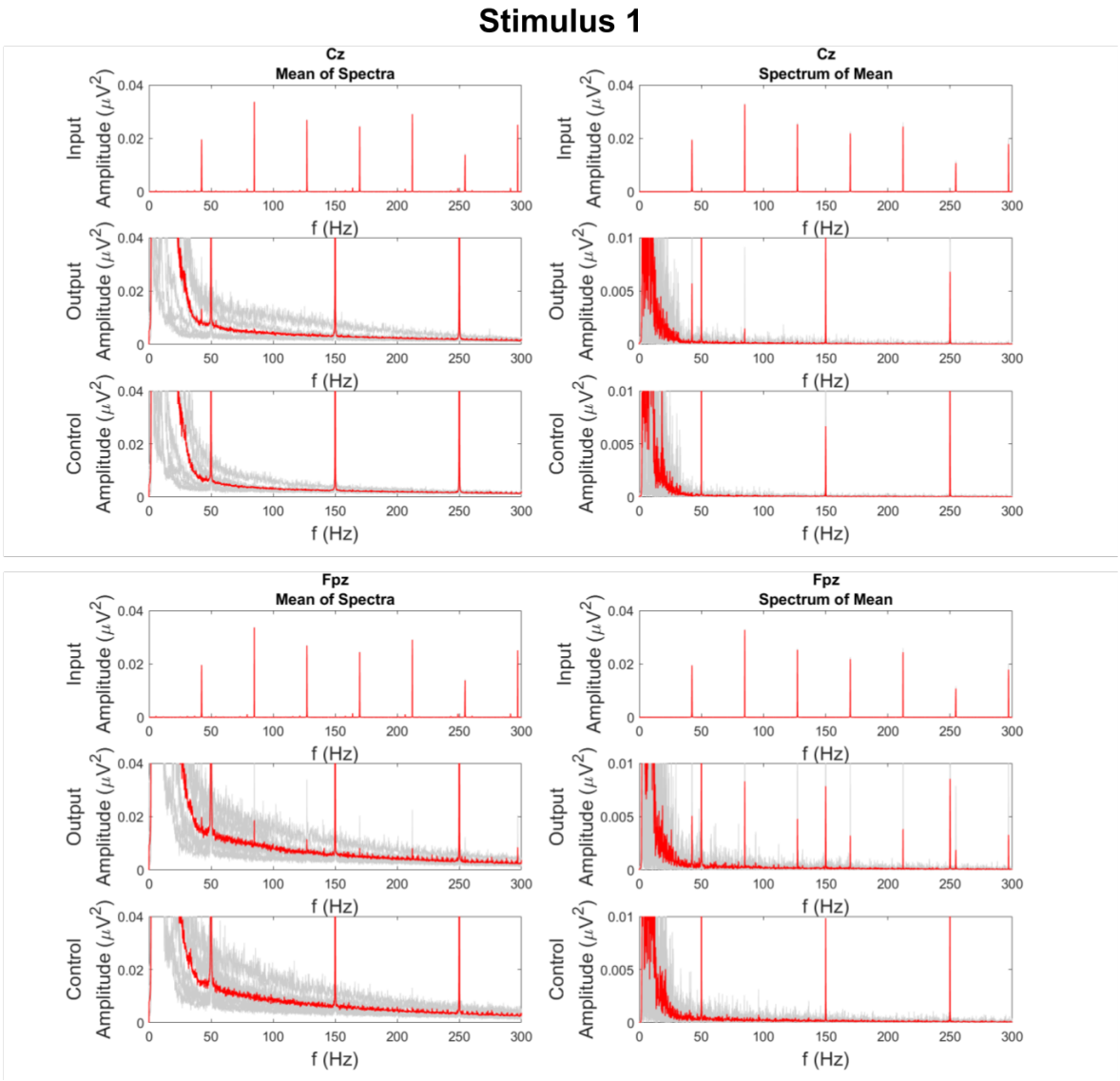

Figure 4.3: Fourier spectrum using Stimulus 1 for channel Cz (top) and Fpz (bottom). The mean of the spectra of all trials is shown on the left while the spectrum of the mean of all trials is shown on the right. In red: average over all subjects, in grey: spectra for rest of the subject

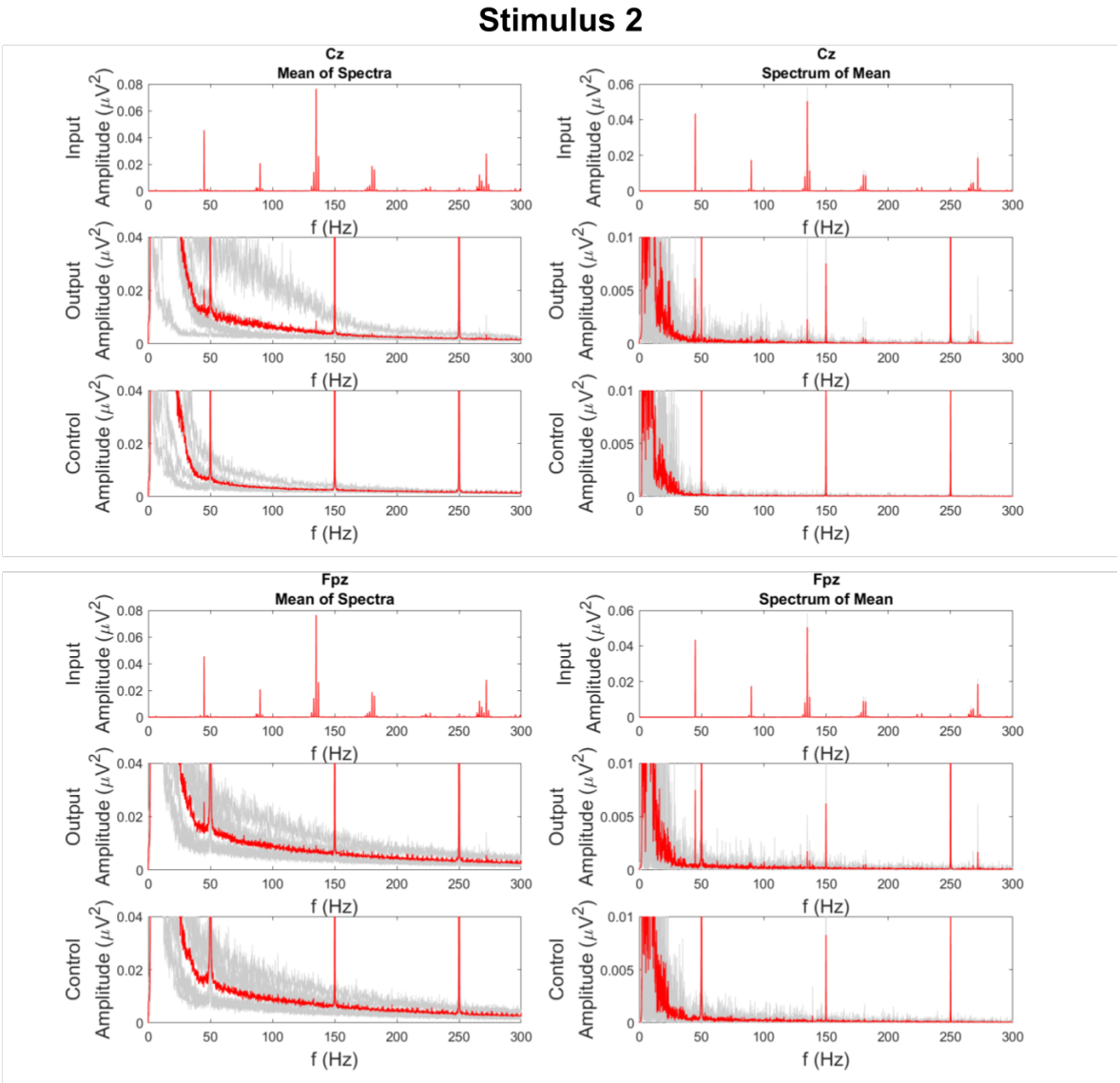

Figure 4.4: Fourier spectrum using Stimulus 2 for channel Cz (top) and Fpz (bottom). The mean of the spectra of all trials is shown on the left while the spectrum of the mean of all trials is shown on the right. In red: average over all subjects, in grey: spectra for rest of the subject

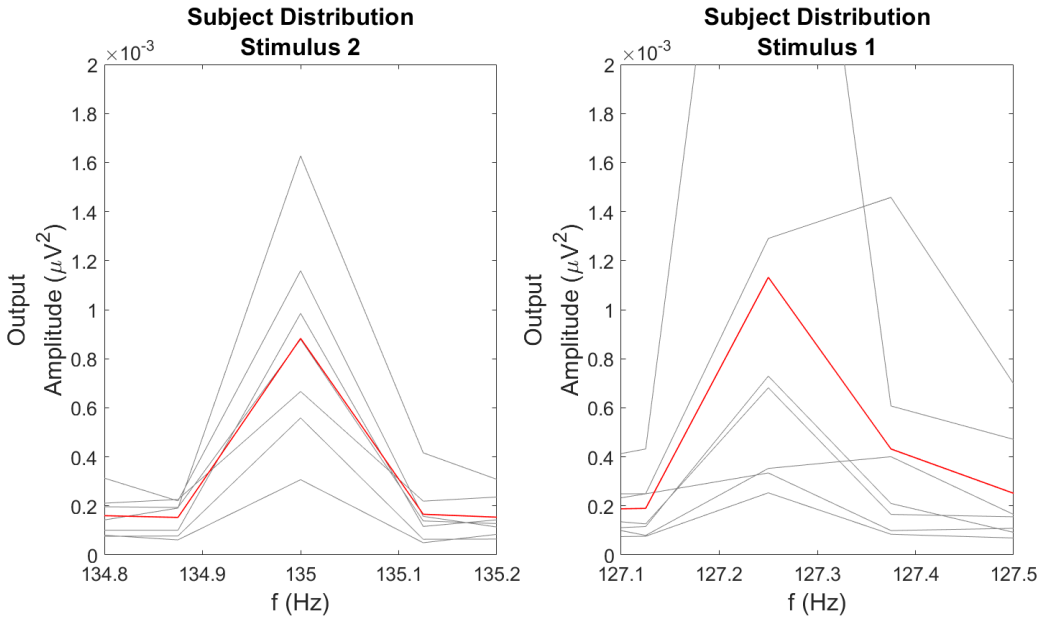

Figure 4.5: Subject-wise distribution of frequency component 3f in the global average for stimulus 1 (right) and Stimulus 2 (left). In red: average over all subjects, in grey: spectra for rest of the subject

4.3 Global Average

The global average over all channels and all subjects is analysed next. The distribution of the spectra over the seven subjects is also observed.

The global average for both stimuli is shown in Figure 4.6. In both cases, the required frequency component is present in the spectra. The fundamental frequency has a higher amplitude for Stimulus 2 ($A_{Stimulus1} = 3.71\times10^{-3}\mu V^2$) than for stimulus 1 ($A_{Stimulus1} = 2.97\times10^{-3}\mu V^2$). However, it is observed that the higher harmonics ($e.g. 2f, 3f, 4f, 5f$) for stimulus 1 have higher amplitudes than these higher harmonics for stimulus 2.

To show the inter-subject differences in the output, the third harmonic is viewed. This is simply because this is the first harmonic component with relatively less noise (which makes the desirable frequency component clearer for closer inspection). A zoomed-in diagram of the third harmonic of desired ($42.38Hz/45Hz$) frequency component in the output is shown for each stimulus in Figure 4.5. It can be seen that the deviation of the amplitudes for the various subjects differs noticeably from the mean, for both stimuli. It should be noted that Stimulus 2 is seen to have one subject for whom the deviation is large. Another interesting observation is that Stimulus 1 shows frequency jitter ($\approx 0.2Hz$).

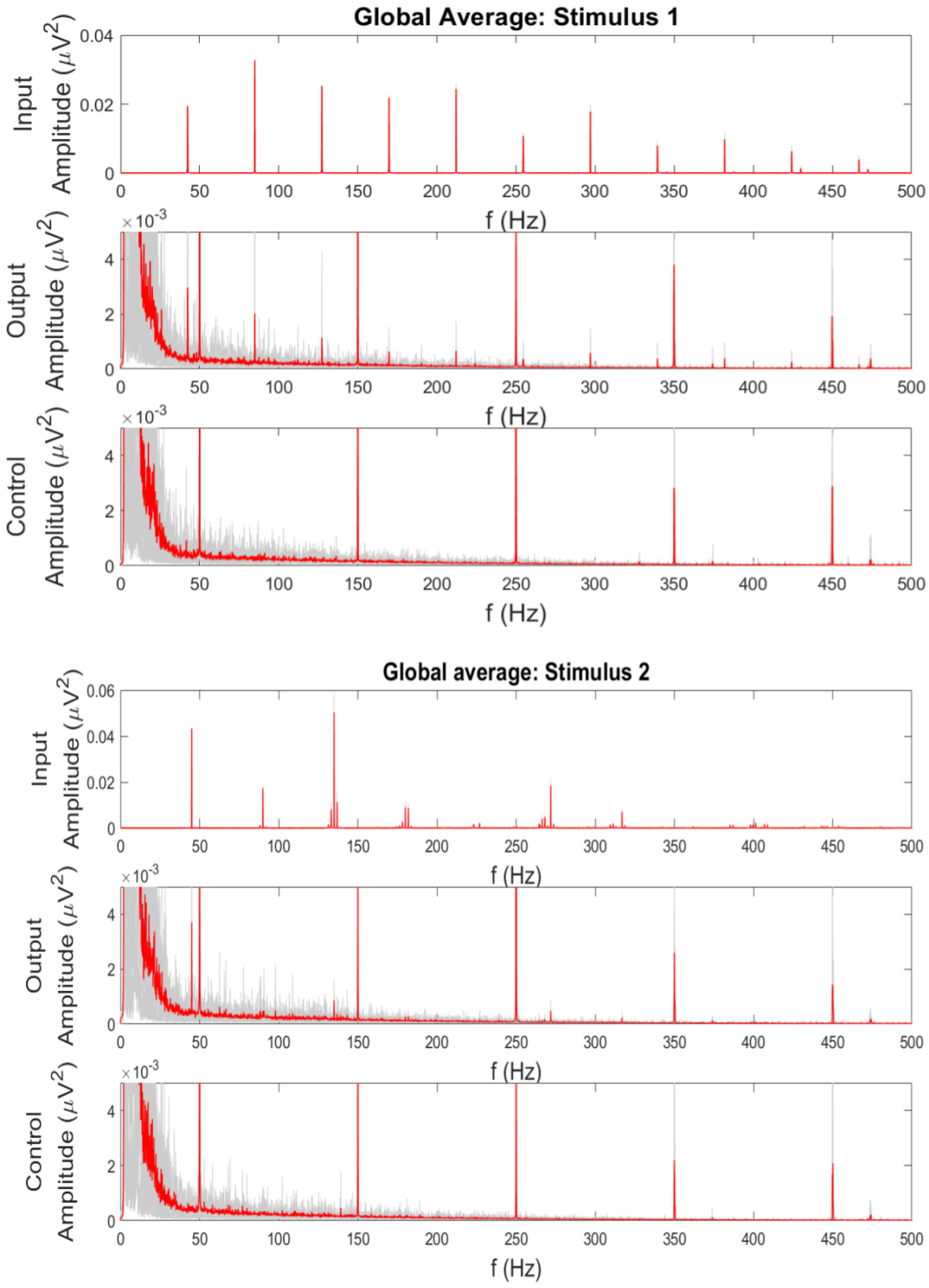

Figure 4.6: Global average over all trials and channels for Stimulus 1 (top) and Stimulus 2 (bottom). In red: average over all subjects, in grey: spectra for rest of the subject

4.4 Cortical Distribution

The distribution of the desired component amplitude over 3 different areas of the head is studied next. The first four harmonics each for 13 channels the frontal region, 13 channels in the middle region and 13 channels at the back of the head are plotted for both stimuli. The mean over all eight subjects for each separate channel is shown, grouped by cortical region.

Figure 4.7 shows the graphs obtained for Stimulus 1 while Figure 4.8 shows the ones obtained for Stimulus 2. Both show the amplitudes of all 29 channels grouped by region and separated by order of harmonic. Figure 4.9 and Figure 4.10 show graph of the median of each box in each row seen in Figure 4.7 and Figure 4.8. In both cases, a general trend of reduction in the amplitude of the first four harmonics of the desired frequency component from the front to the back is clearly present.

The graph shown in Figure 4.10 demonstrates this change in amplitude for Stimulus 2. It is seen that the reduction in amplitude from front to back is significant for the first harmonic (fundamental frequency) and for the third harmonic, but not as much for the second and fourth harmonic. It can also be seen that the first and third harmonics (odd multiples) have higher amplitudes than the second and fourth harmonics (even multiples). If considered separately, the amplitude is seen to reduce with increase in the order of the harmonic for both even and odd harmonics.

The graph in Figure 4.9 indicates that a similar trend is not clear for Stimulus 1. While the fundamental frequency component in the middle area of the head is significantly less than that in the frontal area, the reduction of this amplitude from the middle to the back, while observable, is low. The trend is relatively clearer for the third harmonic. In this case, the trend of the odd harmonics having higher amplitudes than the even harmonics is only seen in the (especially) middle and back regions. For the middle region, the first and third harmonics are higher than the second and fourth, while for the back region, the first harmonic is higher than the second, and the third is higher than the fourth. The frontal region deviates from this trend, and has the second harmonic higher than the fundamental frequency component. It should be noted that the peaks for the fundamental frequency for Stimulus 1 appear to show frequency jitter ($\approx 0.1Hz$).

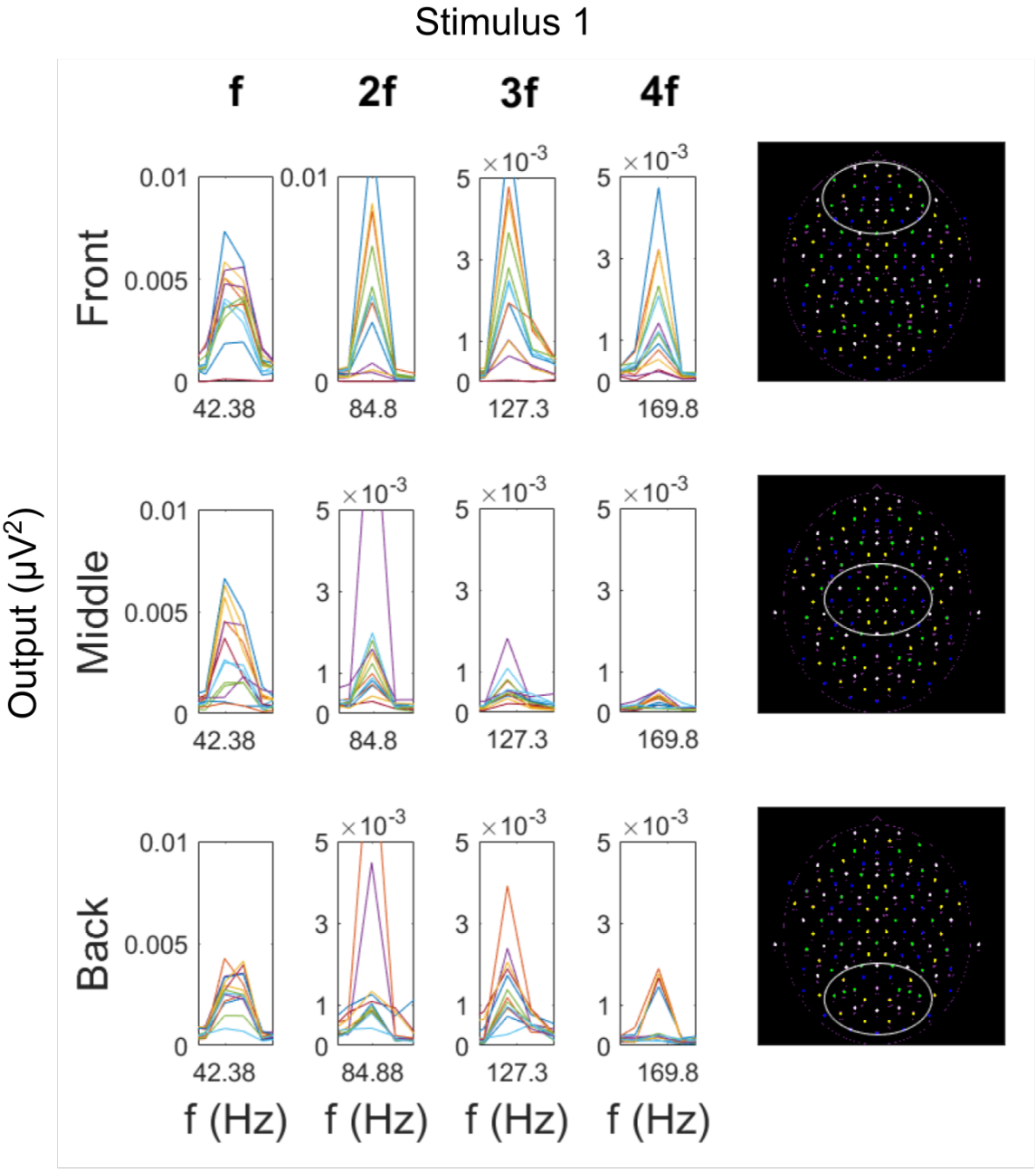

Figure 4.7: Cortical distribution of the first four harmonics output spectrum (averaged over all subjects) for Stimulus 1. The spectral components for: 13 channels in the frontal region of the head are shown in the top row, 13 channels in the middle region are shown in the middle row, 13 channels in the back region are shown in the bottom row. The rightmost column shows the region from which the channels are chosen.

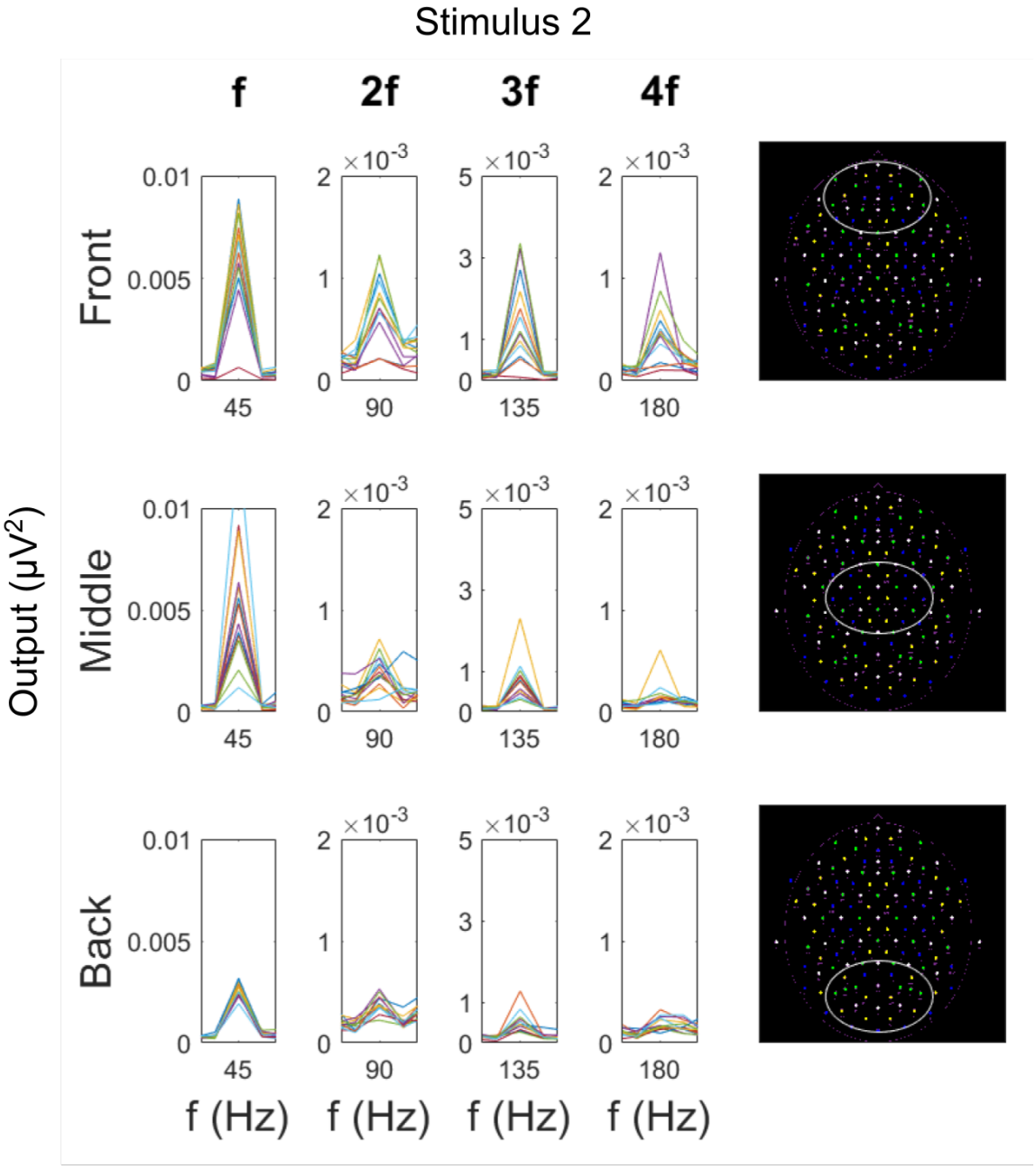

Figure 4.8: Cortical distribution of the first four harmonics output spectrum (averaged over all subjects) for Stimulus 2. The spectral components for: 13 channels in the frontal region of the head are shown in the top row, 13 channels in the middle region are shown in the middle row, 13 channels in the back region are shown in the bottom row. The rightmost column shows the region from which the channels are chosen.

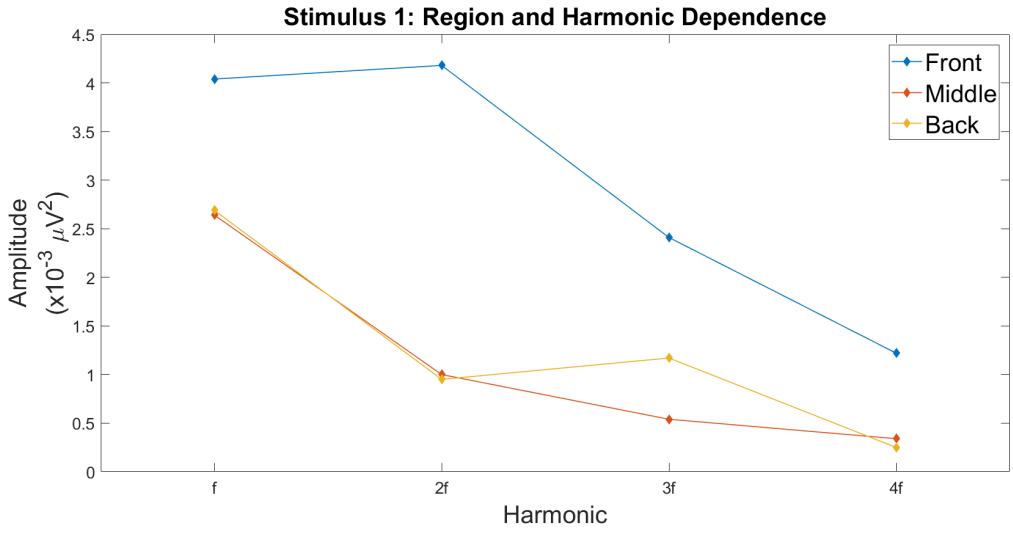

Figure 4.9: Graphs of medians of 13 channels each in the frontal, middle and back regions of the head, for the first four harmonics of the output, in the case of Stimulus 1.

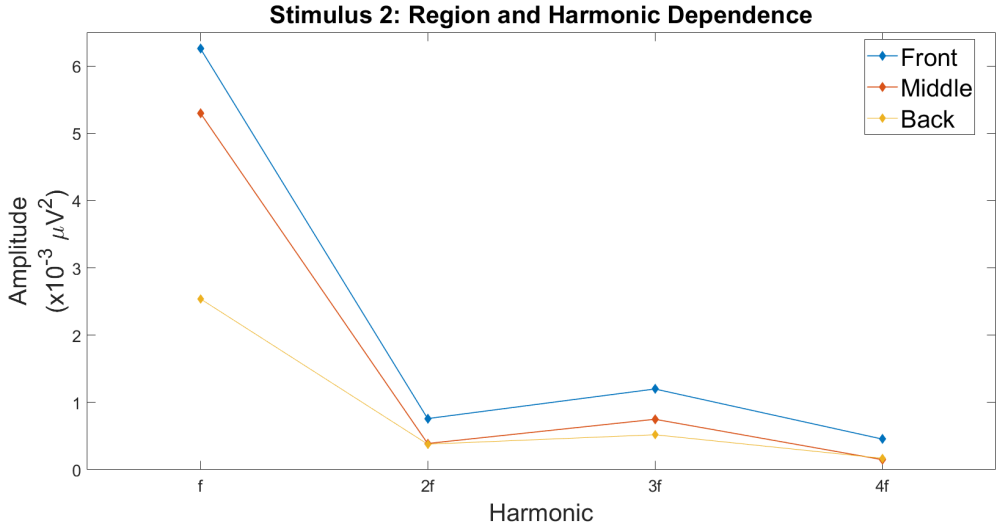

Figure 4.10: Graphs of medians of 13 channels each in the frontal, middle and back regions of the head, for the first four harmonics of the output, in the case of Stimulus 1.

4.5 Area of Perception

The output spectra for the subjects who perceived the stimulus in the vicinity of the electrodes on their arm and for those who perceived it over their entire forearm as well as the average spectrum for both these groups is shown in Figure 8, for both Stimulus 1 and Stimulus 2.

For both stimuli, the desired frequency component of 42.38Hz or 45Hz has a higher amplitude for the subjects who felt it over their entire forearm ($A_{Stimulus1} = 5.68\times10^{-3}\mu V^2, A_{Stimulus2} = 5.12\times10^{-3}\mu V^2$) than for the subjects who felt it around the electrodes ($A_{Stimulus1} = 0.94\times10^{-3}\mu V^2, A_{Stimulus2} = 2.65\times10^{-3}\mu V^2$) . The ratio of the average amplitudes for the localised perception case to the widespread perception case is 1:6 and 1:2, respectively for Stimulus 1 and 2.

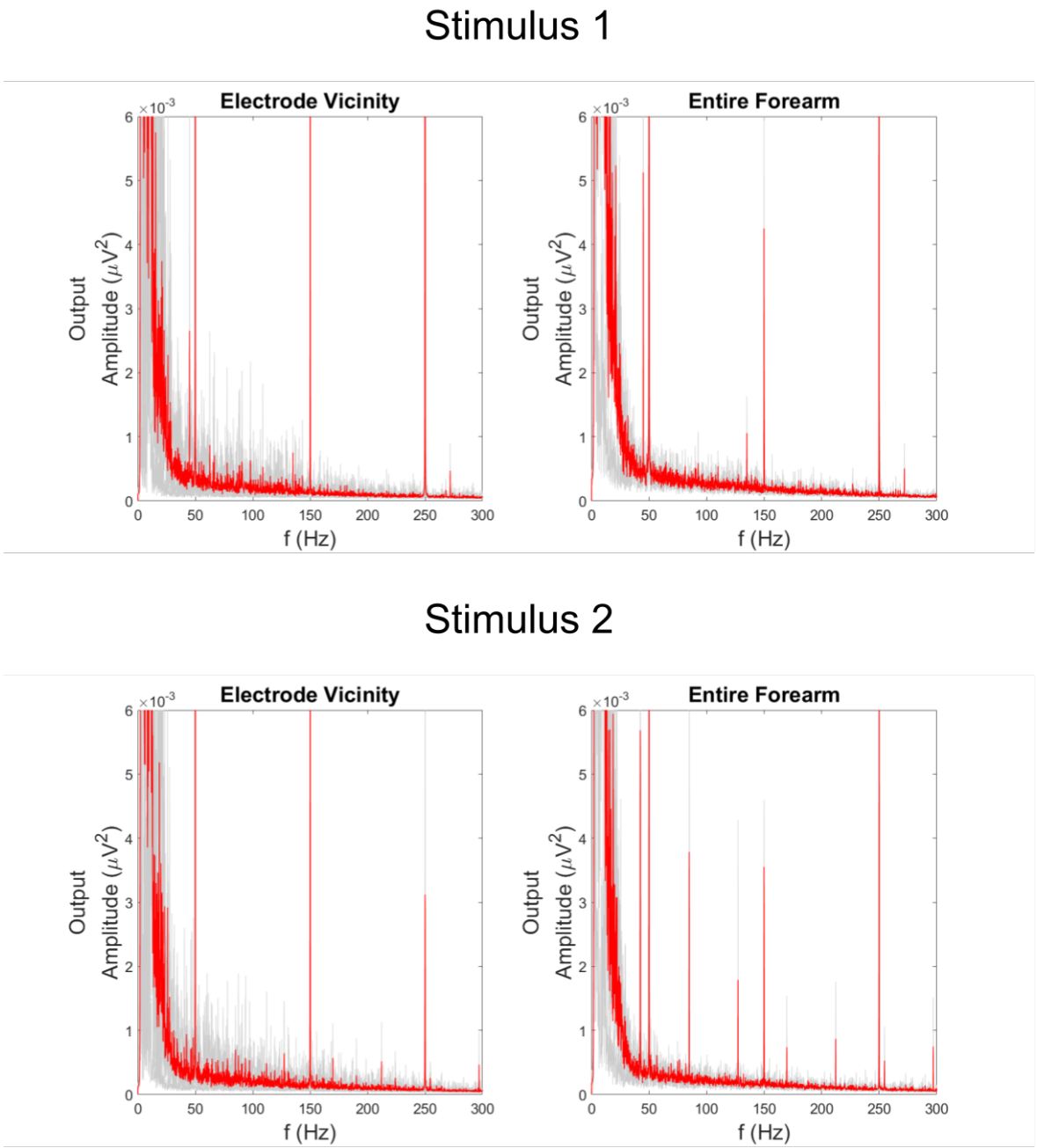

Figure 4.11: For Stimulus 1 (top) and Stimulus 2 (bottom), the spectrum of the output for the subjects who felt the stimulus in the vicinity of the electrodes on their arm (left) and for the subjects who felt the stimulus over their entire forearm (right)

Chapter 5

Discussion

5.1 Frequency Tagging

The primary objective of this study was to show that when a subjects arm is stimulated at a certain frequency, the same frequency can be detected in the EEG data (cortical response) of the subject, thereby tagging the cortical response to the stimulation. In the results of this experiment, the stimulated frequency component was clearly present in all test subjects. However, the possibility that this component could be caused by a stimulus artefact cannot be disregarded (especially for one specific subject, see Section4.3). This is discussed further in Section 5.2. On the other hand, the pre-processing steps require the omission of several trials and several channels for each separate subject, which may have resulted in the loss of data leading to a weaker signal in the output. A lot of noise resulting from background activity of the brain is also seen in the data, which could make the identification of the required frequency component difficult. This is especially notable for higher harmonics which are seen to have progressively lower amplitudes. This would pose a problem if the current detected signal is compounded by stimulus artefact, as the amplitude is likely to reduce if the artefact is successfully removed. However, it was seen that taking the mean of the signals from the various channels and subjects and then plotting the spectrum greatly reduces the noise (compared to taking the average of the spectra of all channels and subjects).

The subject-wise plotting of the spectral data shows a wide distribution with the smallest and largest amplitudes showing significant deviation from the mean. This may reduce the reliability of the study. Secondly, the subject sample size is small (8 subjects analysed) which further reduces the reliability of the results, and as the subjects are restricted to a specific population (students at a university), the effects of disobeying of instructions (lack of good sleep, alcohol consumption) can cause drowsiness and lack of attention during the experiment) cannot be dismissed.

In one result (refer to Figure 4.3), the output amplitudes (shape/envelope) was

seen to follow the input shape exactly. This may be an evidence of the linearity of the sensory system; it is more likely another sign indicating the presence of a strong stimulus artefact, as such an artefact is linearly related to the input. The presence or absence and strength of the harmonics should be also be noted. It is interesting to note that (for stimulus 2 and to an extent for stimulus 1), the odd harmonics were stronger than the even harmonics. This is most likely due to the fact that the box-car function used for the stimuli is essentially a square-wave, and a square wave consists of only the odd harmonics.

5.2 Stimulus Artefact

The analysis of two separate channels showed that a stimulus artefact is indeed present in one subject and was detectable in one of the two channels analysed. The artefact was not visible in the central electrode but was clear in the signal obtained from an electrode at the front. This may be confirmation that the stimulus artefacts are better seen in the frontal electrodes which may imply that these electrodes can be used as better indicators of the presence of such an artefact. However, it should be noted that the artefact is seen in one channel but not in another, which means the successful detection of stimulus artefacts would require looking at the signals from a larger number of electrodes.

Furthermore, the artefacts (stimulus as well as EOG) are detected visually, which is a subjective method and may depend on the expertise of the experimenter. For instance, to an inexperienced experimenter it may be unclear if stimulus artefact is present, or the peaks indicating this presence may be extremely low and thus, missed by the experimenter. In such a situation, the inclusion of the subject would add undesirable data to the results, but its exclusion may lead to loss of desirable data.

The grouped data from electrodes located in the front, middle and back region of the head are in agreement with literature [22] which also showed a higher intensity of signals in the frontal region, progressively decreasing towards the back. This too may, however, simply be an indication of the presence of stimulus artefact as such artefacts may be more detectable at the front.

5.3 Factors Affecting the Output

The temporal analysis of the results suggests that a single-pulse stimulus is noticeably better for the detection of stimulus artefacts. Each single pulse in the input shows a pronounced corresponding peak in the output; the artefact was also present

for the double-pulse in the same specific subject, but not as pronounced in the graph. The reason for this is unclear and should be investigated further.

However, the amplitude of the output components is higher for the the double-pulse input. This is consistent with the behavioural measure wherein the subjects were asked about the degree of perception of the stimulus; each subject reported that the double-pulse stimulus was felt more intensely than the single-pulse one (refer to Section 4.1). Additionally, the output for double-pulse stimulus showed no jitter, as opposed to the output for the single-pulse stimulus. The frequency jitter in Stimulus 1 can be explained by delays in the implementation of the stimulus software by the stimulator. The delay period would no be constant, and would hence cause minor but noticeable differences in the stimulating frequency. The output for Stimulus did, however, show side-lobes, even in the input signal. The reason for this is unknown, but may be related to the simulation device or software.

The results also showed that the amplitude of the output was higher for subjects who perceived the stimulus over the entirety of their forearm than for the ones who felt it in a small area around the electrodes attached to their arm. This is also intuitive as it is likely caused by the accidental stimulation of a bundle of nerves or of the median nerve which is a major nerve passing through the forearm, depending on the position of the electrodes (as this position is only approximately the same for each subject).

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

The pilot study has found sufficient evidence that a signal at the stimulated frequency can be evoked in the brains of the subjects. It has found that factors such as the type of stimulus used and the placement of electrodes on the subjects arm may improve the quality of the result.

The study has shown that a single-pulse stimulus may be better for detecting stimulus artefacts while a double-pulse stimulus results in an output of higher amplitude. It was also found that the output amplitude may be higher when the electrodes are placed such that the subject perceives the stimulus over a larger area of their arm. A study comprising of a larger sample size should be conducted to confirm the influence of these factors on frequency tagging.

The study also found that stimulus artefacts and noise introduced by the background activity in the brain prove to be significant obstructions in such an experiment. Hence, objective methods for the detection and then removal of these artefacts as well as for the removal of the noise are needed.

6.2 Recommendations

The reliability of frequency tagging is greatly affected by the possible undetected presence of stimulus artefacts. The current visual detection method leaves much room for human error, so an objective and accurate method for the detection of these artefact must be devised. In any case, the chosen detection method should be employed for multiple channels in diverse regions of the head to ensure that a stimulus artefact is not missed. A possible area of investigation in this regard is cross-correlation, which has been employed in other unrelated studies for artefact detection and removal [36], [37], and could be adapted for the purpose of studies

similar to this one.

Noise comprised of background activity in the brain is also a nuisance and needs to be removed in a more effective manner. In a larger sample size, issues that could be introduced by the violation of instruction should be removed to ensure better results. For this, an appropriate set of exclusion criteria should be defined and stringently followed.

Based on the evidence found in this study, a combination of single-pulse and double-pulse stimuli can be used in such an experiment, to easily detect stimulus artefacts and to get a higher power in the output, respectively. The location of the electrodes on the subjects arm should be chosen in such a way that they are able to perceive the stimulus over their entire arm.

Apart from the stimuli used in this study, other possibilities can be explored. A novel possibility for a stimulus is a multi-tone signal, which by definition contains multiple excited frequencies within one signal. To observe the behaviour of the system at vastly different frequencies, two or more frequencies can be used simultaneously at the same point. Non/linearity of the system can also be identified using a wave such a multi-tone signal. The stimulus signal in question signal could be multi-sine or a multi-square wave. Several studies consisting of stimuli of different types, shapes and parameters will be needed to characterise the properties of the somatosensory system using the black-box model approach.

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Appendix A

Chronic Pain

Chronic pain is a phenomenon that is distinguished from acute pain [37] in its persistence for an abnormal duration. However, defining chronic pain as a syndrome based on time alone is both insufficient and inconsistent, as chronic pain may be pain lasting for less than a month to over six months, which is the most common definition [37], [38]. Other definitions stipulate the absence of physical findings (uncoupled from bodily injury) or the absence of an identifiable nociceptive process (no apparent cause). Comprehensively, chronic pain is pain that (a) persists beyond the normal healing time for an acute injury or disease, (b) related to a chronic disease or a persistent neurological condition, (c) emerges and persists for months with no conceivable cause [37], [39]. It is often described as electric-like and as evoked by even gentle touch.

The precise cause of chronic pain is somewhat unclear but is often associated with primary afferent nociceptive neuron and corresponding neurons in the dorsal ganglion (see figures) [38]. Normally, as an injury heals, the sensitisation of nerves due to central and peripheral nervous mechanism declines and the pain threshold goes back to the normal level. However, if the afferent neurons and central pathways of the sensory system itself are damaged as a complication of certain diseases, injuries or neurological conditions, then the pain persists into a chronic and difficult-to-treat condition called neuropathic pain [10]. The usual analgesic (pain-relief) medication has little effect with such a condition. The mechanism by which this central sensitisation, during which the sensory system is in a state of high reactivity, can leads to allodynia (heightened sensitivity to touch) and hyperalgesia (heightened sensitivity to pain) is shown in Figure A.1 and Figure A.2, respectively.

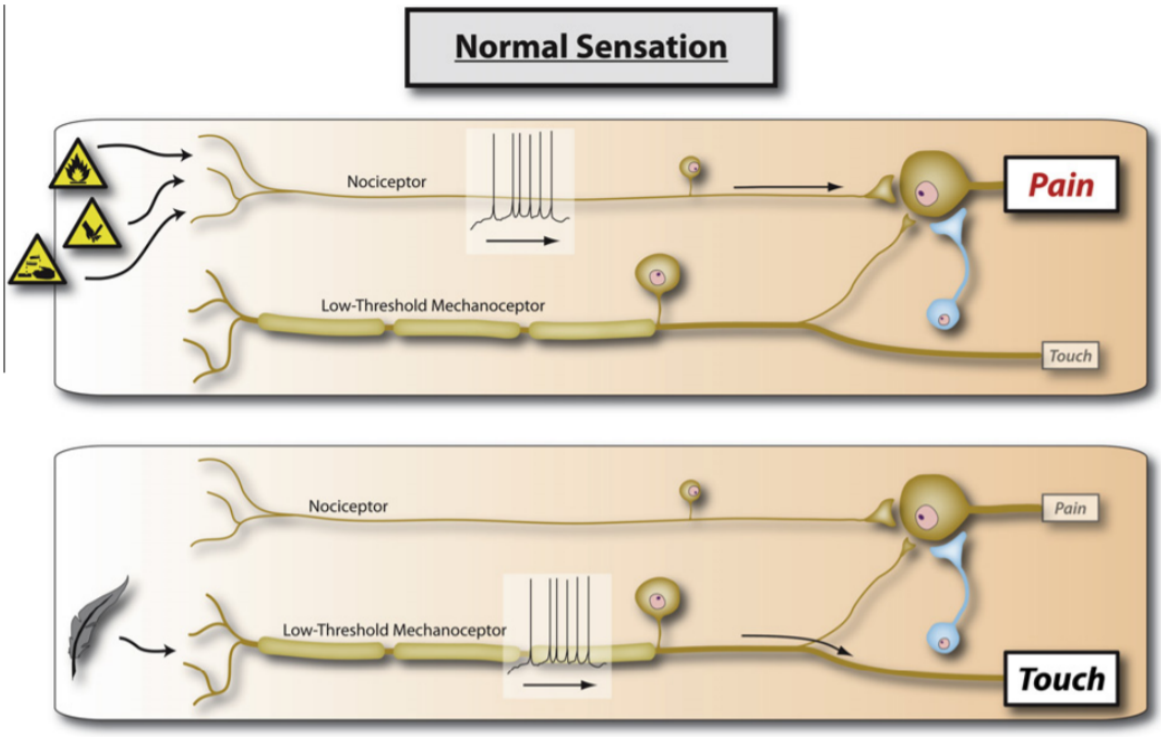


Figure A.1: Normal sensation. Reproduced from [40]

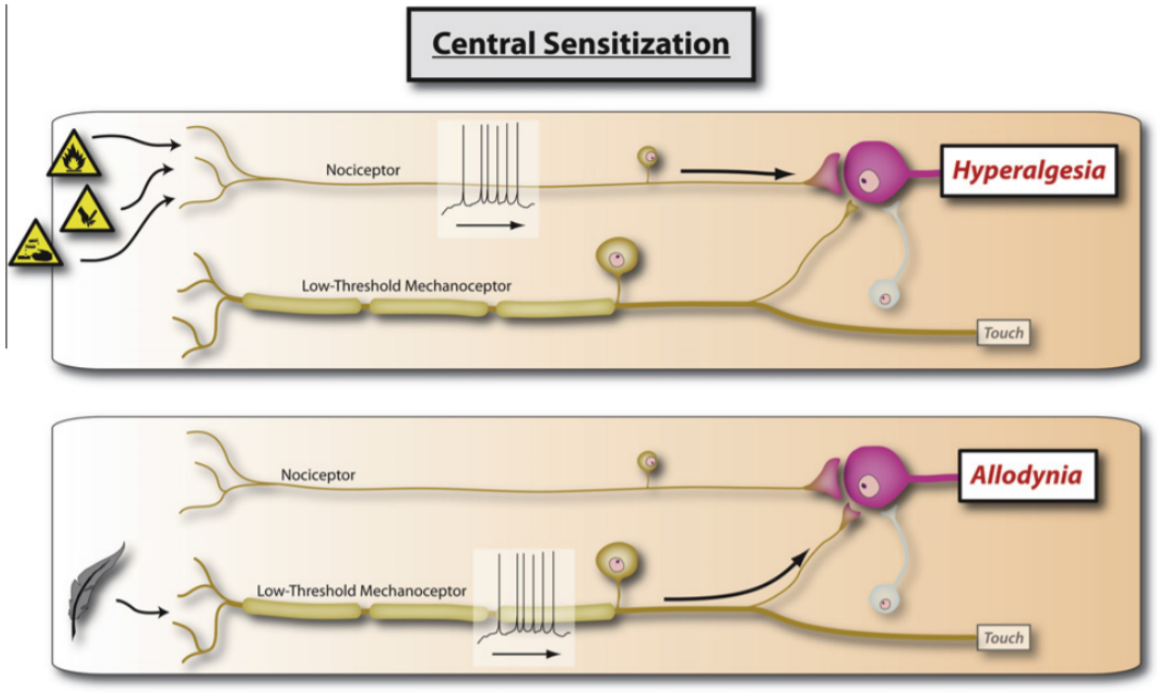


Figure A.2: Central sensitisation. Reproduced from [41]

Appendix B

Experimental Protocol

The latest version of the Experimental Protocol is given next.

University of Twente

Biomedical Signals and Systems

Research theme Nociceptive and Somatosensory Processing

Experimental Protocol

Measurement of Steady-State Evoked Potentials During Transcutaneous Electrical Stimulation: A Pilot Study

Contents

Background

Nociceptive activity can be quantified by recording and analyzing the EEG signal time-locked to the stimuli. Continuous sensory stimulation using periodic modulation of a long-lasting stream of sensory input such as a pulse train allows the continuous investigation of neural processing by eliciting SSEPs. The EEG data in response to such stimuli can be observed to determine if somatosensory processing cortical areas contain these frequencies as well, which can help with the characterisation of neural pathways [8],[17]-[20]. Therefore, it is important to accurately measure the EEG signal to exclude noise and artifacts from external and internal disturbances. The materials required for such an experiment and the step-by-step protocol is described next. The setup of the EEG measurement is illustrated in Figure A.1.

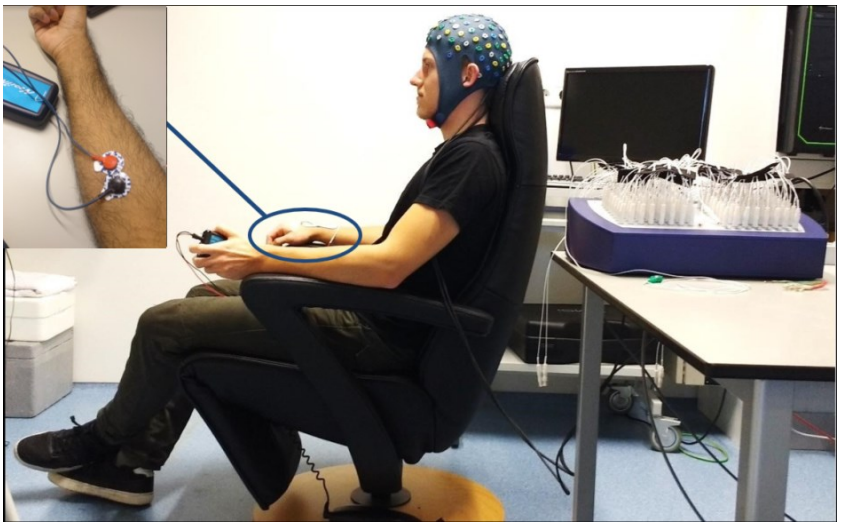


Figure A.1 Measurement setup for EEG measurement. The subject is wearing the EEG cap which is connected to a 128-chennal amplifier. The subject is holding the stimulator in his left hand, while the electrodes are connected to his right arm (upper left corner).

Required Materials

Description	#	Specification
General		
Computers	1	One computer with LabView 2014 or higher, Stimulation software (built in LabView). The computer should have at least the following specifications: - Windows 10 64-bit, or higher - Intel Core i7 3.6 GHz, or higher - 8 GB RAM, or more - Bluetooth adapter One computer with Matlab 2015b or higher, TMSi Polybench Designer, and a custom-made TMSi Polybench recording application. The computer should have at least the following specifications: - Windows 10 64-bit, or higher - Intel Core i7 3.6 GHz, or higher - 8 GB RAM, or more
Medical abrasive gel	2 cl	Medical abrasive gel to remove dead skin cells.

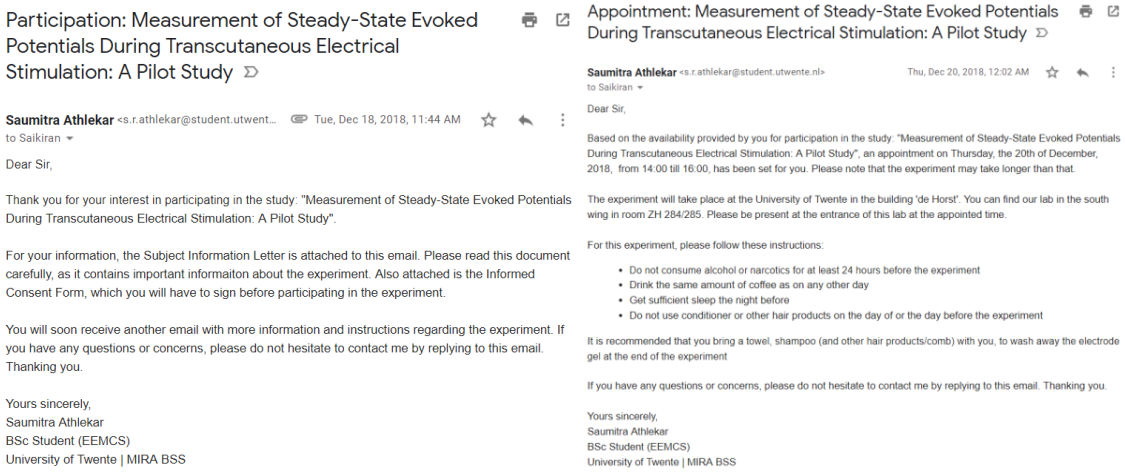
Cleansing tissues	3	Tissues (should preferable not release fibers)
Cleansing sticks	2	Cotton-top cleansing sticks, large
Familiarisation and Multiple Threshold Tracking		
Stimulator	1	NociTRACK AmbuStim single-channel stimulator, capable of generating a minimum current of 8 µA and a maximum current of 16 mA. Shown in Figure 4.
Charger	1	NociTRACK charger for AmbuStim stimulators
Trigger generator	1	Arduino-based trigger generation system, which can be connected to the computer (input) via USB A to B cable, connected to the NociTRACK AmbuStim stimulator (output 1) via a BNC cable and connected to the EEG amplifier via a DB25 parallel cable. Shown in Figure 3 D-E-F.
USB A to B cable	1	Cable for connection of the trigger generator to the computer.
BNC cable	1	Cable for connection of the trigger generator to the NociTRACK AmbuStim stimulator. Should have a length of at least 2 meters.
Parallel cable	1	Cable for connection of the trigger generator to the EEG amplifier. Should have a length of 1-2 meters.
EEG electrodes	2	EEG electrode, which will serve as cathode and ground during the stimulation. Shown in Figure 10.
Stimulator-to-electrode cable	1	A custom-made double cable that connects the stimulation and grounding electrode to the stimulator.
EEG Measurement		
Amplifier	1	TMSI Refa 136-channel amplifier, with 128 unipolar, 4 bipolar and 4 auxiliary input channels.
Medical power supply	1	TMSi power supply with on-off switch, to supply electricity to the EEG amplifier.
Fiber-to-USB converter	1	TMSi optical fiber-to-USB converter.
Optical fiber	1	TMSi optical fiber, used to communicate between the EEG amplifier and the computer via the TMSi fiber-to-USB converter.
USB A to B cable	1	USB A to B cable used to connect the TMSi fiber-to-USB converter to the computer.
EEG caps	2	TMSi 128-channel low-noise actively shielded caps with an EBA multi-connector. A small-medium or medium-large size cap have to be available for different head sizes of the subjects.
EBA multi-connectors	4	EBA multiconnectors from 1-32 Hirose to microcoax cables, to connect the headcap to the amplifier.
Color-coded measurement tape	1	Tape for measuring the required EEG cap size.
Syringes	2	10 ml Luer-Lok tip syringe (B-D Plastipak) for injection of EEG gel into cap electrodes.
Blunt needles	2	Blunt needle (16G) for injection of EEG gel into cap electrodes and scratching the skin.
EEG electrode gel	200 ml	Electro-gel (ECI), for injection in EEG cap electrodes.
Towel	2	Large size towel for covering the shoulders during application of electrode gel.
Power cable	1	Power cable for medical power supply.
Comfortable chair	1	Comfortable chair for participants to relax during the experiment. The chair should especially provide rest to the muscles around the head and neck, since those might disturb the measurement.
Focus image	1	Small image or sign for subjects to look at during the experiment. Shown in Figure A.7.

Procedure

General Preparation

Time: >1 day before experiment

1. Send the potential participants an email containing an official patient information letter. To the ones who confirm their participation, send another email with specific information about the experiment. Examples of these emails are shown in Figure A.2 The second email should inform the participants about:
    - The date, time and location of the experiment
    - Appointment location and contact information
    - Bringing a towel, and shampoo and other hair products to the experiment
    - Abstaining from alcohol for at least 24 hours before the experiment
    - Drinking the same amount of coffee that they would normally drink on any other day
2. If participants with a different first language are expected, make sure to have a suitable translations of the information letter and consent form are available.
3. After dates and times for the experiments have been planned, inform the BHV (Marcel Weusthoff, in this case) about the decided dates and times.
4. Charge the electric chair regularly. However, do not leave the charger connected for more than a few hours, as that might damage the battery of the chair.
5. Make sure that the NociTrack stimulator is also kept charged regularly. Connector the charging cable to the stimulator first, and then to the power supply, as the charger measures the charging level of the battery and determines how much more charging is required.
6. Make sure to be aware of the personnel and phone numbers to be contacted in case of emergencies.



Participation: Measurement of Steady-State Evoked Potentials During Transcutaneous Electrical Stimulation: A Pilot Study

Saumitra Athlekar <s.r.athlekar@student.utwent... Tue, Dec 18, 2018, 11:44 AM
to Saikiran

Dear Sir,

Thank you for your interest in participating in the study: "Measurement of Steady-State Evoked Potentials During Transcutaneous Electrical Stimulation: A Pilot Study".

For your information, the Subject Information Letter is attached to this email. Please read this document carefully, as it contains important informaiton about the experiment. Also attached is the Informed Consent Form, which you will have to sign before participating in the experiment.

You will soon receive another email with more information and instructions regarding the experiment. If you have any questions or concerns, please do not hesitate to contact me by replying to this email. Thanking you.

Yours sincerely,
Saumitra Athlekar
BSc Student (EEMCS)
University of Twente | MIRA BSS

Appointment: Measurement of Steady-State Evoked Potentials During Transcutaneous Electrical Stimulation: A Pilot Study

Saumitra Athlekar <s.r.athlekar@student.utwente.nl> Thu, Dec 20, 2018, 12:02 AM
to Saikiran

Dear Sir,

Based on the availability provided by you for participation in the study: "Measurement of Steady-State Evoked Potentials During Transcutaneous Electrical Stimulation: A Pilot Study", an appointment on Thursday, the 20th of December, 2018, from 14:00 till 16:00, has been set for you. Please note that the experiment may take longer than that.

The experiment will take place at the University of Twente in the building 'de Horst'. You can find our lab in the south wing in room ZH 284/285. Please be present at the entrance of this lab at the appointed time.

For this experiment, please follow these instructions:

- Do not consume alcohol or narcotics for at least 24 hours before the experiment
- Drink the same amount of coffee as on any other day
- Get sufficient sleep the night before
- Do not use conditioner or other hair products on the day of or the day before the experiment

It is recommended that you bring a towel, shampoo (and other hair products/comb) with you, to wash away the electrode gel at the end of the experiment

If you have any questions or concerns, please do not hesitate to contact me by replying to this email. Thanking you.

Yours sincerely,
Saumitra Athlekar
BSc Student (EEMCS)
University of Twente | MIRA BSS

Figure A.2 Example emails sent to subjects

Apparatus Preparation

Time: >0.5 hours before experiment

EEG System:

1. Make sure all the individual inputs of the EEG amplifier are connected and have not been removed. Check that the ground cable is connected too.
2. Attach an image on the wall in front of the chair, as a single point for the subjects to focus on during the experiment (such as the one shown in Figure A.7).

Stimulator:

1. Connect the trigger generator to the stimulator using a BNC cable.
2. Connect the output of the stimulator to the stimulator-to-electrode cable.
3. Do not turn on the stimulator beforehand, so as to conserve battery power.

Materials Preparation

Time: >0.25 hours before experiment

Make sure that the following materials are ready for use and easy to reach:

1. Electrode caps
2. Coloured measurement tape
3. EEG electrode gel and paste
4. Two syringes
5. Blunt needles (unopened, in the package) for gel injection into the electrodes
6. Medical abrasive gel
7. Tissues and cotton sticks
8. ECG electrodes for the stimulator cathode and ground and the ground of the EEG device.

System Startup

1. Turn on the computer.
2. Turn on the EEG amplifier.
3. Open the programme ‘Impedance-Shortcut” which will show the high impedances of the EEG cap electrodes at this stage (red), as seen in Figure A.3.

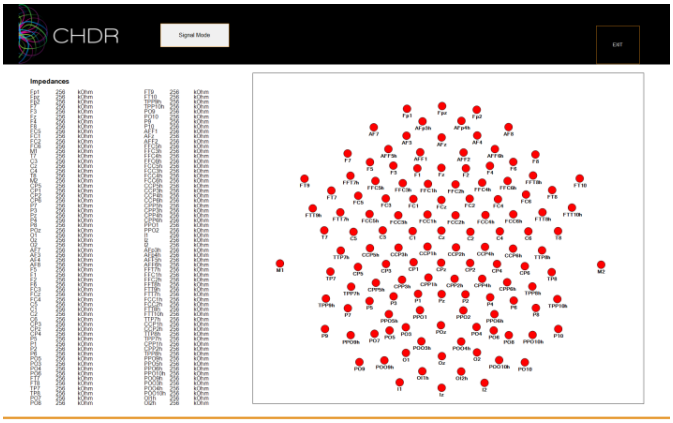


Figure A.3 “Impedance-Shortcut” window showing the impedance of all 128 electrodes on the EEG cap

Subject Reception and Preparation

Time: Start of the session

1. Be present at the lab entrance ten minutes before the session to receive the subject and lead them into the lab.
2. Give the subject a hard copy of the information letter (of which they have received and read a soft-copy in advance, preferably) and the consent form.
3. Ask the subject:
    - **"Have you read the information carefully?"**
    - **"Do you have any questions?"**
4. Instruct the subject to fill in and sign the consent form if they would like to participate in the experiment.
5. Explain to the subject that the experiment will now begin and will take approximately two hours. Instruct the subject:
    - **"Make use of the toilet if you need to, as the session will last for an uninterrupted two hours."**
    - **"Please turn off your phone or put in on Airplane Mode."**

      IMPORTANT: Turn your own phone on airplane mode as well.
6. Seat the subject in the designated chair and instruct them:
    - **"Please sit in a comfortable position. The seat can be adjusted using the buttons on the side."**
    - **"Make sure there is enough space for your legs."**
    - **"Incline the chair backwards to relieve tension in the neck muscles."**
7. Use the coloured measuring tape to determine which size cap is to be used.
8. Place the appropriate electrode cap on the subject's head, pulling it back from the forehead.
9. Instruct the subject:
    - **"Please fasten the Velcro strap under your chin as tightly as comfortably possible."**
    - **"Please use the tightening string given on the side to ensure a better fit."**
10. Measure the distance between the nasion and the inion, and the distance between the two post-auricular points.
11. Check if the Cz electrode is exactly at the centre of the measured distances and adjust the cap if required.
12. Attach the EBA multi-connectors of the EEG cap to the inputs of the EEG amplifier. The connectors are numbered from 1 to 4; connect them such that number 1 is connected the first set of inputs, number 2 and 3 to the next two in that order, and number four to last set, to ensure an accurate special representation of the electrodes of the cap on the computer screen.
13. Use a medical abrasive gel and cotton stick to clean the skin of the forehead area for attaching the ground electrode.
14. Use a dry cotton stick and tissue to wipe off and dry the area and attach the ground electrode.
15. Explain the next part to the subject:
    - **"Gel will now be injected into the electrodes on your head using a blunt needle."**
    - **The needle will be used to scratch your skin and move hair out of the way, for better connectivity. This should not hurt; in case it does hurt, please say so."**
    - **"If you feel any discomfort at any point, you can indicate this immediately.**

16. Take a new needle from the packet and show this to the subject. Attach the needle to the syringe and fill the syringe with gel.
17. Fill each electrode in the EEG cap with gel, by first scratching the skin and moving hair using a large circular movement. Make sure to extract the needle slowly while injecting the gel to avoid gel from squeezing out from the sides and causing cross-connections between electrodes
18. Use the impedance display on the screen (see Figure A.3) to ensure that all impedances are below 5kΩ (yellow, or better, green). Use the back of a narrow cotton stick in a rotational movement inside the electrodes to facilitate better contact, if required.
19. Click on "Signal Mode" (see Figure A.3); the signal window will open (Figure A.4.a. Ask the subject to blink and/or roll their eyes. If the signal is being measured correctly, corresponding variations should be seen on the screen.
20. Explain the next part to the subject:
    - **"Two sticky electrodes will now be connected to you forearm. One is the cathode and the other is the ground."**
21. Attach the electrodes and ask the subjects the press them firmly onto with the skin.
22. Attach the stimulator-to-electrode cables to the two electrodes.
23. Open the programme "FrontPanel-Shortcut", press run first and then enter the subject number and measurement number. The stimulus settings can be selected with the "Settings Path". Pressing continue after this will open the stimulator connection window shown in Figure A.4.b.

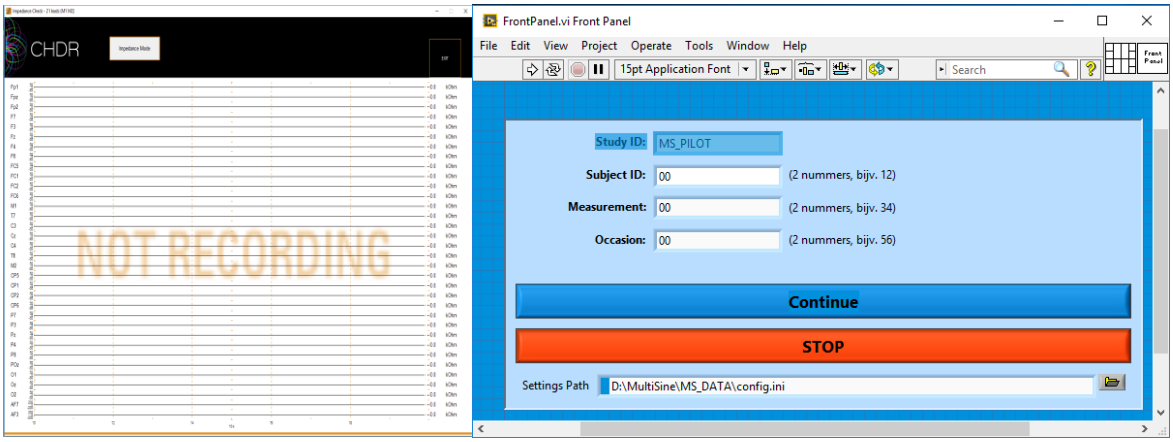


Figure A.4 (a) "Signal Mode" screen to check the signal to see if the EEG cap is properly connected, (b) "FronPanel-Shortcut" window for subject and measurement number input and stimulus setting selection

Familiarisation

Time: ~40 minutes after start of session

1. Explain to the subject:
    - **"For each stage of the experiment, I will press 'start' to begin the process. However, a stimulus will be sent to your arm only when you press the red response button."**
    - **"You must keep the button pressed to keep the experiment going. You can release the button any time if you want to pause or stop the experiment."**

2. Hand the stimulator over to the subject and ask them to hold it in the hand that is opposite to the one being stimulated.
3. Explain the familiarisation procedure to the subject:
    - **“The first part of the experiment is to get you acquainted with the stimulus signal, so that you know what to expect during the actual experiment.”**
    - **“Press and hold the response button until you feel the stimulus clearly. You can continue for as long as you want to; the longer the better, but you can stop if you experience pain. The stimulus will stop automatically if the current amplitude reaches 1mA.”**
4. Turn on the stimulator and click on the ‘Search’ button in the stimulator connection window on the computer (Figure A.5.a). Choose the correct stimulator from the list of Bluetooth devices found (The stimulators have numbers assigned to and written on them).

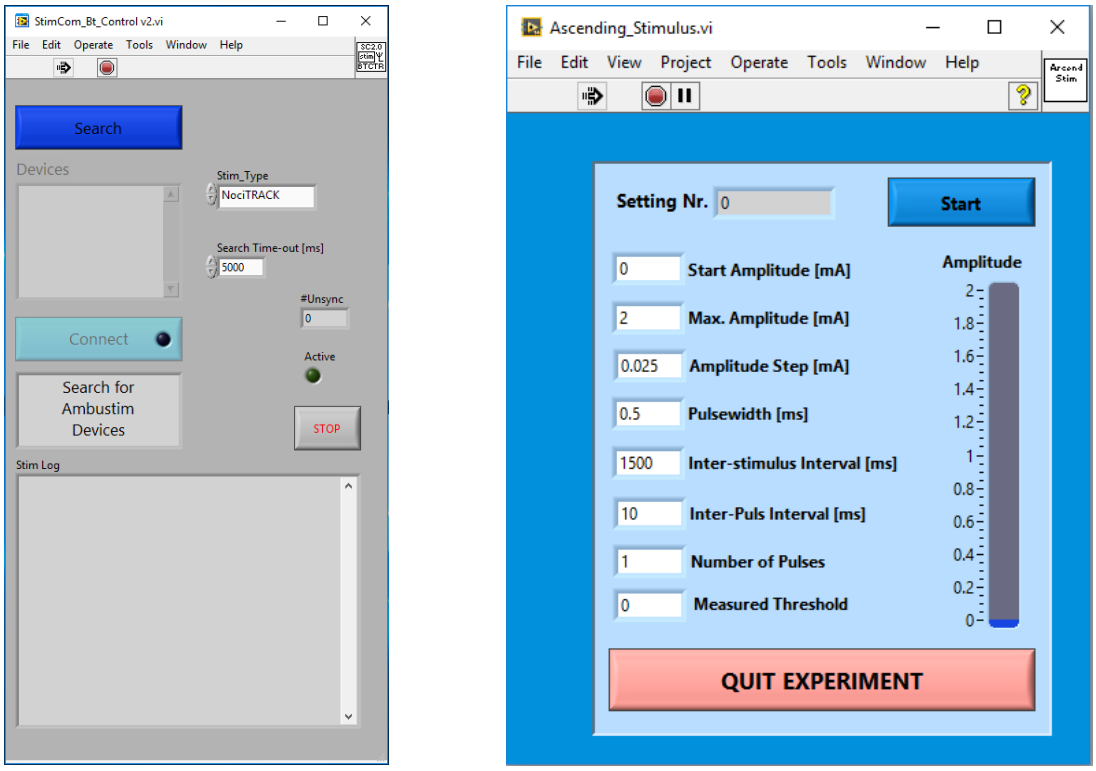


Figure A.5 (a) Window for searching and connecting to the stimulator via Bluetooth, (b) Window for starting the familiarisation or initial threshold detection processes

5. Press ‘Connect’ to connect the stimulator to the computer via Bluetooth. The impedance display will be opened once again, for a check. Upon exiting this window, an interface that is used for familiarisation and initial threshold detection will be opened (Figure A.5.b).
6. When the subject is ready, press ‘Start’ in the stimulation interface window and ask the subject to begin by pressing the response button.
7. Once the subject is familiarised and releases the button, the next stage, i.e., the threshold measurement can begin.

Threshold Measurement

1. Explain the threshold measurement procedure to the subject:
    - **“The next part of the experiment will serve to determine the initial detection threshold. Press and hold the response button until you feel the stimulus.”**
    - **“You must release the button immediately at the first instance that you feel a sensation that you would ascribe to the stimulus.”**
    - **“Upon pressing the button, keep your attention focused on the detection of the stimulus. This will lead to a more accurate determination of the threshold.”**
2. When the subject is ready, ask the subject to begin the measurement by pressing the response button (it is not required to press ‘Start’ again).
3. Once the subject releases the button, confirm that they released the button at the first instant that they felt the stimulus.
4. If required (for instance, in case of uncertainty), this measurement can be repeated by simply pressing ‘Start’ again in the interface window.
5. Once the threshold measurement is successful, press ‘Okay’ to continue. The EEG recording window (Figure A.6.a) and the control interface (Figure A.6.b) for the multiple threshold tracking will be opened, and the final stage of the experiment can begin.

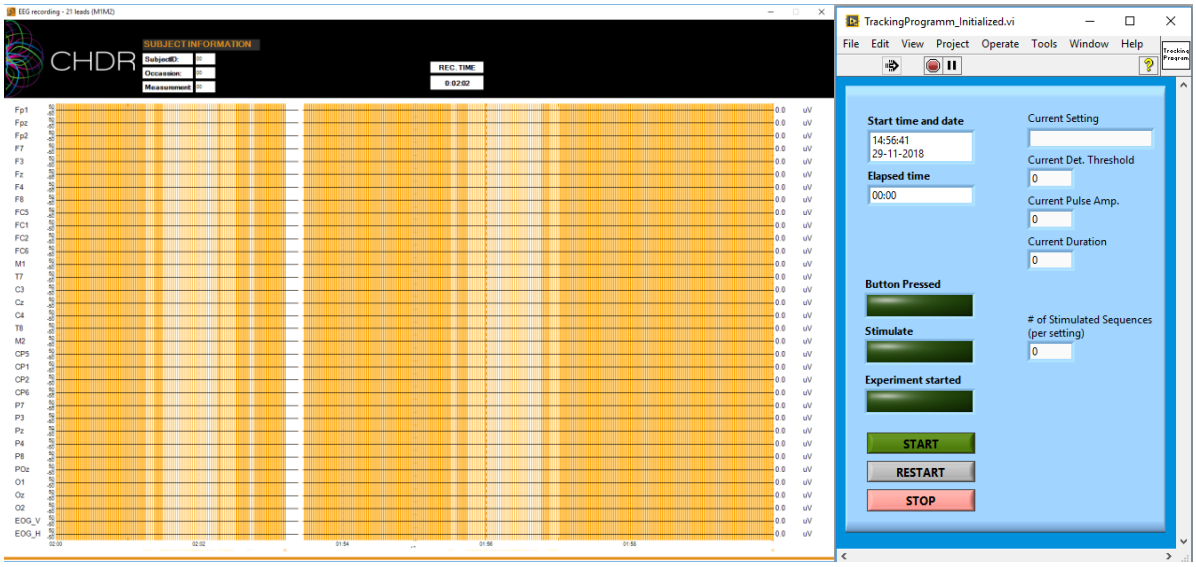


Figure A.6 (a) EEG recording window that shows the signal being measured, the orange background colour appears while the signal is being recorded (b) Control interface to start, restart or stop the experiment, data recording begins when the “Start” button is pressed

Experiment

Time: ~50 minutes after start of session

1. Explain the experimental procedure to the subject:
    - **“To receive the stimulus, you must press the response button and keep it pressed until I ask you to release it.”**
    - **“In case you need a short break or want to talk (ask questions), you can release the button; to continue the experiment, you can re-press the button.”**

2. Instruct the subject:
    - **“Please keeping your gaze fixed on a single point, i.e., on the image in front you while pressing the button. Do not move your eyes, as far as possible.”**
    - **“Try to blink as few times as possible while pressing the button.”**
    - **“Try to relax; also relax your muscles, especially the arm and neck.”**
    - **“Do not talk or move while pressing the button. In case you have/need to, release the button, and wait for the ongoing stimulus to stop.”**
    - **“Following these instructions will greatly enhance the quality of the signals measured, and thereby of the result.”**
3. Press ‘Start’ in the interface window to begin. When the subject is ready, tell them to press the button. The programme records EEG automatically when the response button is pressed.
4. Press ‘Stop’ to stop the experiment and close all the windows involved in the experiment. A ‘Restart’ button is also available in case repeating only this stage of the experiment is needed.
5. This process has to be repeated for each block. After one block is done, the familiarisation step, the threshold measurement and the experiment steps can be repeated. The stimulus can be changed between blocks by using the “Settings Path” option in the window in Figure A.4.

Closure

1. Inform the subject that the experiment was completed successfully.
2. Turn off the stimulator and disconnect the subject from all the cables.
3. Instruct the subject to take off the EEG cap and hand it over to you.
4. Inform the subject:
    - **“You can wash your hair at the sink. A shampoo is available in case you did not bring one with you.**
    - **“There are also showers downstairs where you can take a full shower if you want to (ZH 109, go down the stairs near the red couches).”**
5. Present the subject with the gift that is intended to be a token of appreciation for their participation in the experiment.
6. When the subject is ready to leave, tell the subject:
    - **“Thank you participating in this experiment”.**
    - **“Would you like to be informed of the results of the experiment?”**
    - **“If you have any questions, please do not hesitate to contact me.”**
7. Provide the subject with contact information and lead them out of the lab.

Clean-Up

1. Turn off the software and the EEG amplifier.
2. Clean the cap electrodes directly after the experiment using hot water on spray mode.
3. Hang the cap on the provided fan to dry.
4. Put all the equipment in the proper places.

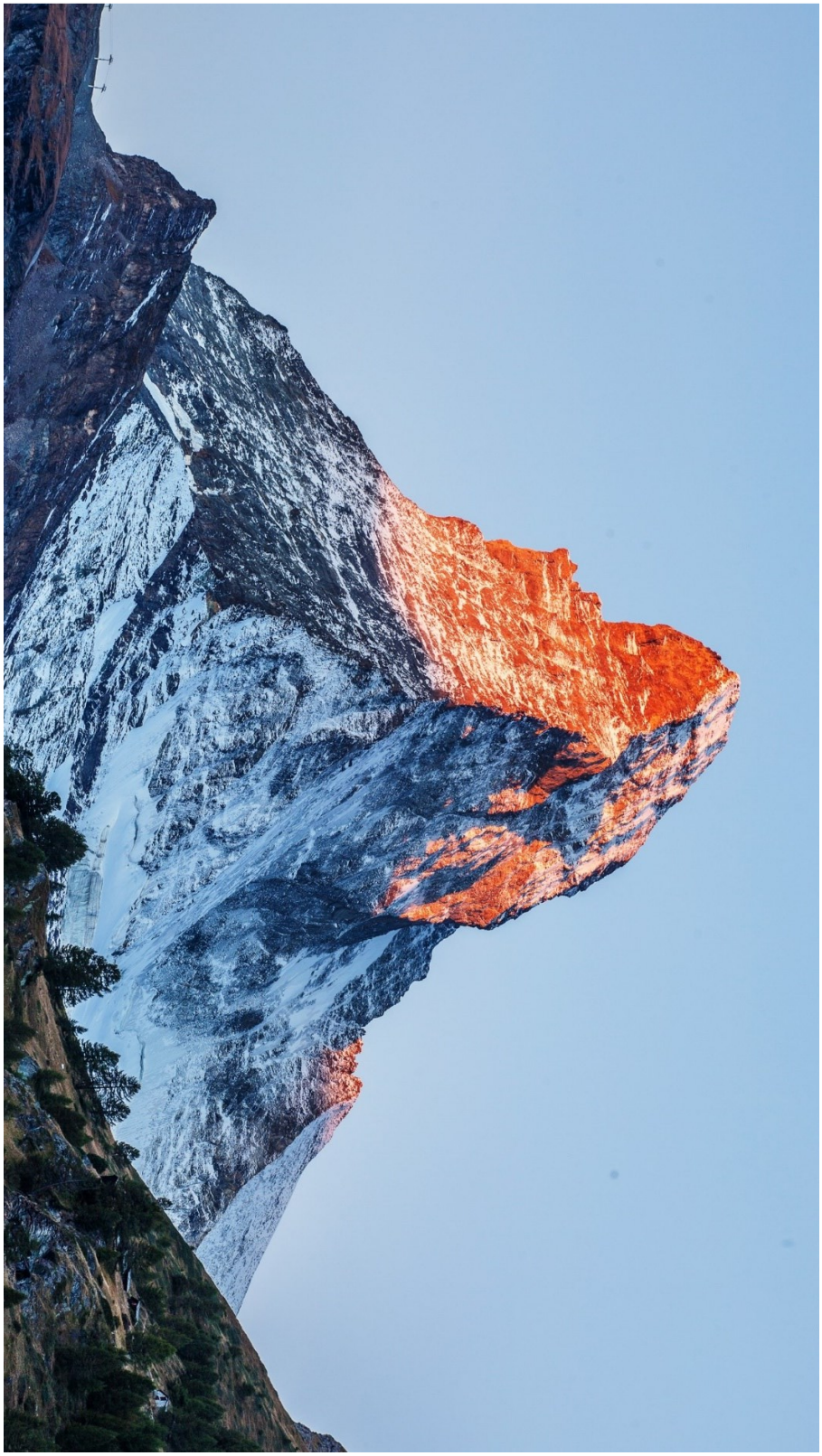


Figure A.7 Figure attached to the wall in front of the subject for focusing gaze

Appendix C

Behavioural Measures

C.1 Questions

Q.1. How would you describe the sensation that you felt during the stimulation?

Q.2. How would you rate the sensation on the following scale:

a. neutral

b. slightly uncomfortable

c.. very uncomfortable

d painful

Q.3. Did you perceive the stimulus-

a. in the area around the electrodes

b. all over your forearm