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COLD PRESSOR INDUCED PAIN MODULATION BY THE ACTIVATION OF DIFFUSE NOXIOUS INHIBITORY CONTROL FOR VALIDATION OF DIAGNOSTIC TOOLS FOR EARLY DETECTION OF CHRONIC PAIN

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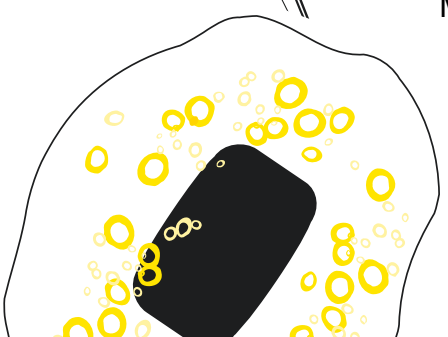

UNIVERSITY OF TWENTE.

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Abstract

Central sensitization is crucial in the development and persistence of chronic pain, which reveals itself as increased sensitivity to nociceptive stimuli. Hence, observation of nociceptive mechanisms may provide insight into this condition and permit early interventions.

This study presents a set up of a pilot experiment for investigation of the effect of diffuse noxious inhibitory control (DNIC) on nociceptive detection threshold (NDT) and evoked potentials (EP). In the experiment, a cold pressor test (CPT) was used as a conditioning stimulus to activate DNIC. Using Multiple Threshold Tracking (MTT) and Intra-epidermal Electrocutaneous Stimulation (IES), the detection threshold of participants was measured. Furthermore, corresponding cortical activity was recorded using electroencephalography. The technical feasibility of the experiment was assessed for two water temperatures, 1°C and 6°C .

As a result, both experiments were found to be technically feasible. For both temperatures, the nociceptive detection thresholds considerably increased during the application of CPT and decreased after its termination independent from stimulus setting. The amplitude of the threshold was considerably higher for single pulse stimuli.

Application of the CPT also reduced the amplitudes of EP components. The stimulus setting did not have a noticeable effect on the cortical response, whereas the participant response showed the highest impact on the EPs. In conclusion, both water temperatures were found to be a suitable conditioning stimulus for use in further researches.

List of Acronyms

BLA Basolateral Amygdala

BSS Biomedical Signals and Systems

CeA Central Nucleus of the Amygdala

CNS Central Nervous System

CPM Conditioned Pain Modulation

CPT Cold Pressor Test

CT Computerized Tomography

DNIC Diffuse Noxious Inhibitory control

DRt Dorsal Reticular nucleus

EEG Electroencephalography

EP Evoked Potentials

ERP Event Related Potentials

fMRI functional MRI

IASP International Association for Study of Pain

IES Intra-epidermal Electrocutaneous Stimulation

LA Lateral Amygdala

LC Locus Coeruleus

MEG Magnetoencephalography

MRI Magnetic Resonance Imaging

MTT Multiple Threshold Tracking

NDT Nociceptive Detection Threshold

NoP Number of Pulses

PAG Periaqueductal Gray Region

RVM Rostral Ventromedial Medulla

SRP Stimulus-Response Pairs

STT Spinothalamic tract

VAS Visual Analogue Scale

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1 Introduction

Chronic pain is a long-term pain that has lasted for more than six months, which can be initiated by an injury and prolong after the time that healing was expected to have occurred. In other cases, when pain occurs for no apparent reasons and seems to serve no useful purpose, it is classified as chronic pain. It is usually thought to be a result of the disturbed processes in the central nervous system of the body. [1]

According to the studies by Harald Breivik et al.(2006) [2] and Bekkering et al. (2011) [3] 1 in 5 adults suffers from chronic pain in Europe, equating up to 95 millions of adult population. Furthermore, 54% of these patients report not being able to function normally. It is also estimated that an average chronic pain patient has a threefold increase in medical consumption [4] , [5]. This proves that chronic pain is not only a physical and mental burden for the patients, but also it is a severe social and economic burden for the society that requires an effective solution.

Central sensitization plays an essential role during the development and persistence of this debilitating condition, which reveals itself as increased sensitivity to nociceptive stimuli. As a response to disease, injury or other medical condition maladaptive changes can be induced in several ascending and descending pathways where pain perception is modulated. These changes may lead to an imbalance, which can cause the aforementioned increased pain sensitivity [6]. Clinical observation of nociceptive pathways is limited at present. However, it could give a better insight into the development of chronic pain, provide better understanding and permit early selections of interventions of this condition.

In 2018, at the department of Biomedical Signals and Systems (BSS) of University of Twente, a novel method of Multiple Threshold Tracking (MTT) in combination with Evoked Potentials (EP) had been developed by B. van den Berg. With this method, the properties of nociceptive processing can be observed by analyzing the objective neurophysiological responses to nociceptive-specific electrocutaneus stimulation. This is done by providing a subject with temporarily defined stimuli with a varying number of pulses and inter-pulse interval. Further processing of the Stimulus-Response Pairs (SRP) and Electroencephalography (EEG) data into estimating Nociceptive Detection Threshold (NDT) and EP in the brain may provide information about the properties of the nociceptive system of the subject [7].

One important step in the validation of the MTT-EP method is the assessment of observability of the changes in the nociceptive functions, which are hypothesized to play a crucial role in the development and maintenance of chronic pain. This can be achieved by analyzing the neurophysiological responses corresponding to a Conditioned Pain Modulation (CPM), which would induce a well-characterized centrally mediated change in the nociceptive system. One of the popular CPM methods extensively in a lot of scientific researches is the Cold Pressor Test (CPT), which is conducted by immersing an extremity (hand or foot)

into ice-cold water. Other research groups have demonstrated that CPT can activate central mechanisms such as Diffuse Noxious Inhibitory control (DNIC) which can be observed by a lower sensitivity to nociceptive stimuli after CPT [8][9]. There are also other studies showing that activation of DNIC also can modulate EPs obtained from electrocutaneus stimulation[10][11][12].

Taking into consideration that CPT has been used consistently and extensively in other studies as a pain model for the activation of DNIC, it can be seen as a promising technique for validation of MTT-EP method. However, one problem that can be faced during the combination of CPT with MTT-EP could be the fact that DNIC is a short-lived mechanism. The effect is maximal during the application of conditioning stimulus and returns to the baseline within approximately 5 minutes after the cessation [9]. Therefore, it is considered more reliable to perform the measurements in parallel with conditioning stimulus. Another problem is that it is still unknown is whether time available during the application of the CPT will be sufficient for gathering sufficient amount of MTT-EP data.

Therefore, it is of importance to assess the technical feasibility of implementing CPT in combination with MTT-EP before using it as a validation tool for this method. Hence, this paper aims to perform a technical pilot in order to reach the following objectives:

- **Primary Objective:** To assess technical compatibility of CPT with the MTT-EP method.
- **Secondary Objective:** To evaluate the effect of the CPT on the Nociceptive Detection Threshold (NDT) and the Evoked Potentials (EP).

The remaining of this paper is organized as follows. Chapter 2 gives the reader a brief background on nociception, MTT-EP method and Cold Pressor Test. Subsequently, the experimental and analysis methods are discussed in Chapter 3, followed by the results and discussion in chapter 4. Finally, Chapter 5 gives recommendations for further research and concludes the paper.

2 | Background

2.1 Pain Perception and Nociception

2.1.1 Pain Perception

Pain is a subjective experience, a percept that has been defined by the International Association for Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage"[13]. It is a sub-modality of somatic sensations with urgent and primitive quality which mainly serves as a protective function. One of the most general classifications of pain is the division into persistent and chronic pain.

The chronic pain seems to serve no useful purpose and only makes the patients suffer, whereas persistent pain can characterize many clinical conditions and warn of injury that should be avoided or treated. The persistent pain is further subdivided into neuropathic and nociceptive pains. Neuropathic pain often has a burning and electric sensation that results from direct injury to nerves in the central or peripheral nervous system. Nociceptive pain results from the direct stimulation of nociceptors, pain-sensing nerve cells that are activated by noxious insults to peripheral tissues[7] [14]. The more detailed discussion of this process and nociceptive pathways will follow further in this chapter.

2.1.2 Nociceptive Pathways

Nociception is a subsystem of the somatosensory system that is involved in pain perception. Under normal conditions, nociception starts when pain stimuli are detected by nociceptors - specialized high threshold, sensory, bare nerve endings located in the skin and other tissue. Nociceptors signal the Central Nervous System (CNS) about the occurrence of stimuli that threaten or actually produce tissue damage. These nerve afferents are categorized as C-fibers causing slow, poorly localized pain and as $A\delta$-fibers causing fast, sharp and well-localized pain sensations. C-fibers are unmyelinated polymodal fibers that have a diameter less than $2\mu m$ and conduction velocity below $1.0ms^{-1}$, whereas $A\delta$ -fibers are thinly myelinated with a diameter around $3\mu m$ that are conducting at a velocity ranging from 5.0 to $30.0ms^{-1}$. Nociceptors are mainly located on the superficial layers of dermis and epidermis. Besides nociceptors, there are also mechanoreceptors contained in the deeper layers of the skin. Mechanoreceptors innervated by $A\beta$ fibers are of particular interest because even though they do not directly respond to noxious stimuli, they contribute to the perception of the stimulus quality. The activity in the large diameter $A\beta$ fibers can not only modify but can

also attenuate the pain perception [14].

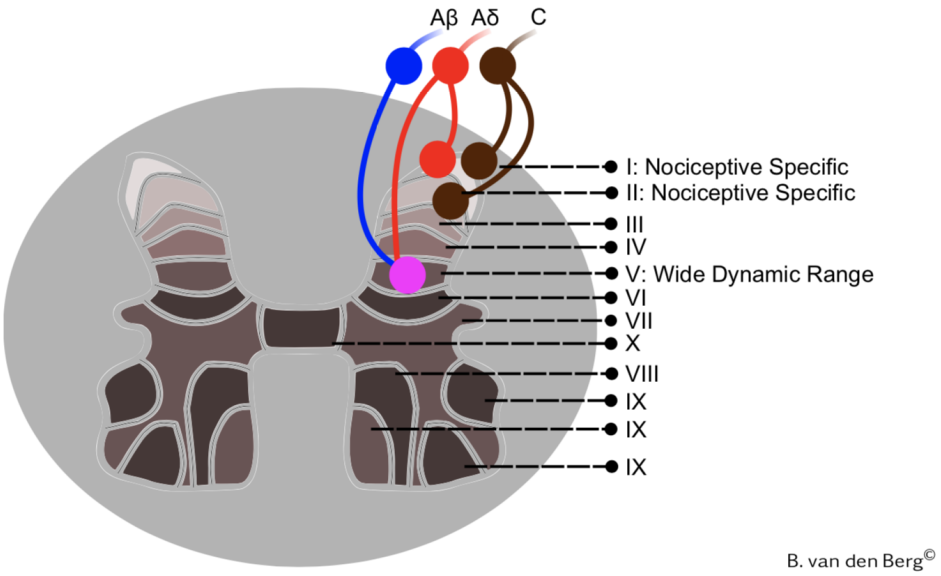


Figure 2.1: Sensory receptors terminating in the Raxed Laminae dorsal horn of the spinal cord. [7]

Once nociceptors get activated by noxious stimuli, they propagate the information about pain through the sensory fibers at the dorsal horn of the spinal cord, to the so-called Raxed laminae. The figure 2.1 shows where exactly in Raxed laminae each type of afferent nerve fiber tends to terminate. $A\delta$-fibers terminate in laminae I and V, whereas C-fibers mainly terminate in laminae I and II. $A\beta$-fibers, on the other hand are terminating in laminae III to VI. The nociceptors terminating in lamina V are believed to connect to wide dynamic range (WDR) neurons, activated by both $A\delta$ and $A\beta$- fibers.

In the Raxed Laminae, nociceptors would then activate second-order neurons (also referred to as transmission neurons or the projection fibers) by releasing chemical pain neurotransmitters, such as substance p or glutamate. Even though signals from nociceptors are conducted to the higher centers in the spinal cord via several pathways, the majority of these second-order neurons transmit their action potentials via Spinothalamic tract (STT) to the thalamus. In the thalamus, the information is passed from second order neuron to a third order neuron which transmits the signal further to part of somatosensory cortex corresponding to the location of the noxious stimulus (e.g. injury). Other major ascending pathways involved in nociception are Spinorecticular and Spinosencephalic tracts. The more detailed overview of these three pathways can be found in figure 2.2 [7][14].

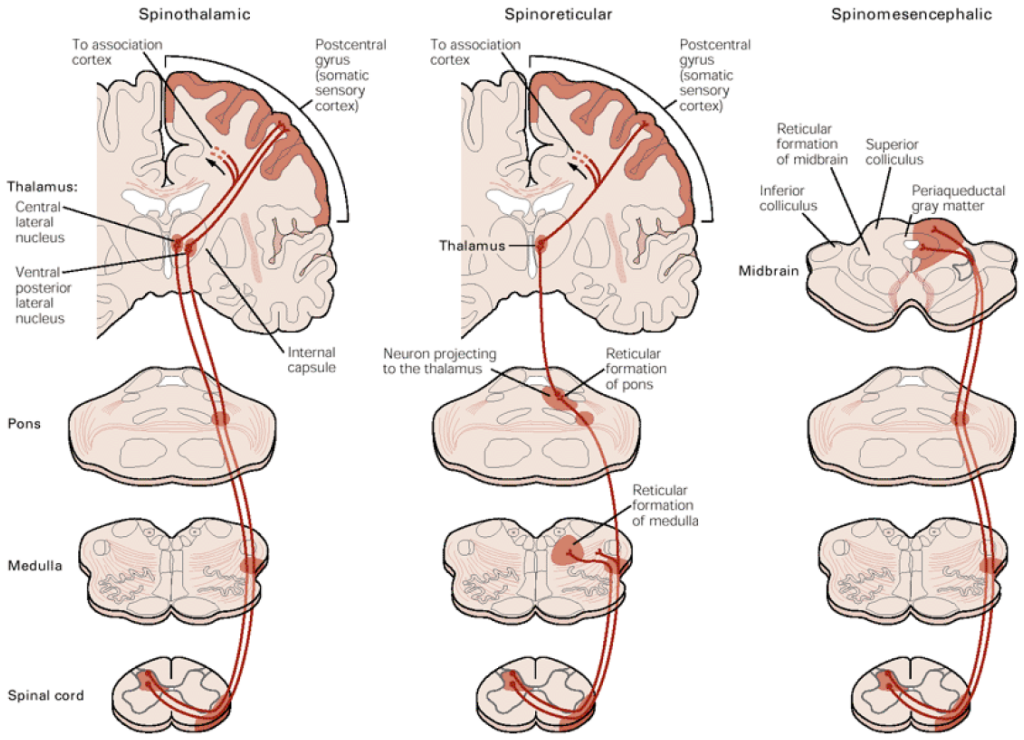


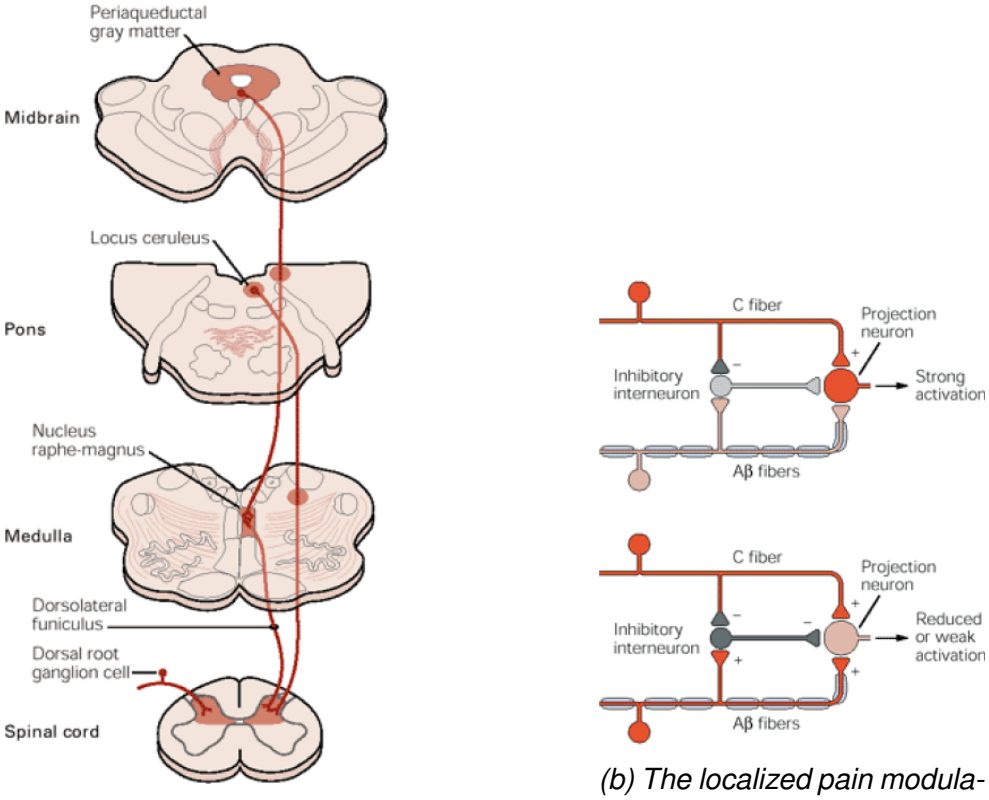
Figure 2.2: Three major ascending pathways that transmit nociceptive information from spinal cord to higher centers. Spinothalamic tract is the most prominent (Image source: Principles of Neural Science, 2000, Figure 24-8 p. 482 [14])

2.1.3 Pain Control System

One of the most remarkable findings of pain research is the modulatory circuits of the brain whose primary purpose is to regulate pain perception. There are several modulatory systems (descending pathways) within the CNS that inhibit or excite the brain response to noxious stimuli by controlling the input signal to the Raxed Laminae. The working principle of this pain modulatory circuitry can be explained using the figures 2.3. As it can be seen from the picture on figure 2.3a, the descending pathway arises in the Periaqueductal Gray Region (PAG) of midbrain and projects to the serotonergic nuclei, such as raphe magnus and others. Then through the dorsolateral funiculus, it projects further to the dorsal horn of the spinal cord. The PAG also inputs the signal to the nucleus paragigantocellularis and the noradrenergic cell groups in pons and medulla, where additional spinal projections arise. In the dorsal horn of the spinal cord, these pathways inhibit the nociceptive projection neurons through direct connections and interneurons (see figure 2.3b). [14]

Diffuse Noxious Inhibitory Control

The WDR neurons and the trigeminal nuclei caudalis and oralis that are primarily located in the dorsal horn, specifically in superficial and deeper layers of lamina V, get the input from primary nociceptive and non-nociceptive neurons. These interneurons are involved in transmitting information to ascending pathways and to polysynaptic reflexes. The WDR neurons can be inhibited by applying noxious conditioning stimuli to a different area of the body. This supraspinal pain inhibition mechanism is known as diffuse noxious inhibitory control (DNIC).



(a) Descending pathways

(b) The localized pain modulation

Figure 2.3: Pain modulation circuitry (Image source: Principles of Neural Science, 2000, Figure 24-10 and Figure 24-11 p. 486 [14])

The concept was first formulated from the observation of the response of the spinal dorsal horn units to peripheral stimuli applied to various body parts or electrical stimulation of peripheral nerves in anaesthetized rats [15][16]. The peripheral noxious stimuli could suppress the neuronal responses of convergent dorsal horn units to both electrical stimulations of C-fibers and to the application of noxious heat. The working principle of DNIC is often described as "pain inhibits pain" counter-irritation. Application of conditioning stimulation causes the activation of the descending inhibitory mechanisms described in figure 2.3 and decreases pain response to the second noxious stimuli. DNIC can be evoked by application of stimuli to various parts of the body, and, therefore is diffuse in nature. [15][16] It has been suggested that DNIC functions as a filter, allowing the system to focus on a painful stimulus in a background of basic somesthetic activity and amplification of that signal in the transmission system.[9][17]

There are different approaches and numerous kind of experimental settings to evoke DNIC effect in humans in laboratory conditions. The topical review by Pud et al. (2009) summarizes the commonly used experimental methods for activating DNIC effect in young, healthy subjects. One of the thermal modality that is frequently employed as a conditioning stimulus is noxious heat, by using a contact thermode or hot water bath. Injection of hypertonic saline into the anterior tibialis muscle is a more rarely used conditioning stimulation type utilized in experimental conditions. The most commonly implemented paradigm to activate DNIC effect is the application of a noxious conditioning cold stimulus, known as the cold pressor test (CPT). This test is carried out by immersing extremity into ice-cold water [8].

In this study, CPT is implemented as a tool to evoke the DNIC mechanism. A more detailed

review of CPT and its methods will follow in chapter 2.3. As it comes to the test-pain stimulation, there is a wide range of different experimental pain modalities in use, such as thermal, electrical, mechanical and chemical noxious simulation. The electrical noxious stimulus is used for this research (See section 2.2.4).

It has also been found that the DNIC effect fades gradually along with the time (5-8 minutes) after the application of conditioning stimulus. Therefore, the simultaneous application of test-pain and conditioning stimuli leads to higher DNIC magnitude than sequential paradigms [8][9].

2.2 Multiple Threshold Tracking and Evoked Potentials

2.2.1 EP: Measurement of Nociceptive Cortical Activity

There are various methods to record brain activity such as Computerized Tomography (CT), Magnetic Resonance Imaging (MRI), functional MRI (fMRI), Magnetoencephalography (MEG) etc. However, all mentioned methods require heavy and specialized equipment and, therefore, are beyond the scope of this research. Another widely used method for monitoring cortical activity is known as Electroencephalography (EEG). It involves placing a large set of non-invasive electrodes along the scalp and measuring the current corresponding to fluctuations caused by synaptic connections in deeper layers of the cortex. The main advantage of EEG is its high temporal resolution, which allows changes to be followed in the millisecond range. This property and its simplicity make EEG suitable for cortical activity observation, despite the important limitation of low spatial resolution. Usually, in EEG research, so-called evoked potentials (EP) are of particular interest. EP are parts of EEG signals, that are evoked by some event, such as stimulus, activity or thought. These are transient responses of the cortex to a stimulus measured by phase-locked activity after stimulus processing [18]. EPs are generally analyzed in terms of components which are classified based on the polarity (P for positive; N for negative) and the order of the appearance (1,2,3 etc.) of the maximum amplitudes. Based on the literature, essential components of nociceptive EPs are N1, N2 and P2, since those components are thought to represent sensory stimuli processing in the brain. The early negative components N1 and N2, which are believed to originate from the activity in the somatosensory cortex, are best measured in the lateral part of the scalp. N1 is thought to represent early sensory processing because it is the first component activated in EEG that emerges after stimulation. N2 component, on the other hand, was shown to modulate perception of the stimulus with respect to attention. Another significant component, P2 originates from anterior cingulate cortex and can be measured at central locations of the head [19] [20]. It is often related arousal and attention[21] [22] [23]. All of these peaks are not specific to nociceptive stimuli because they are related to both sensory and nociceptive stimuli. For this reason, these are a rather modulated reflection of the signals received from nociceptors[7]. For the example of EP, please see figure 2.4.

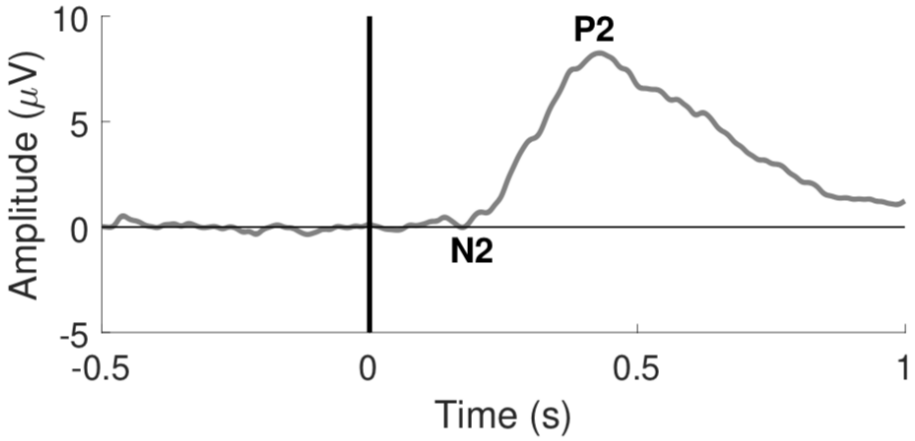


Figure 2.4: Evoked potential at the CPz-M1M2 derivation (with a band-pass filter of 0.1 Hz to 40.0 Hz) in response to a nociceptive stimulus, averaged over 12 subjects [7].

The main difficulty in the analysis of EPs is signal-to-noise ratio far below one. Therefore, statistical methods such as Time-locked Averaging, Linear Regression, Linear Mixed Models, etc. are required in order to extract significant information from the EEG data. This paper will not treat these statistical analysis methods further because it would deviate from the main objectives of this study. If interested in a more detailed discussion of relevant statistical techniques, the reader is advised to refer to the paper of B. van den Berg [7].

Undesirable components in EEG signal and their elimination

As mentioned above, EEG suffers from certain drawbacks, such as low SNR and variety of noise sources affecting it. The typical range of scalp potentials is between $20-100\mu V$, which means the signal of interest is likely to be buried in noise and interfering electrical activity. Averaging the signal recorded over repeated occasions is the simplest and one of the most effective ways of improving the reliability of the results. However, this method relies on the assumption that the noise in the data is random and symmetric in relation to the interesting events. Other types of noise can be reduced by using the methods discussed further in this chapter.

The most common biological artefacts that can be observed in EEG signals are EMG (muscle activity) and EOG (eye blink, eye movement) activities. It is possible to significantly reduce those artefacts by properly setting up the experimental conditions. For example, eye movement can be reduced by providing the subject with a focus image, and EMG activity can be significantly reduced by making sure that the subject is comfortable and relaxed during the measurement. However, this would not help to avoid the interference altogether, because some components such as eye blinks cannot be prevented. These artefacts are usually visually recognizable since the amplitude of potentials is much higher than that of EEG. Hence, they can be removed by removing the source signal in which such artefacts are observed. Another method of accurately removing nuisance signals is by using independent component analysis (ICA) which uses various measures of statistical independence to separate the measured signal into a number of source signals [24].

The noise caused by skin potential artefact, depending on the conductance of skin can

be reduced by using an abrasive cream, removing dead skin cells and scratching the skin area before placing the electrodes. High pass filter can be implemented to remove noise caused by sweating and drifts in electrode impedance; a cut-off frequency of 0.01Hz is recommended [24].

There is also a number of non-biological components that can affect the EEG signal. The most problematic type of artefact is stimulus artefacts, which are time-locked to the stimulus. These are caused by electromagnetic coupling between the stimulator and recording electronic devices. The presence of these components can be misleading because the cortical response may be evoked due to the stimulus, or it may be a mixture of the response and stimulus artefact. Unfortunately, the only way of making sure that the measured data represents only the cortical stimulus-response, is preventing such artefact from the beginning. This can be done by testing the equipment beforehand by comparing the recorded EEG signal with the stimulus inputs. Cross-correlation can also be a method of checking the similarity between two signals. If stimulus artefacts are present, glitches (voltage spikes) present in the stimulus can be removed by adapting and improving the internal electronics of the stimulator.

Other potential noise sources that can be present in EEG data are the high-frequency white noise, high-frequency noise from the amplifier, or a 50 Hz noise caused by the coupling with AC power lines in the experiment room. Specific frequency noise, such as the AC power line signal can be avoided by grounding all the items in the experimental room that may cause coupling or can be removed using a notch filter[24].

Rejecting trials or channels with a high incidence of noise can be another method of increasing the reliability of EEG data. This can be done by observing abnormal trends and outliers in the variances over all trials and channels and excluding ones with too much noise. The main disadvantage of that method is that often some desirable information is lost during this process[24].

2.2.2 MTT: Psychophysical measurement of nociception

Psychophysics is a study dealing with quantitative relations between physical stimuli and mental phenomena. Psychophysical methods are extensively applied to different perceptual systems for determining essential properties of those (for example, perception threshold) [25]. Modern applications of psychophysics rely on adaptive paradigms often statistically optimized to converge to the true value. The stimulus-response behaviour of nociception can also be measured using this type of approach. For example, a paradigm based on the method of limits was developed by Doll et al. (2015) [26] for continuous tracking of a non-stationary psychophysical threshold. In the original method of limits, each consecutive stimulus is increased (ascending trials) or decreased (descending trials) until the subject can perceive the stimulus. In contrast, in the method introduced by Doll et al. the stimulus is randomized at every step. This is an effective way to prevent identification of the method by the subject and minimize bias in the data. See figure 2.5,

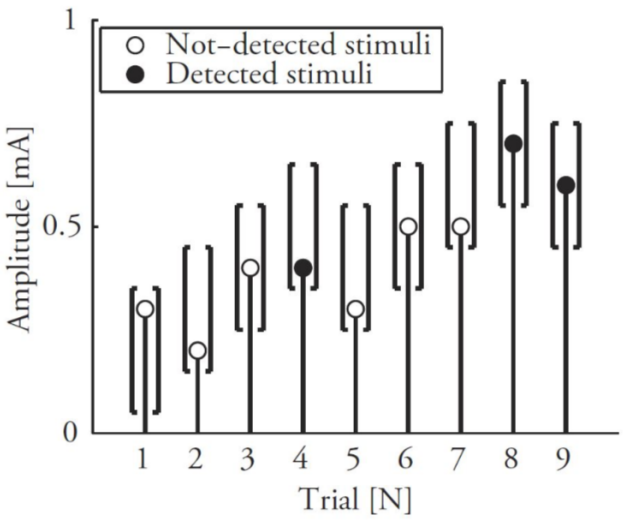


Figure 2.5: The method of threshold tracking based on method of limits from Doll et al. The expected value of the randomized stimulus is increased/decreased during every step based on the response. (Image source: Doll et al. (2015) [26])

In further research, Doll et al. (2016) also showed that this new method could be used to track multiple psychometric thresholds. This can be done by applying the threshold tracking paradigm to different types of stimuli, with random variation in order. The main benefit of this method, which will further be referred as Multiple Threshold Tracking (MTT) method, is that it is possible in test multiple types of stimuli, such as stimuli with different pulse numbers within a single experimental session (Figure 2.6). This is particularly useful property, since it was proven by R.J. Doll (2016) that variation in temporal properties of an intra-epiderma electrical stimuli result in variation in nociceptive processing which opens up new perspectives in nociception research [27]. For more theoretical and technical information about MTT please refer to [28].

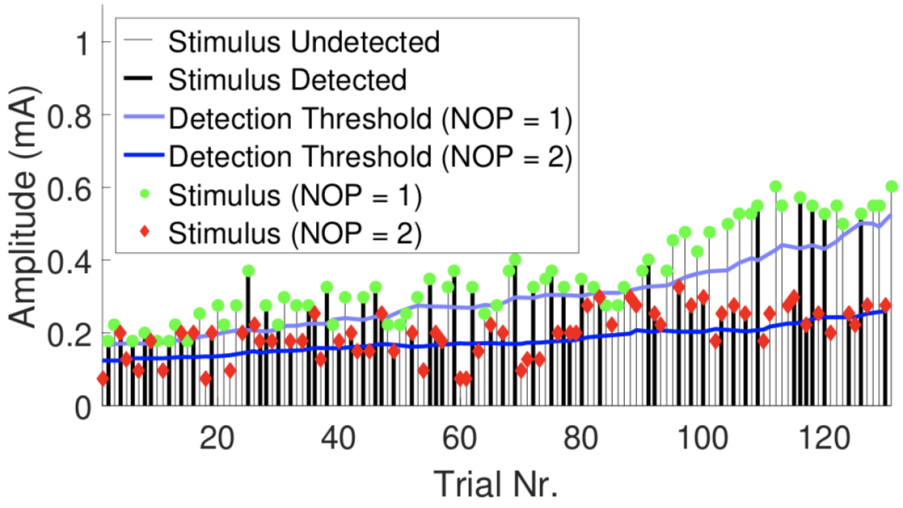


Figure 2.6: The MTT measurement method applies multiple types of stimuli in a randomized sequence. By measuring the responses of subjects a psychophysical threshold can be determined by generalized linear regression. For more information, please refer to Doll et al.(2016) [28])

2.2.3 Intra-epidermal electrocutaneous stimulation

A fundamental method to investigate the pain mechanisms is through stimulation of nociceptive afferent nerve fibers. There is a wide variety of methods available, including mechanical (e.g. pinprick), thermal (e.g. CPT), electrical (e.g. IES) and chemical (e.g. capsaicin) methods. It is important that the method is safe, reproducible and quantifiable. Furthermore, in order to study nociception without interference from other systems, a method to specifically stimulate superficial afferent fibers. Intra-epidermal electrocutaneous stimulation (IES) is a good example of pain stimulation that fulfils all of these requirements. It acts as a point current and selectively activates the most superficial nociceptors without producing any skin damage. When used at low intensity this method can be implemented for activating specifically $A\delta$ fibers [29][7]. This nociceptive stimulation method was used in this research.

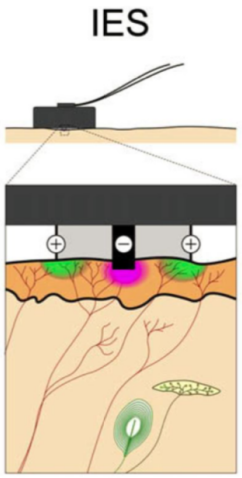


Figure 2.7: Schematic representation of intra-epidermal needle electrode for nociceptive stimulation. IES acts as a point current source, and is suitable to exclusively stimulate the most superficial nociceptive afferents. At low intesity this method can be for activating specifically $A\delta$ fibers [29] [7].

2.2.4 MTT-EP

In the research by B. van den Berg (2018), a novel method was developed by effectively combining MTT with EP. In this research, it was proven that MTT, which is shown to be an effective tool for measuring the effect of stimulus parameters of nociceptive detection, in combination with EP creates a new way for observing the properties of nociception. This study used the MTT-EP setup adapted from the one developed by M. Schooneman (2015) [30]. For this setup, instrumentation of an EEG setup was linked with the instrumentation and protocols of the MTT setup available at BSS group of University of Twente, which used the procedure developed by Doll et al. [28]. NociTrack Ambustim stimulator was used to generate square current pulses for cathodal intra-epidermal stimulation with Intra-epidermal Electrocutaneous Stimulation (IES) electrode displayed on figure 2.7. This electrode was used for its ability to selectively activate the nociceptive $A\delta$ fibres based on the fact that these fibres are found in the superficial portion of the skin[29].

The overview of the setup can be found in figure 2.8. The experimental protocol of MTT-EP

developed by B. van den Berg has been used a basis for setting up the protocol for this technical pilot [7].

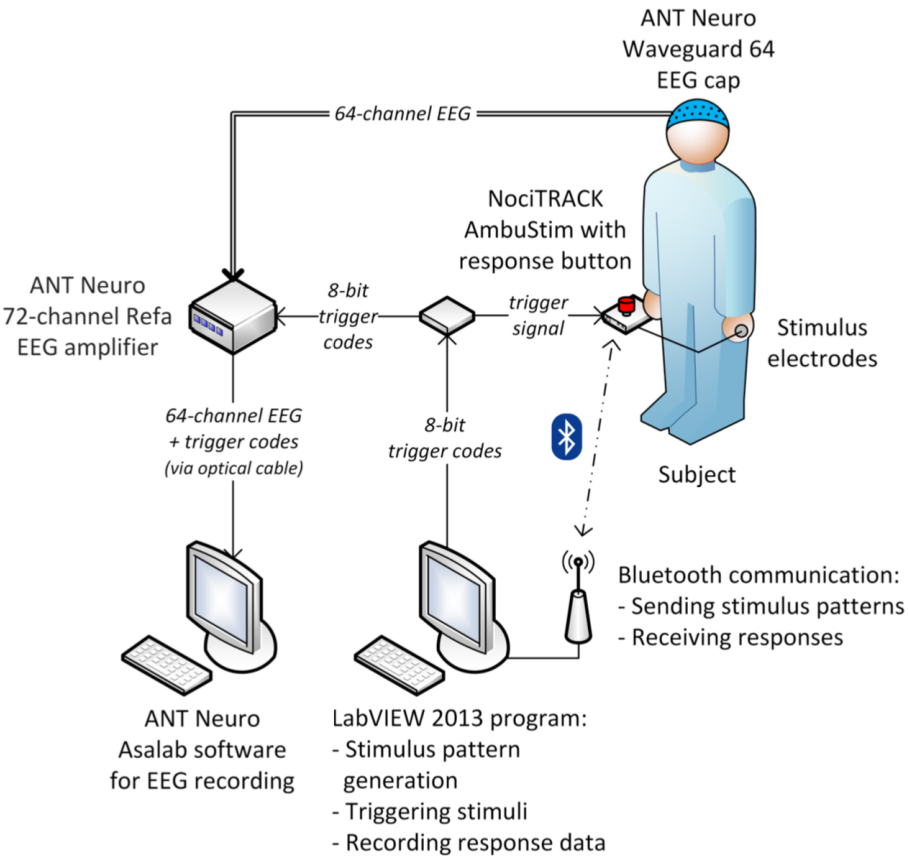


Figure 2.8: The MTT-EP setup. Adapted from Schooneman by B. van den Berg [30] [7].

2.3 Cold Pressor Test

As already mentioned earlier in the paper, CPT is conducted by immersion of hand or foot into the cold water. It is among the most commonly used laboratory stressors, used extensively in the fields of cardiovascular and pain researches. For this paper, CPT is going to be used as a tool for activation of the DNIC mechanism, that has been discussed in chapter 2.1.3. Even though the CPT is widely used in different research fields, it is not consistent in its method. There are many variations between different studies in the methodologies, equipment and parameters of the test. Consequently, this creates difficulties in comparing the results of these researches. In the paper by Mitchell et al. (2004), the main objective of which was to standardise the CPT methods, several important aspects that affect the results have been described [31]. The research concluded that even temperature variations of 2 $^{\circ}C$ can result in significantly different pain perception for both men and women. At 1 $^{\circ}C$ water bath, the Visual Analogue Scale (VAS) score was significantly higher than at 3-7 $^{\circ}C$ (See figure 2.9). This suggests that 1 $^{\circ}C$ is more effective to activate the DNIC effect.

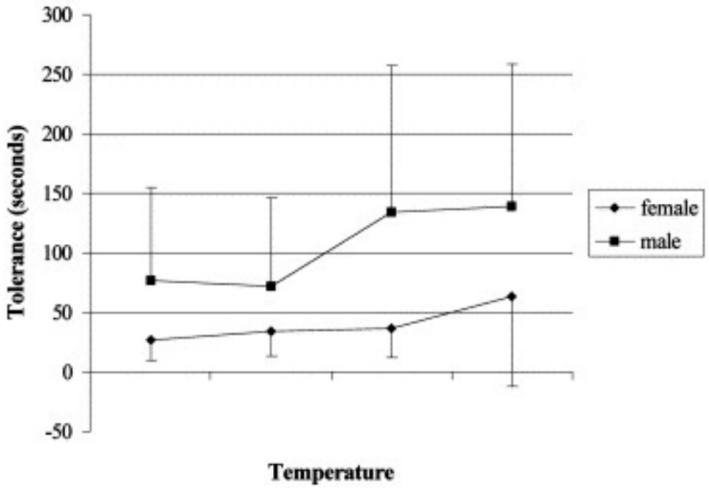


Figure 2.9: Mean VAS ratings of men and women in 1, 3, 5 and 7 $^{\circ}C$ CPT [31]

However, figure 2.10 shows that there is a trade-off between the intensity of the conditioning stimulus (e.g. temperature) and the tolerance time, which is another critical parameter for this study. As mentioned in section 2.1.3, magnitude of the DNIC effect is strongest during the application of the conditioning stimuli, and, therefore is best to be applied simultaneously with test-pain measurements. For this case, this means the MTT-EP measurements should be conducted in parallel with CPT. The main difficulty in combining these methods is that it is still unknown whether the tolerance time of participants will be sufficient to gather enough data for reliable results. Using a circulating water bath is another widely used method to avoid heat building up near the extremity. Studies which used circulated water generally have a lower average tolerance range (28.65-119.75 sec) than those using stationary water (37.11-190.3 sec)[31]. Hence, circulating water is important to make sure that the intensity of the stimulation is constant throughout the testing.

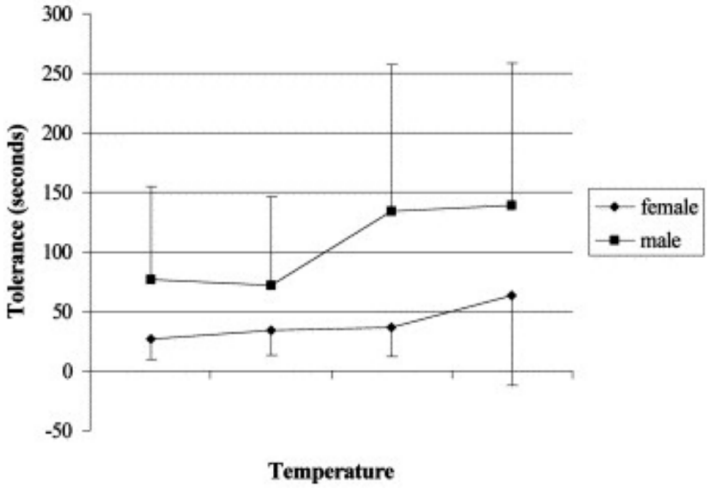


Figure 2.10: Mean tolerance times of men and women in 1, 3, 5 and 7 $^{\circ}C$ CPT [31]

2.3.1 Reproducibility and Test-Retest stability

In a study by Fasano et al. (1996), the reproducibility of the Cold Pressor Test was investigated. The researchers worked with two groups of healthy participants. The first group had a test and two times a retest on the same day, whereas the second group had the same procedures on three consecutive days. Heart rate and blood pressure of the participants were measured and compared to each other, to see whether the values were similar during the retests. The difference in results was observed for both groups between test and retest, but group 2 showed slightly more difference. Another result was that the effect of the CPT was higher during the first step [32]. This suggests that for a maximal effect, CPT sessions should have sufficient time between each other.

Another study by Koenig et al.(2013) had assessed 2-week test-retest stability of CPT with two different temperatures (4 °C and 6 °C), and as a measure of pain threshold and tolerance [33]. As it can be seen the results on figure 2.11 demonstrate excellent 2-week test-retest reliability for measures of pain threshold and pain tolerance using the CPT as pain stimulus in healthy subjects [33]. Hence, in this research, at least two-week time interval will be used between the experimental sessions.

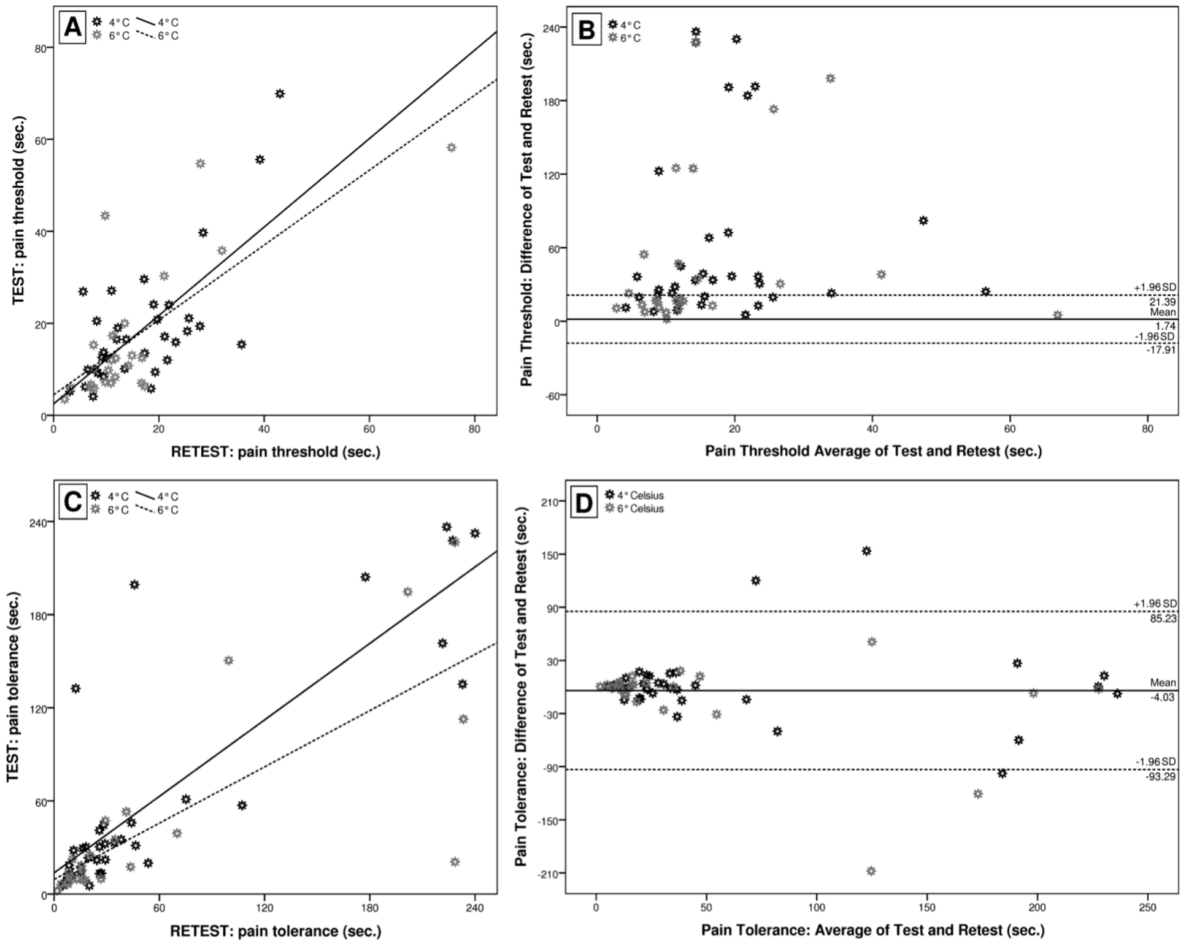


Figure 2.11: Visual inspection of test–retest stability by Koenig et al. [33].

2.3.2 Effect of cold pressor test on EP

The effect of CPT on the neurophysiological response has already been evaluated in other studies. For example, in the research by Torta et al. (2015) CPT was used as a conditioning stimulus to activate DNIC. [12] In this study different types of test stimuli were used to activate specific sensory receptors ($A\delta$, C, $A\beta$ -fibers). EPs were measured on central channels of EEG, before, during and after the application of the conditioning stimulus. For $A\delta$-fibers at a water temperature of 5°C, they obtained the results displayed in figure 2.12 . The magnitude of N2 and P2 components were reduced during CPT in comparison with before and after [12].

Another study from Höffken et al. (2017) which used 10 °C water as a conditioning stimulus and recorded brain response over central electrode (Cz) had observed similar trend (see figure 2.13) [11]. Note that the result displayed on the figure is for one participant, and, hence, it is not known to what extent this result can be considered to be a statistically accurate representation of nociceptive cortical activity with respect to CPT.

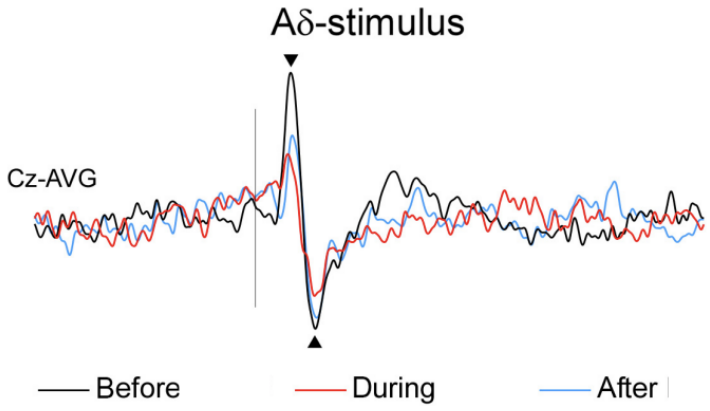


Figure 2.12: Evoked potentials (N2-P2). Grand-average waveforms of the vertex EPs elicited by $A\delta$ stimuli. [12].

2.3.3 Effect of cold pressor test on NDT

The effect of CPT on NDT has also been investigated by R. J. Doll (2016), using two different types (single and double pulse) of intra-epidermal stimulation as test-pain stimuli [28]. 14 healthy subjects performed psychophysical yes-no detection task. Five minutes after the start of the experiment, the cold pressor was applied for two minutes. Thresholds for both stimulus types showed an increase over time during CPT and decreased after its termination. Even though the trends in thresholds were similar, the values were significantly higher for single pulse stimuli. Moreover, a prolonged elevation was present in both stimulus type after termination of CPT. The author suggests that this can either be explained by the fact that CPT effect is sustained for 2-3 minutes after application, or by the limitation of multiple threshold tracking methods, more specifically due to the length of tracking window[28].

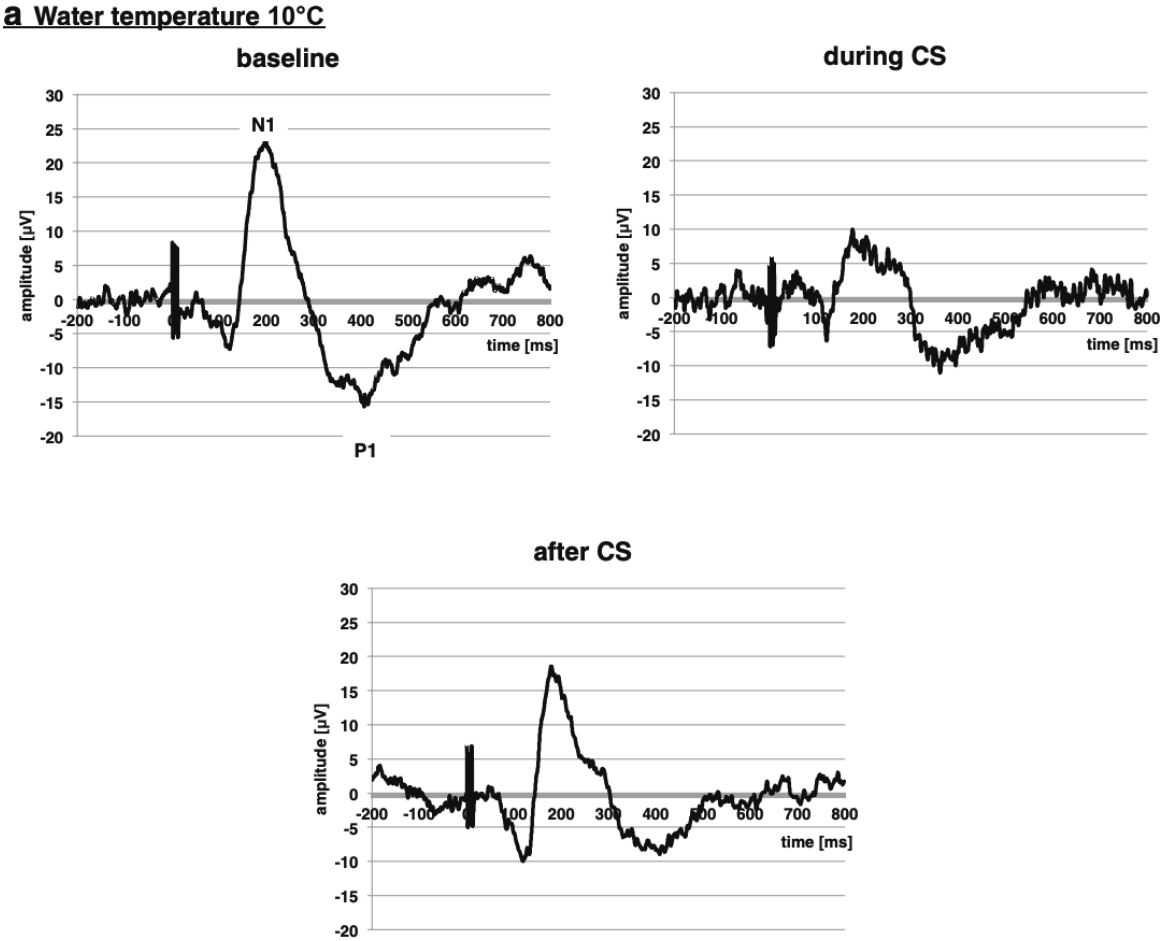


Figure 2.13: Evoked potentials of one participant before, during and after application of conditioning stimuli [11].

3 Method

3.1 Participants

Young, healthy participants (N = 6) were recruited for the experiment at the University of Twente, the Netherlands. All 6 participants were males, even though the gender of the participant was not a factor in the inclusion criteria. The mean age of the subjects was 23.3, with a standard deviation of 2.25. The participants were contacted via email and given information about the research and the experiment. After accepting the request to participate in the research, they were given further instructions. Participants were instructed to avoid consumption of alcohol and narcotics, and over-consumption of coffee for 24 hours prior to the experiment. They were also recommended to get a sufficient amount of sleep and good breakfast and/or lunch before the experiment. The participants were asked to fill in the questionnaire before the session to provide the general information about their age, gender, body weight, substance consumption as well as the history of neurological or chronic pain disorders, or other significant health issues. See A.3 for the template of the questionnaire. In the case of chronic pain history or other significant health issues, the participant would have been excluded from the research (See inclusion criteria in Section A.2). Moreover, a written informed consent form was obtained from every participant. At the end of the experimental session, subjects were given financial compensation for participation in the research.

Every participant participated in 2 experimental sessions, where the difference was in the water temperature of the CPT. On the first appointment CPT of 1 °C bath was conducted, whereas on the second session 6 °C bath was implemented.

3.2 Study Parameters

Primary parameters

- **Nociceptive Detection Threshold** [mA] was measured using the MTT method, by measuring the response (detected or undetected) to two types of stimuli (single and double-pulse) with varying amplitudes.
- **Evoked Potentials** was measured using EEG. Electric signals reflecting the neuro-physiological activity related to the stimulus were extracted at a fixed interval around every stimulus. (0.5 seconds before and 1 second after each stimulus).
- **Immersion time** - Time that extremity was kept in the cold water bath was recorded.

- **Water temperature** - Two water temperature were tested during this study, 1 °C and 6 °C. The stability of 1 °C bath was ensured by having sufficient amount of ice in the water. The stability of 6 °C bath was controlled by measuring the temperature directly before and after immersion using a mercury thermometer.

Secondary parameters

Secondary parameters were obtained using the participant questionnaire in the appendix A.3:

- **Age, sex** - Age and sex are factors that can influence the NDT and the tolerance time. This information was obtained through a questionnaire.
- **Present medication intake and substance consumption** was recorded in order to detect any confounding factors during the experiment.

3.3 Equipment and Experimental Setup

3.3.1 EEG

In order to record the EPs, the scalp EEG was continuously recorded with a sampling frequency of 1KHz, using an ANT neuro Waveguard EEG cap with 128 Ag/AgCl electrodes and 136-channel TMSi Refa EEG amplifier. TMSi Polybench software was used to register the EEG data and trigger codes on a dedicated desktop computer. Since this study is a technical pilot for assessing the technical feasibility of the setup, only selected set of channels were recorded to reduce the preparation time. Based on the results of the previous studies, 12 electrodes that give the best overview of the nociceptive brain activity were selected. These electrodes are Fpz, T7, T8, Cpz, M1, M2, Cz, Fz, C3, C4, C5 and C6. Electro-gel was injected into these electrodes to keep the impedance below 5 $K\Omega$(See the impedance interface on figure 3.1 for the overview of the measured channels). The ground electrode was attached to the center of participants forehead. To reduce the occurrence of the EOG artefacts, a focus image was attached in front of the participants, and they were asked to keep their eyes fixed on that image. The subjects were seated in an electric chair and were allowed to adjust the position to make sure they feel relaxed and comfortable. They were also instructed to blink as few times as possible and to maximally avoid any muscle movement during the experiment to improve the signal quality.

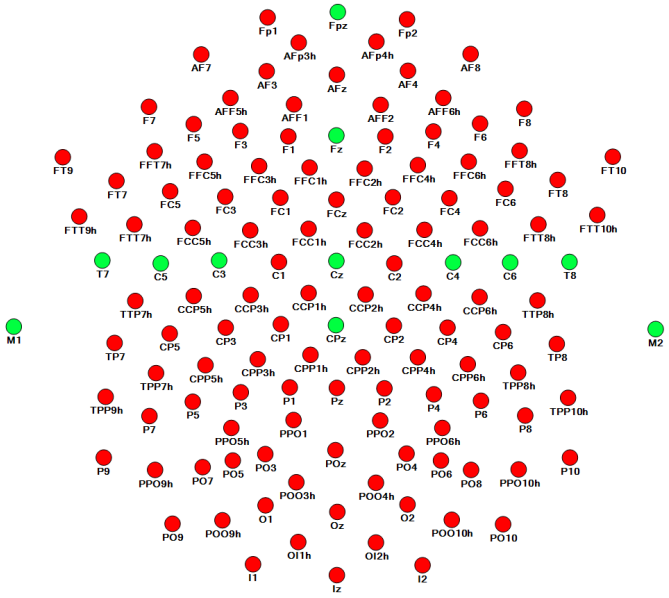


Figure 3.1: The impedance interface that gives the overview of the channels recorded during the experiment (in green).

3.3.2 MTT

Stimulus

The stimuli were selected according to the MTT procedure developed by Doll et al. [28]. The first stimulus type consisted of one cathodic, square-wave electrical current pulse with a pulse width of 0.21 ms and the second type is two cathodic, square-wave electrical current pulse with a pulse width of 0.21 ms and inter-pulse interval of 0.10 ms (see figure 3.2). The order of stimulus types was selected for the same number of times but in a randomized order. The amplitudes were also randomly adjusted according to the estimated detection threshold of the subject. This resulted in 2 simultaneously tracked NDTs.

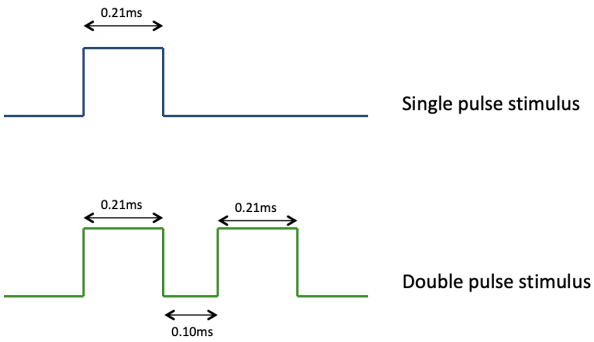


Figure 3.2: The two stimulus types used during the experiment

NociTrack AmbuStim stimulator and IES-5 electrode

The stimuli described in the previous subsection were delivered to the IES-5 electrode using an electrical 1-channel NociTRACK AmbuStim stimulator, developed and thoroughly tested by the BSS group at the University of Twente. The stimulator was controlled by a software developed on LabView interface. The IES-5 electrode, which is displayed on figure 3.3, was attached to the back of the participant's hand. The participant was grounded using the adhesive ground electrode also attached to the back of the hand, next to IES-5 electrode. Low currents (up to 1.4 mA) were used, as a stimulus of more than 2.5mA could also activate non-nociceptive $A\beta$ fibres (Mouraux (2010) [29]).

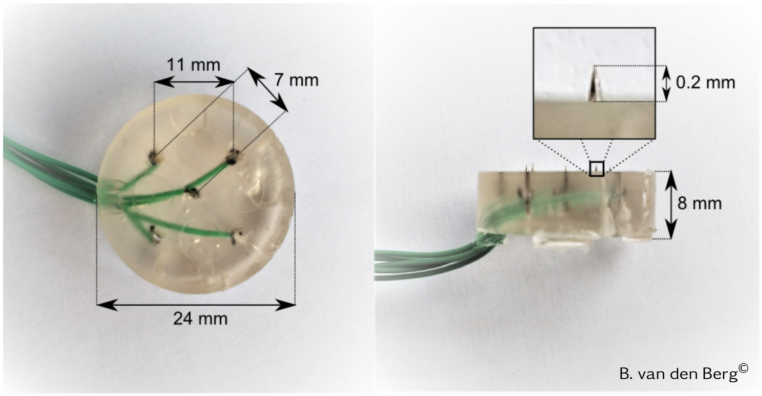


Figure 3.3: Intra-epidermal needle electrode for nociceptive stimulation. The needles have a length of approximately 0.2 mm, enabling penetration of the stratum corneum and invasion of the most superficial layers in the epidermis. Due to its ability to specifically stimulate nociceptive $A\delta$- and C-fiber afferents, this electrode is used in this study. [7]

3.3.3 CPT

Even though at first simple polystyrene water tanks were used to conduct the cold pressor test, later the tank was replaced by a plastic cool box of the size 35x34x24cm. The reason for that was the leakage of a polystyrene tank during one of the experiments, which caused 50 Hz noise in the EEG data by creating grounding problems. Hence, for CPT, it is important that the water tank is made from an insulating, waterproof material. The cool box was filled by 1 °C for the first and 6 °C water for the second experimental sessions. The temperature of 1 °C bath was kept stable by making sure there is enough ice in the water throughout the experiment. The temperature for 6 °C water was controlled before and after immersion using a mercury thermometer to test the temperature stability of the cool box. The test was conducted by immersing left foot of the participant into the cold water since IES-5 electrode was attached to the right hand and participant had to hold the stimulator on the left hand. The subjects were instructed to hold their foot in the water for as long they can tolerate, and were allowed to withdraw foot anytime they want. Maximum allowed immersion time for 1 °C bath and 6 °baths were 7 and 10 minutes respectively. The reason for testing 2 temperatures was that the participants were not expected to tolerate 1 °C for a sufficiently long time to get enough NDT and EEG data. Water circulating equipment was not used. However, for 6 °C, the participants were asked to move their foot in the water every 3 minutes of the immersion.

3.4 Procedure

Both experimental sessions were conducted almost identically, with the only difference being the temperature of the CPT. The time interval between the experimental session was set to a minimum of two weeks. However, one exception had to be made, and one participant had a one-week interval due to lack of time. The sessions lasted about 65-80 minutes and consisted of:

- **Introduction (10 minutes):** The subject was provided with information about the experiment and asked for consent to participate in the study.
- **Questionnaires (5 minutes):** The subject was asked to fill in a questionnaire related to the secondary study parameters.
- **Preparation (15 minutes):** The subject was connected to the EEG equipment and the nociceptive stimulation device.
- **Familiarization (5 minutes):** The subject was familiarized with the stimuli. In this phase, the subject could get used to determine whether a stimulus exceeds the stimulation threshold and learn how to behave during the experiment.
- **Pre-CPT/Baseline measurements (10 minutes):** The actual experiment, in which nociceptive stimulus-response pairs was measured for a variety of nociceptive stimuli without CPT. This was done until 80 SRP per stimulus type were tested.
- **CPT measurements (5-10 minutes):** The subject would continue with the experiment, but now with left foot immersed in the cold water.
- **Post-CPT measurements (10 minutes):** The subject would no longer keep the foot immersed in the cold water. The experiment would be continued.
- **Round-up (5 minutes):** The subject was disconnected from the equipment and debriefed.

For a detailed standard operating procedure of this experiment, please refer to the appendix A.4.

3.5 Data analysis

3.5.1 Preprocessing and analysis of the EEG data

The EEG data were recorded using the PolyBench programme by TMSi, the Netherlands. All data (pre-)processing steps were done using Matlab (Version: 2017b) (Mathworks, USA) and the FieldTrip toolbox (Radboud University, the Netherlands). In pre-processing all the frequencies below 0.01 Hz and above 40 Hz were filtered out. The low-pass filter was used to reduce noise caused by skin potential artefacts, and high pass filter removed the frequencies that were out of the range of interest. This also filtered out most of the 50Hz noise that was

present in the data because of the capacitive coupling with AC power lines (more details about this issue will be discussed in the next chapter). After this, a plot of the variance of all the channels and trials for every participant was inspected. The channels and trials that have high variance were rejected, and, hence, excluded from further processing (see figure 3.4 for examples). Because Fpz was showing high variance for every participant (mainly due to EOG artefacts) and also because we wanted to keep consistency in the averaging per subject, the channel was rejected from all the available data.

Cz - M1, M2 (Cz with reference to M1 and M2) and T7-Fz channel derivations were chosen to analyze the evoked potentials. Cz - M1, M2 derivation would represent the central cortical activity, and hence, the obtained results could be compared to the literature data presented in section 2.3.2. T7-Fz was added to the analysis because contralateral EPs would give a better overview of early negative components. Next, the grand averages over all channels and participants were computed and plotted against various study parameters, such as CPT condition, response or stimulus type.

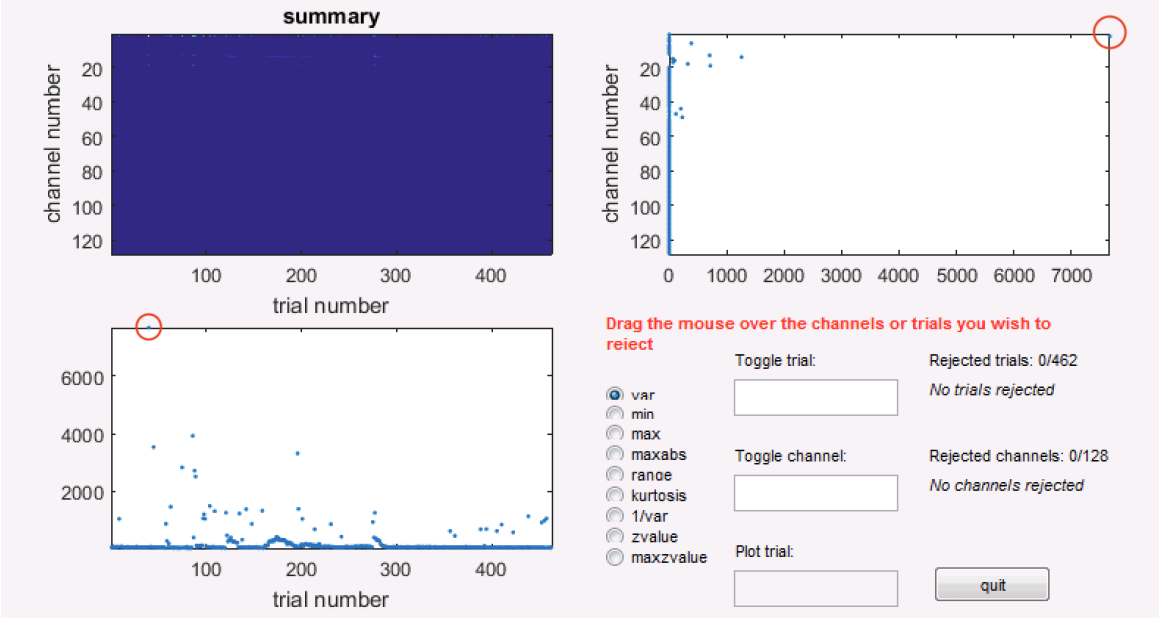


Figure 3.4: Left bottom: trial vs variance plot. Right top: channels vs variance plot. Examples of parameters with high variance circled in red.

3.5.2 Analysis of the MTT data

By using the same method used by Doll et al. (2016) [28] to compute the psychometric function, generalized linear regression was implemented (See equation (3.1) for generalized formula).

$$\ln \frac{p(y_j)}{1-p(y_j)} = \beta_0 + \sum_{k=1}^{p} \beta_k x_{jk} + \eta_j \tag{3.1}$$

The model predictors were CPT condition (pre-CPT, CPT or post-CPT), stimulus setting (single or double) and stimulus amplitude. The statistical model used (Wilkinson notation) can be found in the equation (3.2), where:

- D is the response of the participant (Detected or Undetected)
- amp is the noxious stimulus amplitude in mA
- $type$ is the stimulus setting (single or double pulse)
- cpt is the CPT condition (Pre-CPT; CPT or Post-CPT)

$$D \sim 1 + amp * type * cpt + (1 + amp * type * cpt)|subject \tag{3.2}$$

After the β coefficients of equation (3.1) were computed on Matlab using $fitglme$ function, the threshold amplitude (NDT) and corresponding slopes could be computed by substituting $p(y_j)$ with 0.5. This would result in equations (3.3) and (3.4) (for derivation please refer to appendix A.1).

$$amp = \frac{-(\beta_0+\beta_2\times type+\beta_3\times cpt_1+\beta_4\times cpt_2+\beta_8\times type\times cpt_1+\beta_9\times type\times cpt_2)}{(\beta_1+\beta_5\times type+\beta_6\times cpt_1+\beta_7\times cpt_2+\beta_{10}\times type\times cpt_1+\beta_{11}\times type\times cpt_2)} \tag{3.3}$$

$$slope = \beta_1 + \beta_5 \times type + \beta_6 \times cpt_1 + \beta_7 \times cpt_2 + \beta_{10} \times type \times cpt_1 + \beta_{11} \times type \times cpt_2 \tag{3.4}$$

By entering those equations in Matlab and NDT and slopes can be plotted for every CPT condition and each pulse type.

4 Results and Discussion

4.1 Feasibility of the Experimental Procedure

4.1.1 MMT-EP with 1°C Cold Pressor Test

Immersion time

Despite the initial expectation, the 1 °C CPT seems to be compatible with MTT-EP experiment. It was expected that the participants would not be able to tolerate the pain induced by CPT for more than 2 minutes. However, 4 out of 6 participants showed a tolerance time of 7 minutes, which was the maximum time participants were allowed to keep their foot in the cold water. Those participants have indicated that the pain is most intense during the first 2 minutes of immersion, and becomes more tolerable afterwards. They have also mentioned that they would be able to continue CPT for more than 7 minutes if required. Nevertheless, there were still other subjects (2 out of 6) who showed the tolerance time less than 2 minutes. The amount of SRPs collected from these participants were approximately 10 stimuli per type, which is not sufficient for obtaining reliable results. During 7 minutes of immersion, around 50-60 stimuli per type could be tested (see table 4.1).

Technical aspects

In some of the experimental sessions, high amplitude 50 Hz noise was observed on EEG recordings. The investigation of this problem revealed the following reasons for this coupling with AC power lines:

- **Improper cleaning of the EEG cap:**
  It was observed that even a small amount of dry gel on the electrode might cause significant errors in common mode rejection while using selected channels (only 12 in this case). Hence, it is necessary to thoroughly wash the cap, especially the electrodes that were used during the experiment as well as neighbouring electrodes.
- **Leakage of the CPT bath:**
  Another reason that could cause the grounding issues, and, hence, result in 50 Hz noise on EEG data is the leakage of the CPT container. For this reason, the polystyrene container was eventually replaced with a plastic waterproof coolbox.
- **Wet towel:**
  The participants were allowed to dry their foot with the towel right after CPT. For some

cases the towel was left on the floor and participants would put their barefoot on it. This is another potential source of grounding problems, and, therefore, the towel should be removed before continuing with post-CPT measurements.

- **The metal equipment in the lab:**
  The position of metal equipment, such as a table, cupboard, etc. could also amplify the 50 Hz signal that is present on the EEG data. Hence, it was necessary to ground all equipment that could potentially cause capacitive coupling with power lines.

However, it should be noted that the cleaning of the EEG cap was the primary reason. The other secondary reasons stated above could amplify the 50 Hz noise that is already present due to the dry gel on the electrodes. In the case the cap is properly cleaned, the other three factors would not have a significant influence on the signal because common mode rejection would work correctly.

4.1.2 MMT-EP with 6°C Cold Pressor Test

Temperature stability

6°C CPT with MTT-EP is also a technically feasible combination. The coolbox used for CPT was stable enough to maintain a constant temperature ($\pm$ 1°C) during the immersion (see figure 4.1). The average temperatures before and after immersion were $5.92 \pm 0.35°C$ and $6.49 \pm 0.38°C$, respectively.

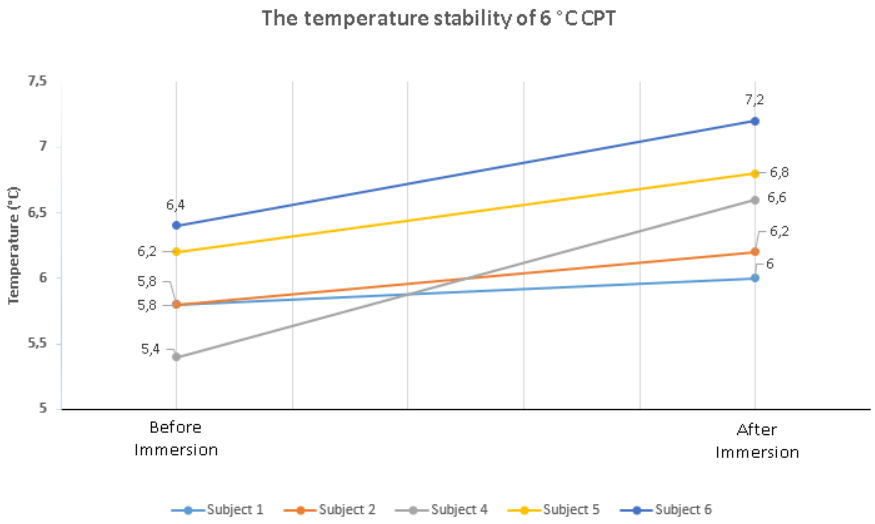


Figure 4.1: The line graph representation of temperature stability for 6° CPT.

Immersion time

The tolerance time for participants did not significantly differ from 1 °C bath. The participants who could tolerate the 1°C water for 7 minutes (maximum allowed immersion time for the first condition) could tolerate the 6 °C water for 10 minutes (maximum allowed time for the second condition). From the two participants who had tolerance times less than 2 minutes

in the first session, only one showed a significant improvement for the second session (10 minutes). It is thought that this improvement was mainly caused by the fact that the subject was informed that the pain intensity is expected to decrease by time eventually. The second participant showed a minor improvement (from 1 minute to 4 minutes), even though the same information was shared with him as well (see figure 4.2). This suggests that the immersion time mainly depends on the participant's individual pain tolerance and change in temperature (in this case from 1 °C to 6°C) has only a minor effect on it.

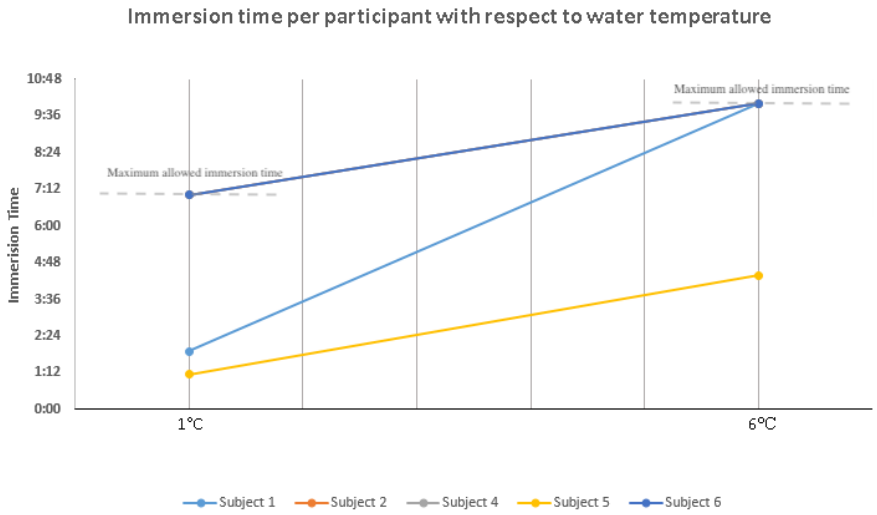


Figure 4.2: The line graph representation of immersion time for both CPT temperatures. (Note: The lines for subjects 2, 3 and 6 are overlapping.)

4.2 Results of MTT-EP data analysis

For the analysis of the collected data, one participant was excluded for both sessions and one was excluded only from 6°C session because of the issues with the Bluetooth connection of the NociTrack stimulator (interrupted session) and loose connection of the IES-5 electrode (incorrect MTT data). As a result, 5 and 4 participants were analysed for 1°C and 6°C CPTs, respectively. For the overview of the data sets used further please refer to tables 4.1 and 4.2.

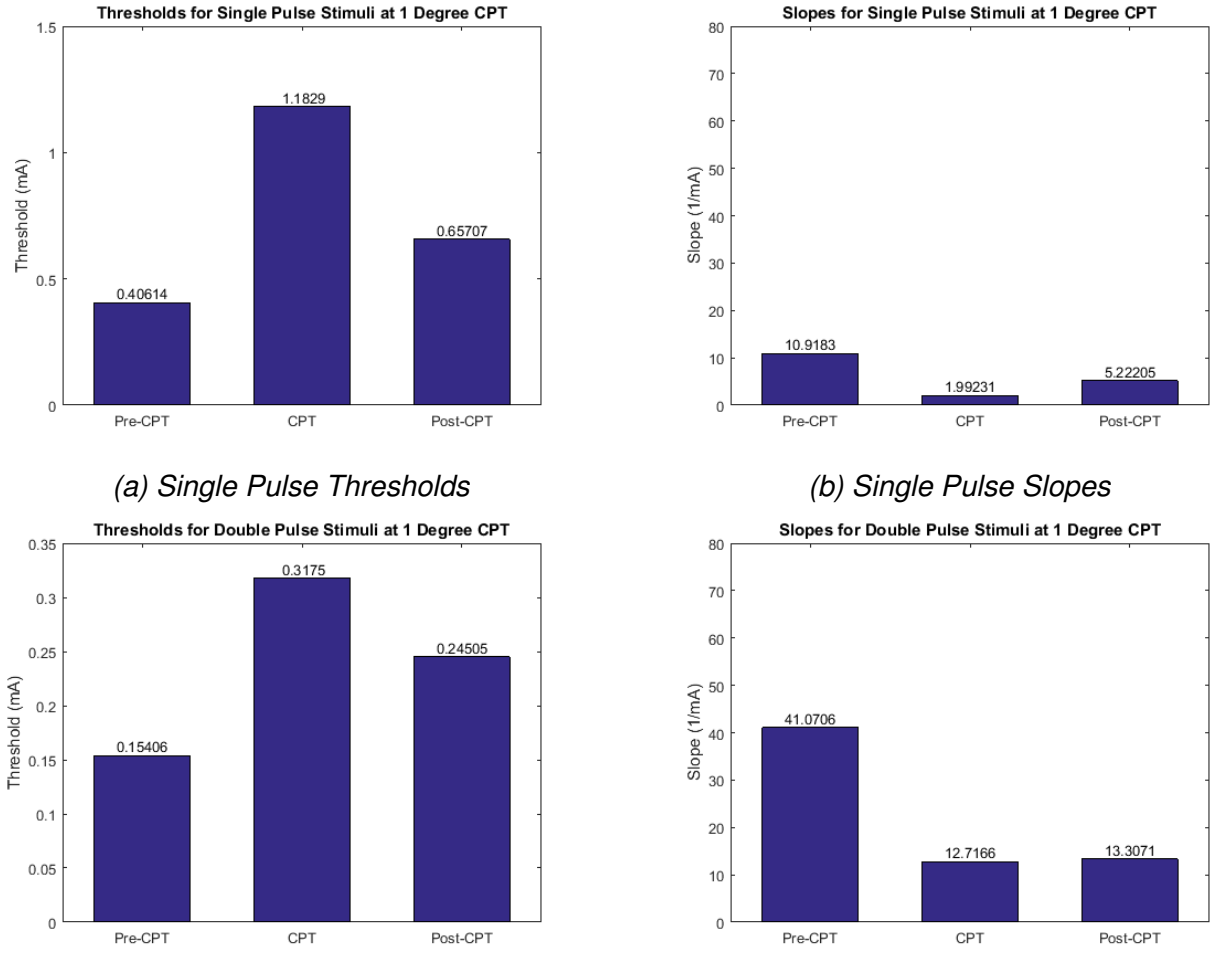
Table 4.1: The overview of the data set for 1° C CPT

Subject Code	Immersion Time	Pre-CPT trial number (per stimulus setting)	CPT trial number (per stimulus setting)	Post-CPT trial number (per stimulus setting)	Total number of trials (per stimulus setting)	Water temperature
01	1:54	80	9	62	151	1°C
02	7:00	81	56	94	231	1°C
04	7:01	81	51	98	230	1°C
05	1:09	81	8	91	180	1°C
06	7:00	80	60	90	230	1°C

Table 4.2: The overview of the data set for 6° C CPT

Subject Code	Immersion Time	Pre-CPT trial number (per stimulus setting)	CPT trial number (per stimulus setting)	Post-CPT trial number (per stimulus setting)	Total number of trials (per stimulus setting)	Water temperature (Before immersion)	Water temperature (After immersion)
01	10:00	81	73	76	230	5,8°C	6°C
02	10:00	81	99	50	230	5,8°C	6,2°C
05	4:23	83	33	114	230	6,2°C	6,8°C
06	10:00	80	71	79	230	6,4°C	7,2°C

4.2.1 Analysis of the Nociceptive Detection Thresholds



(a) Single Pulse Thresholds

(b) Single Pulse Slopes

(c) Double Pulse Thresholds

(d) Double Pulse Slopes

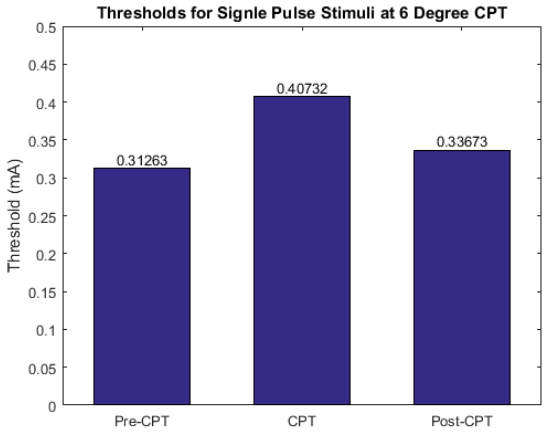
Figure 4.3: The estimated thresholds and slopes per CPT condition for at 1 ° C CPT

The results of generalized linear regression estimation for a single pulse and double pulse at 1°CPT are presented in figures 4.3. It can be seen that both stimulus settings show the same trends in threshold (figure 4.3a; 4.3c) and slope estimations (figure 4.3b; 4.3d). The thresholds for both stimuli considerably increases during CPT and reduced after termination of the test. The NDT value for single pulse almost tripled during CPT, and for Post-CPT was still higher than Pre-CPT. The values for the threshold amplitudes of the single pulse are in general lower for double pulse stimuli, which is consistent with the results of Doll et al. (2016) [28] discussed in the section 2.3.3. Furthermore, the figures 4.3b and 4.3d show that the slope of the psychometric function is higher before CPT, and decreases significantly

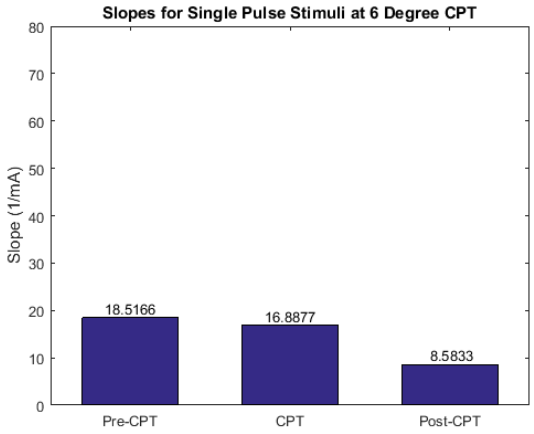
after its application, which means that the participants were more certain about whether or not they felt the stimuli around the estimated thresholds before the CPT. However, in contrast to thresholds, the slopes for double pulse was considerably higher than that of single pulse stimuli.

The similar trends could also be observed at 6 °C bath, (see figure 4.4). Application of CPT also caused an increase in the values of estimated thresholds (figures 4.4a and 4.4c); however, the increase was not as sharp as for 1°C. The slopes for 6 °C are also higher for pre-CPT and show minor decrease afterwards.

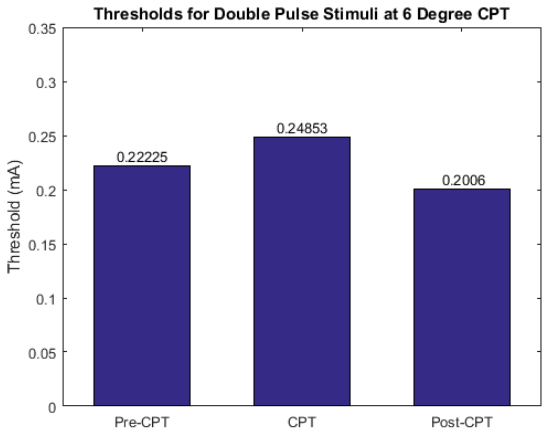
As a summary, the bar charts of estimated thresholds and slopes on figures 4.3 and 4.4 suggest that DNIC effect can be observed on MTT data for both temperatures. However, the effect appears to be strongest for 1°CPT.



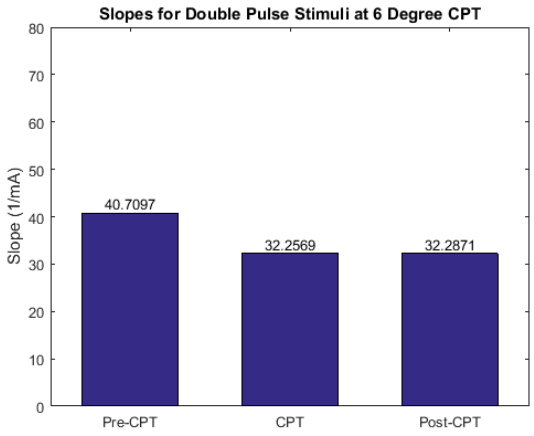
(a) Single Pulse Thresholds



(b) Single Pulse Slopes



(c) Double Pulse Thresholds



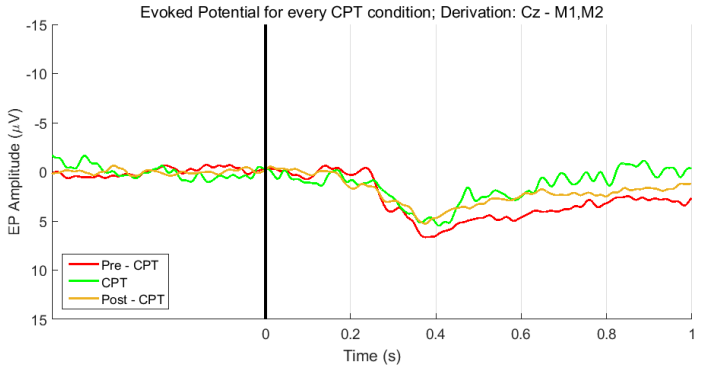
(d) Double Pulse Slopes

Figure 4.4: The estimated thresholds and slopes per CPT condition for at 6 °C CPT

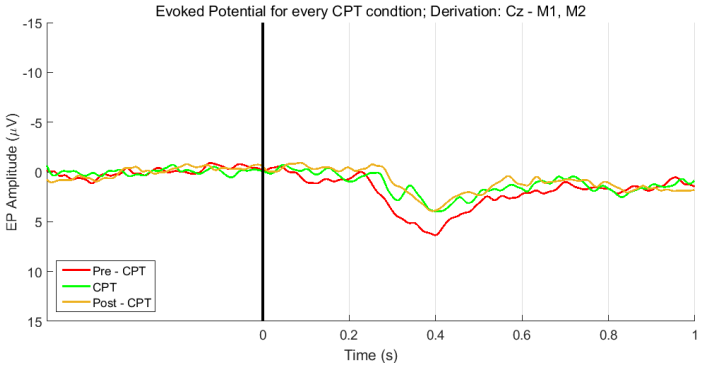
4.2.2 Analysis of the Evoked Potentials

The effect of CPT on EP

The plots of grand average of EP vs time over Cz-M1,M2, for three CPT conditions are shown in figures 4.5a and 4.5b for 1°C and 6°C. From both figures it can be seen that the amplitude of positive component present at 0.4s is reduced during CPT and stays approximately the same after termination of CPT. This effect resembles the results of Torta et al. (2015) and Höffken et al. (2017) presented in figures 2.12 and 2.13 [12] [11].



(a) At 1° C CPT

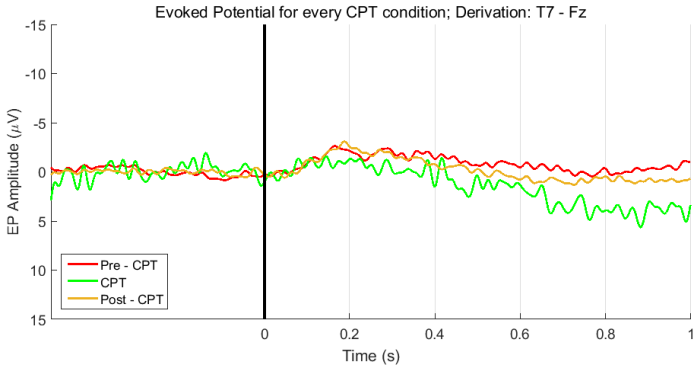


(b) At 6° C CPT

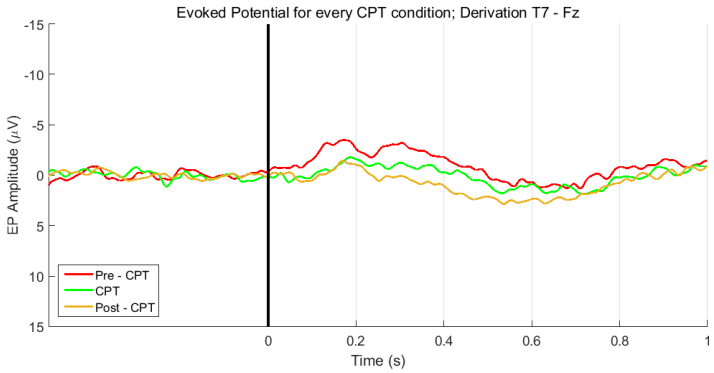
Figure 4.5: The grand average of EPs for every CPT condition over Cz - M1, M2;

A similar effect can be observed on the contralateral cortical activity displayed in figure 4.6. As it can be seen from the figures, the amplitude of early negative latency (N2) is also decreasing after application of CPT.

However, unlike the MTT results, in the case of EPs, the effect of CPT is more visible for 6°C bath. The reason for that could be the fact that the EEG data collected during 6°C session was of better quality since the noise problems mentioned earlier (see section 4.1.1) were already solved.

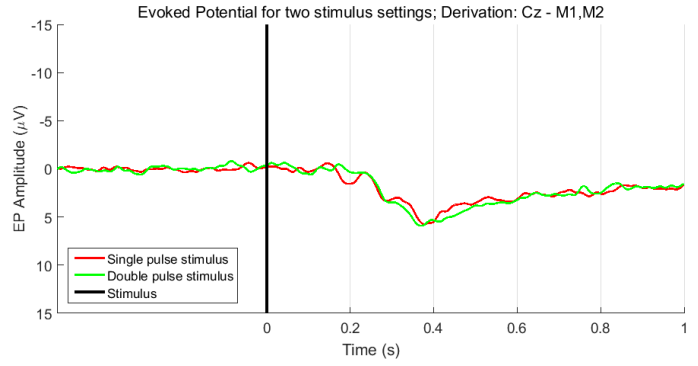


(a) At 1° C CPT

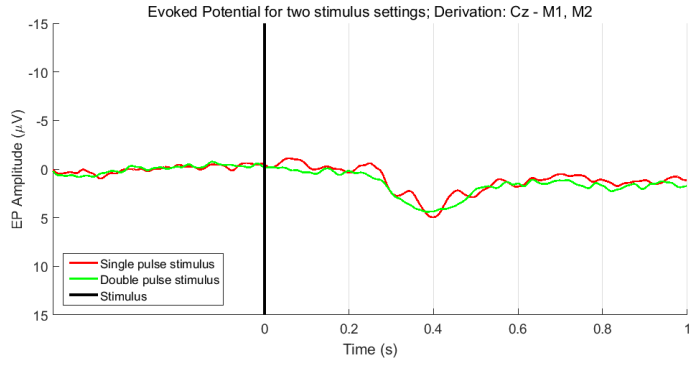


(b) At 6° C CPT

Figure 4.6: The grand average of EPs for every CPT condition over T7-Fz;



(a) At 1° C CPT



(b) At 6° C CPT

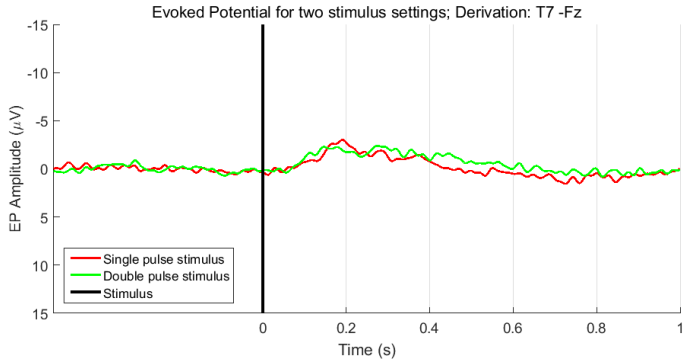
Figure 4.7: The grand average of EPs for single and double pulse over Cz - M1, M2

The effect of stimulus type on EP

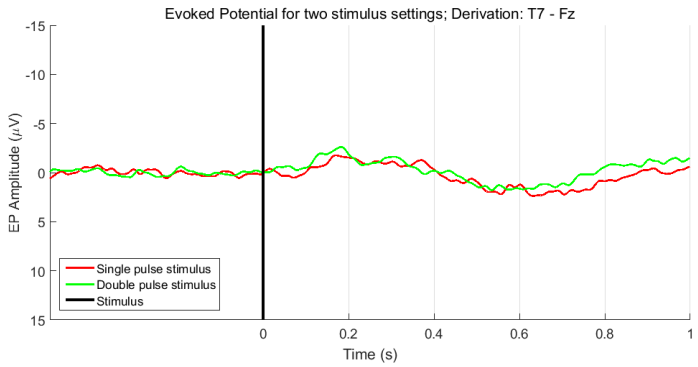
Based on figures 4.7, which displays the EPs for two stimulus settings over Cz-M1, M2, the number of pulses does not have a noticeable effect on the amplitude of the P2 component (at 0.4 seconds). The same can be concluded about N2 components, based on figure 4.8. This leads to the conclusion that even though the stimulus setting is an essential parameter in MTT results, it does not have the same significance for EPs.

The effect of response on EP

According to figures 4.9 and 4.10 the parameter that appears to have the biggest effect on the evoked potentials is the response of the participant. It can be seen that the EEG data almost remains at the baseline when the stimuli were not detected and shows clear positive component at 0.4 seconds on figures 4.9a and 4.9b and clear N2 component at 0.2 seconds on figures 4.10a and 4.10b, respectively.

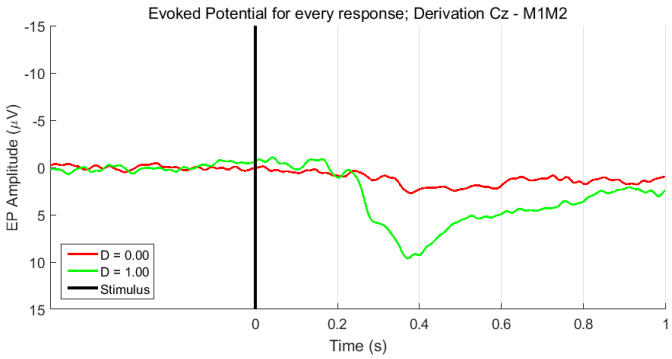


(a) At 1° C CPT

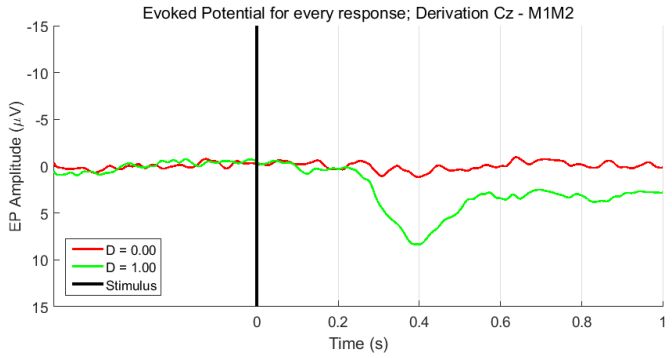


(b) At 6° C CPT

Figure 4.8: The grand average of EPs for single and double pulse over T7-Fz

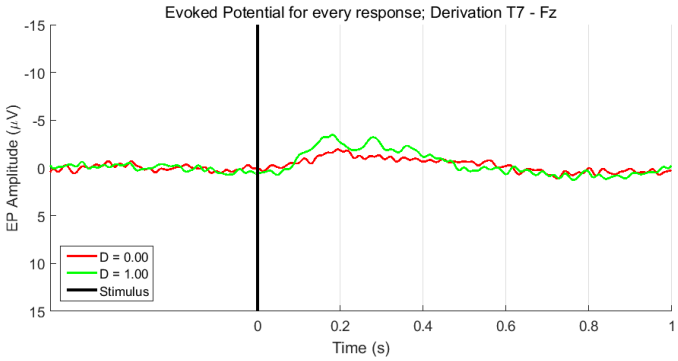


(a) At 1° C CPT

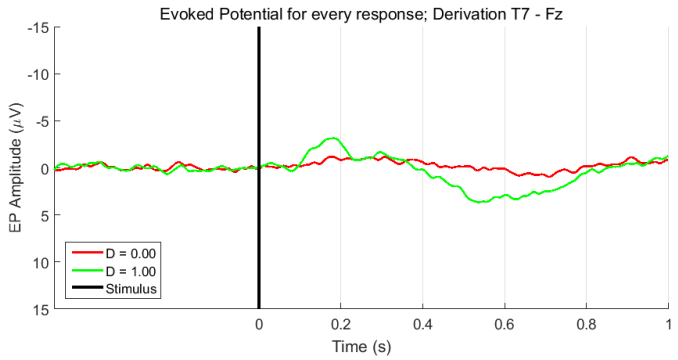


(b) At 6° C CPT

Figure 4.9: The grand average of EPs for every response over Cz - M1, M2



(a) At 1° C CPT



(b) At 6° C CPT

Figure 4.10: The grand average of EPs for each response over T7-Fz

4.3 Summary

Both experiment sessions were found to be technically feasible when taking into account the aspects discussed in section 4.1.1. The tolerance time of the participants was not a significant obstacle in conducting the experiment. Despite the initial expectation, most of the participants could tolerate the cold water baths for a sufficiently long time to collect enough data. The cool box used for the experiment was proven to maintain a stable temperature throughout the immersion, and, therefore, can be used for CPT independent on the chosen water temperature. An important point to note is that only males participated in this research. However, it has been shown by Mitchell et al. (2004) [31] that the gender of the participant plays a role in the tolerance time. Females usually tend to have lower pain tolerance than males (see figure 2.10).

MTT data analysis showed that the DNIC effect could be observed for both stimuli settings for both CPT temperatures. During the application of conditioning stimulus, the estimated NDT went up and decreased after termination of CPT. These results are consistent with the research of Doll et al. (2016)[28]. The threshold for single pulse (figure 4.3a has extremely high value during CPT (1.829 mA), which is caused by the fact that out of five participants included in the analysis two did not have sufficient amount of SRPs for CPT condition (Table 4.1), and other two indeed showed much higher thresholds than the rest of the participants. This suggests that the NDT values and slopes during CPT can be biased, and hence should be confirmed by testing more participants.

For every condition slopes of the psychometric functions were highest at pre-CPT and decreased during and after CPT, which means that the thresholds were more well-defined before CPT. Some values for psychometric slopes for double pulse stimuli were much higher than those presented by Doll et al. (2016), however, these values should also be confirmed by recruiting more subjects in the future.

Based on the EEG data, it was observed that CPT decreases the amplitude of EP components. The number of pulses did not show any noticeable effect on the EPs, whereas the response was found to be the main determining parameter.

Most of the participants indicated that moving their foot in the water at 6°C bath, increased the intensity of the perceived pain. This suggests that water circulation is an important factor for maintaining constant stimulus intensity throughout CPT.

5 | Conclusion and Further Recommendations

This pilot study assessed the technical feasibility of the MTT-EP in combination with CPT. It was found that these set-ups are compatible with each other, and a standard operating procedure for MTT-EP experiment with CPT was established (See appendix A.4). Hence, the primary objective of this research was achieved. The secondary objective was also met through the analysis of the data collected through this experiment and lead to the following findings:

- The difference between 1 °C and 6 °C appears to be too little to cause a substantial difference in the immersion time. The immersion time of participants also depends on their individual pain perception. 4 out of 6 participants had an immersion time of 7-10 minutes.
- The effect of DNIC on NDT is best observed for 1°C CPT. However, both temperatures can be used to investigate DNIC effect.
- For the evoked potentials, DNIC effect can also be observed for both temperatures.
- Stimulus setting has a considerable effect on the NDT but does not show any noticeable effect on EP.
- Among all analyzed factors subject's response is the most important predictor of EP.

Based on these findings, the study concludes that both temperatures can be used as a conditioning stimulus to evoke the DNIC. At 1°C CPT it is easier to maintain a stable temperature than at 6°C. However, 6 °C shows slightly higher immersion time, and, hence, permits more data to be collected.

For further research, it may be useful to add a water circulation equipment to the set-up. Moreover, the maximum allowed immersion time can be extended to 15 minutes such that more data is collected during CPT. The amount of participants recruited for these pilot experiments is not sufficient to provide statistically significant results. Therefore, it is important to conduct a study comprising of larger sample size (including both males and females) to confirm the results presented in this paper. Using all channels of the EEG cap would give a better overview of the nociceptive cortical activity, and, hence, should also be considered in further experiments. Furthermore, more advanced statistical techniques such as linear mixed models, time lock averaging, etc. can also be implemented for a broader analysis of the EP data.

6 | Bibliography

[1] "Chronic pain: Backgrounder," *Boston Scientific: Advanced science for life*, 2012. [Online]. Available: https://pae-eu.eu/wp-content/uploads/2015/03/NM-86505-AA_INTL-Chronic-Pain-Backgrounder_Final-2.pdf

[2] H. Breivik, B. Collett, V. Ventafridda, R. Cohen, and D. Gallacher, "Survey of chronic pain in europe: Prevalence, impact on daily life, and treatment," *European Journal of Pain*, 2006. [Online]. Available: http://www.nascholingnoord.nl/presentaties/2012_02_02_Breivik_et_al___Survey_of_chronic_pain_in_Europe.pdf

[3] E. Bekkering, M. Bala, K. Reid, E. Kellen, J. Harker, R. Riemsma, J. Huygen, and J. Kleijnen, "Epidemiology of chronic pain and its treatment in the netherlands," *The Netherlands journal of medicine.*, 2011.

[4] A. Berger, E. Dukes, and G. Oster, "Clinical characteristics and economic costs of patients with painful neuropathic disorders." *Journal of pain: official journal of the American Pain society*, 2004.

[5] M. Gore, A. Sadosky, B. Stacey, K. Tai, and D. Leslie, "The burden of chronic low back pain: clinical comorbidities, treatment patterns, and health care costs in usual care settings." 2012.

[6] M. Curatolo, M. Müller, A. Ashra, Y. Neziri, A, K. Streitberger, K. Andersen, O, and L. Arendt-Nielsen, "Pain hypersensitivity and spinal nociceptive hypersensitivity in chronic pain: prevalence and associated factors." 2015.

[7] B. van den Berg, "Stimulus related evoked potentials around the nociceptive detection threshold," *University of Twente*, 2018.

[8] D.Pud, Y.Granovsky, and D. Yarnitsky, "The methodology of experimentally induced diffuse noxious inhibitory control (dnic)-like effect in humans." 2009.

[9] G. van Wijk and D. Veldhuijzen, "Perspective on diffuse noxious inhibitory controls as a model of endogenous pain modulation in clinical pain syndromes." 2018.

[10] L. Eitner, Ö.S.Özgül, Enax-Krumova, J. Vollert, C. Maier, and O. Höffken, "Conditioned pain modulation using painful cutaneous electrical stimulation or simply habituation?" *European Journal of Pain*, 2018.

[11] M. Tegenthoff, Ö.S.Özgül, Enax-Krumova, J. Vollert, C. Maier, and O. Höffken, "Evoked potentials after painful cutaneous electrical stimulation depict pain relief during a conditioned pain modulation." *BMC Neurology*, 2017.

[12] D.Torta, M. Churyukanov, L. Plaghki, and A. Mouraux, "The effect of heterotopic noxious conditioning stimulation on aδ-, c- and aβ-fibre brain responses in humans." *European Journal of Neuroscience*, 2015.

[13] H. Merskey, *Part III: Pain Terms, A Current List with Definitions and Notes on Usage, Classification of Chronic Pain*, 2nd ed. Seattle: IASP Press, 1994.

[14] E. R. Kandel, J. H. Schwartz, and T. M. Jessel, *Principles of Neural Science*, 4th ed. USA: McGraw-Hill companies inc., 2000.

[15] D. Le-Bars, A. H. Dickenson, and J. M. Besson, "Diffuse noxious inhibitory controls (dnic). i. effects on dorsal horn convergent neurones in the rat." *Pain vol. 6, Issue 3, p.305-327*, 1979.

[16] ——, "Diffuse noxious inhibitory controls (dnic). ii. lack of effect on non-convergent neurones, supraspinal involvement and theoretical implications," *Pain vol. 6, Issue 3, pp.305-327*, 1979.

[17] D. Le-Bars, "The whole body receptive field of dorsal horn multireceptive neurones," *Brain Research Reviews, vol. 40, no. 13, pp.29-44*, 2002.

[18] O. David, J. Klinker, and K. Friston, "Mechanisms of evoked and induced responses in meg/eeg," *NeuroImage vol. 31 no. 4 pp. 1580-1591*, 2006.

[19] L. Garcia-Larrea, M. Frot, and M. Valeriani, "Brain generators of laser-evoked potentials: From dipoles to functional significance," *Neurophysiologie Clinique vol. 33 no. 6 pp. 279-292*, 2003.

[20] M. C. Lee, A. Mouraux, and G. D. Iannetti, "Characterizing the cortical activity through which pain emerges from nociception," *Journal of Neuroscience vol. 29 no. 24 pp. 7909-7916*, 2009.

[21] V.Legrain, J.M.Gueri, and a. R.Bruyer, "Attentional modulation of the nociceptive processing into the human brain: Selective spatial attention, probability of stimulus occurrence, and target detection effects on laser evoked potentials," *Pain vol. 99 no. 1-2 pp. 21-39*, 2002.

[22] R. Parasuramani, "The psychobiology of sustained attention," *Sustained Attention in Human Performance pp. 61-101*, 1984.

[23] M. I. Posner, "Orienting of attention," *The Quarterly journal of experimental psychology vol. 32 no. 1 pp. 3-25*, 1980.

[24] G. Repovs, "Dealing with noise in eeg recording and data analysis„" *Informatica Medica Slovenica vol. 15 pp. 18-25*, 2010.

[25] F. Kingdom and N. Prins, *Psychophysics*. Academic Press, 2010.

[26] R. J. Doll, P. H. Veltink, and J. Buitenweg, "Observation of time-dependent psychophysical functions and accounting for threshold drifts," *Attention, Perception, and Phsychophysics vol. 77 no. 4 pp. 1140-1447*, 2015.

[27] R. Doll, P. Veltink, and J. Buitenweg, "Effect of temporal stimulus properties on the nociceptive detection probability using intra-epidermal electrical stimulation," *Experimental Brain Research 234(1) pp. 219-227*, 2016.

[28] R. J. Doll, P. H. Veltink, and J. Buitenweg, “Characterization of psychophysical method for simultaneous tracking of multiple non stationary thresholds,” 2016.

[29] A. Mouraux, G. Iannetti, and L. Plaghki, “Low intensity intra-epidermal electrical stimulation can activate $a\delta$-nociceptors selectively,” *Pain*, 2010.

[30] M. Schooneman, “Measurement of evoked potentials during multiple threshold tracking of nociceptive electrocutaneous stimuli,” *Thesis*, 2015.

[31] L. A. Mitchell, R. A. MacDonald, and E. E. Brodie, “Temperature and the cold pressor test,” *Journal of Pain, vol. 5, no. 4, pp. 233 – 237*, 2004.

[32] M. L. Fasano, T. Sand, A. O. Brubakk, P. Kruszewski, C. Bordini, and O. Sjaastad, “Reproducibility of the cold pressor test: studies in normal subjects,” *Clinical Autonomic Research, vol. 6, no. 5, pp. 249–253*, 1996.

[33] J. Koenig, M. N. Jarczok, R. J. Ellis, C. Bach, J. F. Thayer, T. K. Hillecke, and S. Hum, “Two-week test–retest stability of the cold pressor task procedure at two different temperatures as a measure of pain threshold and tolerance,” *Pain Practice*, 2013.

A | Appendices

A.1 Calculation of thresholds and slopes

$$\ln \frac{p(y_j)}{1-p(y_j)} = \\ = \beta_0 + \beta_1 \times amp + \beta_2 \times type + \beta_3 \times cpt_1 + \beta_4 \times cpt_2 + \\ + \beta_5 \times amp \times type + \beta_6 \times amplitude \times cpt_1 + \\ + \beta_7 \times amp \times cpt_2 + \beta_8 \times type \times cpt_1 + \beta_9 \times type \times cpt_2 + \\ + \beta_{10} \times amp \times type \times cpt_1 + \beta_{11} \times amplitude \times type \times cpt_2$$

For $p(y_j) = 0.5$:

$$0 = \\ = \beta_0 + \beta_1 \times amp + \beta_2 \times type + \beta_3 \times cpt_1 + \beta_4 \times cpt_2 + \\ + \beta_5 \times amp \times type + \beta_6 \times amplitude \times cpt_1 + \\ + \beta_7 \times amp \times cpt_2 + \beta_8 \times type \times cpt_1 + \beta_9 \times type \times cpt_2 + \\ + \beta_{10} \times amp \times type \times cpt_1 + \beta_{11} \times amp \times type \times cpt_2$$

Rearranging the equation:

$$amp = \\ \frac{-(\beta_0 + \beta_2 \times type + \beta_3 \times cpt_1 + \beta_4 \times cpt_2 + \beta_8 \times type \times cpt_1 + \beta_9 \times type \times cpt_2)}{(\beta_1 + \beta_5 \times type + \beta_6 \times cpt_1 + \beta_7 \times cpt_2 + \beta_{10} \times type \times cpt_1 + \beta_{11} \times type \times cpt_2)}$$

$$slope = \\ \beta_1 + \beta_5 \times type + \beta_6 \times cpt_1 + \beta_7 \times cpt_2 + \beta_{10} \times type \times cpt_1 + \beta_{11} \times type \times cpt_2$$

A.2 Participant inclusion criteria

The following inclusion criteria were applied:

- A signed, written informed consent.
- Be in good health.
- Aged 18-40

A potential subject who was meeting any of the following criteria were excluded from participation in this study:

- Refusal to participate before/during the study;
- Skin problems at the site of the pain sensitivity measurement;
- Language problems;
- Implanted stimulation device (e.g. pacemaker, neuromodulation device);
- Pregnancy;
- Pain complaints at the time of the experiment;
- A medical history of chronic pain;
- Cardiac arrhythmia
- Heart valve defects
- Heart muscle diseases
- Open wound on the foot to be immersed

A.3 Participant Questionnaire

General Information

Gender:	O male	O female	O other	
Age:	……			
Body length:	…… m			
Body weight:	…… kg			
Employment status:	O unemployed	O student	O part-time	O full-time

Medication

Are you currently using any medication?

O yes O no

If so, please list the medication you are using:

Name	Frequency	Dosage

Substance Consumption

How many units of alcohol do you consume per week, on average?

……

How many units[1] of alcohol did you consume during the past 24 hours?

……

How many cigarettes do you smoke per week, on average?

……

How many cigarettes did you smoke during the past 24 hours?

……

Did you use any other recreational drugs during the past 24 hours?

1) One unit of alcohol is defined as 250 ml of beer, 100 ml of wine or 35 ml of liquor.
2) Chronic pain is defined by 3 months of persistent pain in one or more anatomical regions that is unexplainable by another pain condition.

……

Physical Activity

How many hours per week do you exercise, on average?

……

How many hours did you exercise during the past 24 hours?

……

Sleep

How many hours per night do you sleep, on average?

……

How many hours did you sleep last night?

……

Pain

Did you suffer any injury or illness during the past year?

O yes O no

On a scale of 0 (no pain) to 10 (worst imaginable pain), what grade would you give to the worst pain intensity experienced during these injuries/illnesses?

……

Do you have any history of chronic pain*?

O yes O no

If so, please list describe the type(s) of chronic pain[2] you were experiencing:

Description	**Frequency**	**Pain Intensity (0 to 10)**
	……	……
	……	……
	……	……
	……	……

1) One unit of alcohol is defined as 250 ml of beer, 100 ml of wine or 35 ml of liquor.
2) Chronic pain is defined by 3 months of persistent pain in one or more anatomical regions that is unexplainable by another pain condition.

A.4 Experimental Protocol

University of Twente

Biomedical Signals and Systems

Research theme Nociceptive and Somatosensory Processing

Standard Operating Procedure

MTT-EP Experiment with CPT

Written by: Fidan Mammadli

Initial version: 06-05-2019

Last revision: 13-06-2019

Contents

Background

Sensitivity of ascending pathways in the nociceptive system is reflected in nociceptive thresholds in human subjects. Thresholds can be estimated using electrical stimulation of nociception specific nerve fibers. Electrocutaneous stimulation using a needle electrode has been shown to selectively stimulate nociception related Aδ-fibers with currents lower than twice the detection threshold [1-3]. Varying stimulus parameters (e.g. number of pulses, pulse duration and inter pulse interval) result in different thresholds [4]. A needle electrode [3, 5] is placed on the right hand. Electrical stimuli are generated with a custom-built stimulator (University of Twente). Stimuli are applied with a frequency of approximately 0.3 Hz to the subject, which should release a button-switch to indicate perception of the stimuli.

Nociceptive activity can be quantified by recording and analyzing the EEG signal time-locked to the stimuli. The average time-locked signal, referred to as the evoked potential (EP) describes transient synchronized activity of large neural networks within the cortex. Additionally, non-phaselocked activity can be quantified using the wavelet-transformed signals, and refers to synchronization and desynchronization of local neural networks. Therefore, it is important to accurately measure the EEG signal to exclude noise and artifacts from external and internal disturbances.

To measure the EEG signal during nociceptive threshold measurement, a setup for EEG measurement can be combined with a setup for threshold measurement, as is depicted in Figure 1.

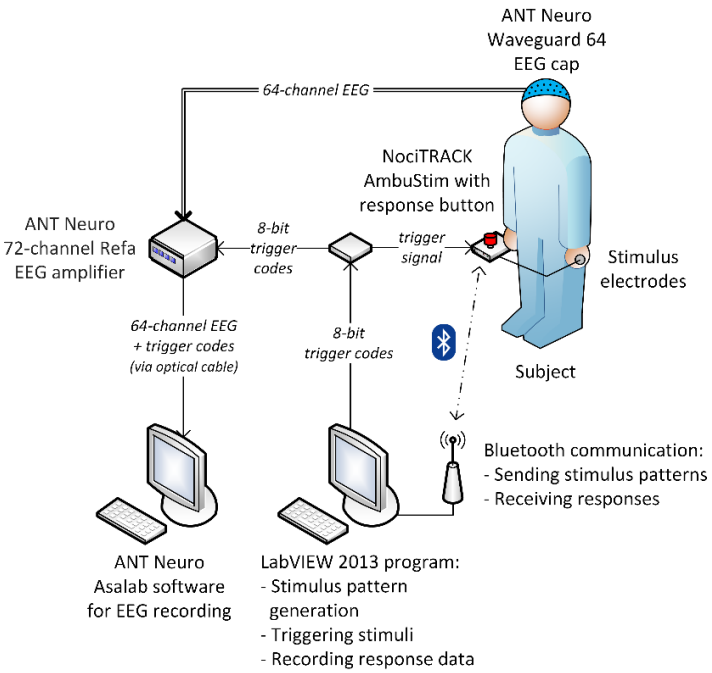


Figure 1: Example setup combining EEG measurement with nociceptive stimulation and nociceptive threshold measurement.

Cold Pressor Test is a potential technique for the validation of the NDT-EP method, and has been used extensively and consistently by other research groups as a pain model for the activation of diffuse noxious inhibitory control. One problem that the CPT however has, is that the activation of DNIC is short-lived: it is maximal during the application of the conditioning stimulus and returns to baseline within about 5 minutes after (van Wijk & Veldhuijzen, 2010). As such, for the best possible measurements, it is considered best to perform measurements *during* the application of the conditioning stimulus. This paper gives the experimental protocol for the assessment of the technical feasibility of implementing CPT together with ND-EP method by using 1℃ and/or 6℃ degree CPTs. Once the method is proven to be feasible, the induced effect of CPT on the response to electrical and stimuli will be evaluated.

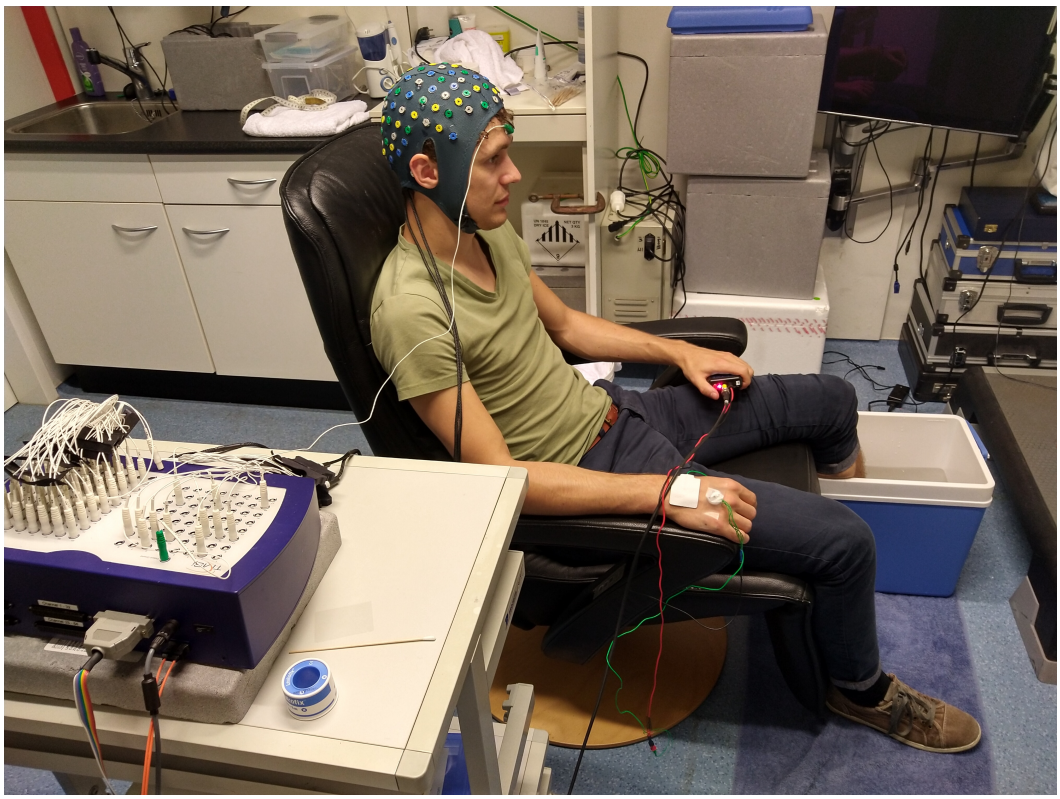


Figure 2. Showcase of MTT-EP-CPT experiment

Required Materials

Description	#	Specification
General		
Computer	1	One computer with LabView 2013 SP or higher, MTT-EP stimulation software (build in LabView). The computer should have at least the following specifications: - Windows 7 64-bit, or higher - Intel Core i5 1.6 GHz, or higher - 8 GB RAM, or more - Bluetooth adapter
Tape	0.5 m	Non-allergenic skin-friendly medical tape. E.g. Leukofix.
Medical abrasive gel	2 cl	Medical abrasive gel to remove dead skin cells.
Cleansing liquid	2 cl	Alcohol, 70 % Ethanol
Cleansing tissues	2	Tissues (should preferable not release fibers)
Cleansing sticks	2	Cotton-top cleansing sticks
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.
Stimulation electrode	1	Sterile IES-5 electrode for intra-epidermal electrocutaneous stimulation. Shown in Figure 8.
Grounding electrode	1	Adhesive electrode, which will serve as a ground during the stimulation. Shown in Figure 9.
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	3	Large size towel for covering the shoulders during application of electrode gel, and 2 medium size towels for subjects to dry the hair after measurement.
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 13.
Cold Pressor test		
Cool box	1	35x34X24cm. From insulating material, preferably plastic. See figure 5. Important: Do not use polystyrene container. The coolbox should not be leaking! Even a minor leakage may cause grounding issues during cold pressor test.
Ice	5 kg	2 containers filled with ice
Water	6l	Tap water should be suitable
Thermometer	1	Mercury or digital thermometer with a range of at least -4 to 30 degrees.

Procedure

A. General Preparation

Time: > 1 hour before session

1. Send potential participants an information e-mail containing the official patient information letter. After they confirm their participation, send them another e-mail with specific information about the experiment. These e-mails can contain a text like the one shown in Figure 3 The second mail should tell potential participants to:
   - The date, time and location of the experiment.
   - Where to meet the researcher and contact information.
   - Preferably wear lenses to the experiment if they have the option to choose between lenses and glasses.
   - No alcohol is allowed within 24h before the experiment.
   - Drink the same amount of coffee as they normally do.
2. If you expect participants with another language, make sure translations are available.
3. Plan an experimental date with potential participants via e-mail or telephone.
4. After a date has been planned, inform the emergency backup person about the date and time of the experiment. Register the participant on the 'Subject Registration Form'.
5. Regularly charge the electric chair. However, do not leave the charger connected for more than a few hours, since this might damage the battery.
6. **Always make sure that sufficient sterilized electrodes are available**. If this is not the case, sterilize a batch of electrodes according to "*SOP - Sterilization of IES-5 and BiModEl Electrodes*".
7. Make sure that you know in which lab the experiment takes place and which numbers to call in case of an emergency.

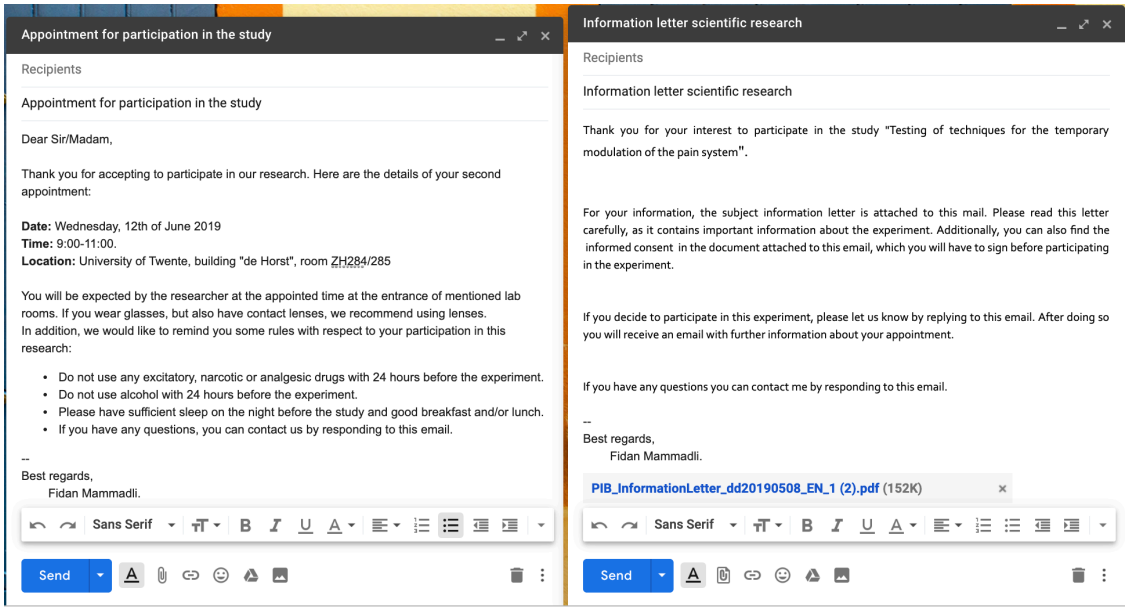


Figure 3: Example e-mails.

B. EEG System Preparation

Time: > 1 hour before session

1. Check that all of the following cables are connected properly and have not been removed:
   a) EEG amplifier to the power adapter.
   b) Fiber-to-EEG converter via the optic cable to the EEG amplifier and via a USB A to B cable to the EEG computer.
   c) Trigger generator to the computer using the USB A to B cable.
   d) Trigger generator to the EEG amplifier using the parallel cable.
   e) The EBA multi-connectors 1 and 2 to the following EEG channels: 2, 6, 17, 18, 19, 13, 14, 15, 16, 44, 47, 49

2. Attach a small sign or image in front of the chair, for subjects to focus on a single point during the experiment.

For more information about the connections, please refer to Figure 4.

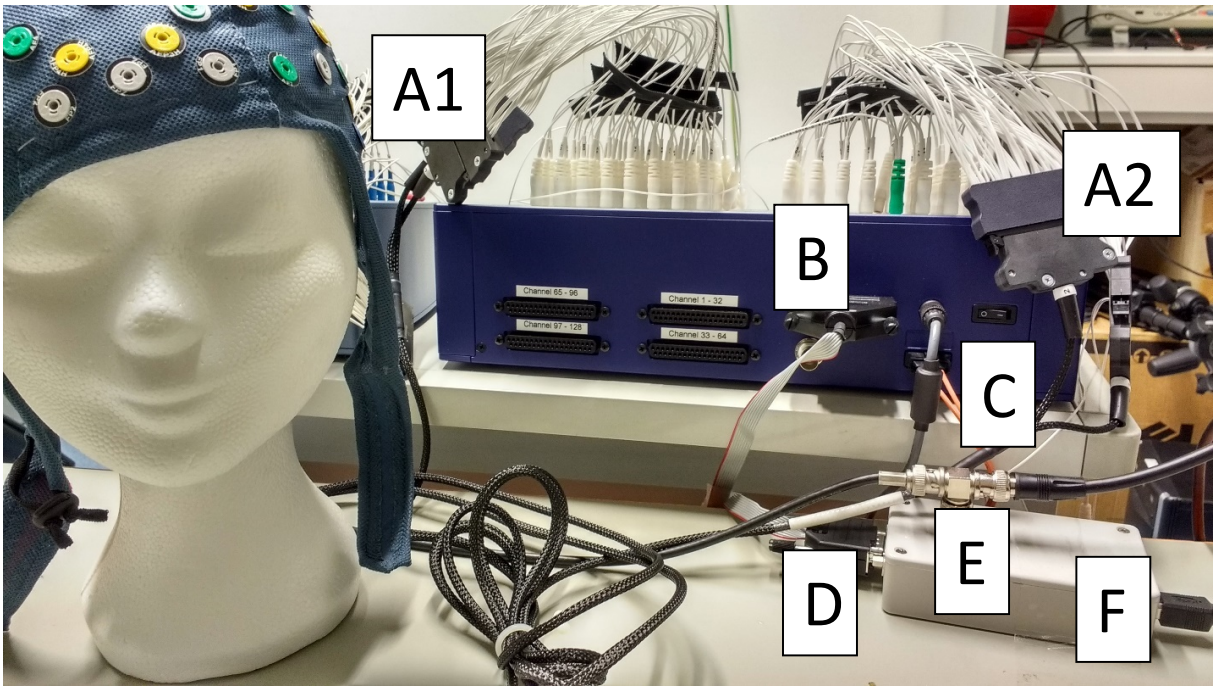


Figure 4: Connections between EEG system, trigger generator and EEG cap. A1 and A2 are EBA multi-connectors connecting the cap to individual inputs of the EEG amplifier. B is the parallel input of the EEG amplifier, which should be attached to D, the parallel output of the trigger generator. The orange double cable at C is the optical output of the EEG amplifier, which should be connected to the EEG computer via the TMSi fiber-to-USB adapter. The gray cable above the optical fiber is the power cable, which should be connected to the power via the TMSi medical power supply. E is the BNC output of the trigger generator, which should be connected to the BNC input of the stimulator, and optionally to the oscilloscope, using a splitter. F is the USB input to the trigger generator, which should be connected to the LabView computer.

C. Stimulator System Preparation

Time: > 1 hour before session

1. Connect the trigger generator to the stimulator via the BNC cable.
2. Connect the output of the stimulator to the stimulator to electrode cable
3. Charge the stimulator **with the stimulator turned off**, using the NociTRACK charger.
4. Do not turn on the stimulator beforehand, to conserve the battery.

For more information about the connections, please refer to Figure 5.

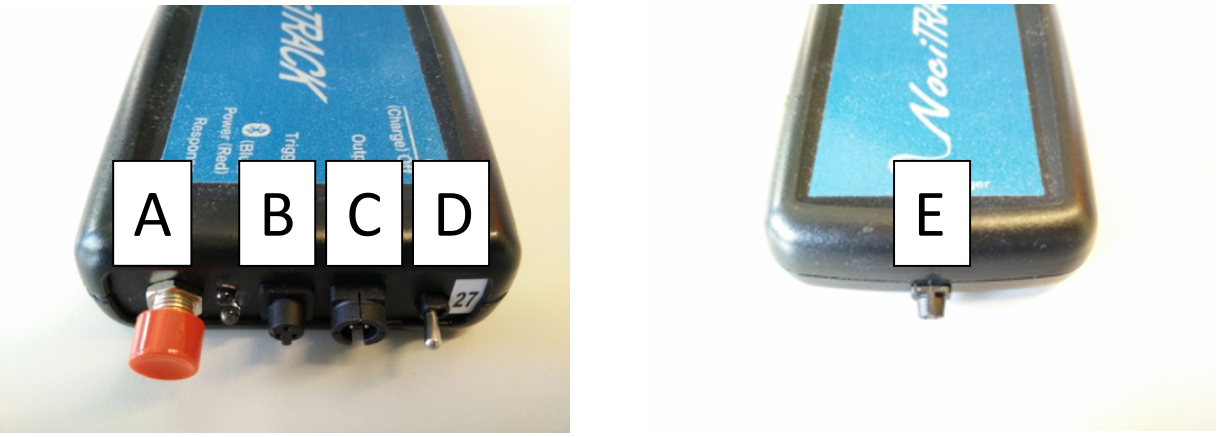


Figure 5: Inputs and outputs of the NociTRACK AmbuStim stimulator. A is a response button, which is actuated by the subject to indicate if the stimulus is perceived. B is a connection to the stimulator cable. C is an input for the trigger signal. D is the power switch. E is the input for the charger. The stimulator should be switched off while charging.

D. Materials Preparation

Time: > 20 minutes before session

1. Make sure that the following copies/ papers are available in the lab:
   a) A copy of Information letter
   b) A copy of an Informed consent
   c) A copy of blank participant questionnaire
   d) A copy of compensation declaration & the compensation itself.
   e) Pen

2. Get the signature of the emergency backup person on the 'Subject Registration Form'. Clearly indicate the starting time, ending time, subject code, name of the backup person and phone number on the form.

3. Make sure that the following materials are ready for use and easy to reach:
   a) Electrode caps (properly washed and dried beforehand)
   b) Colored measurement tape
   c) Standard measurement tape
   d) EEG electrode gel

e) Syringe
f) Blunt needles (in the package) for gel injection into the electrodes
g) Medical abrasive gel
h) Tissues and cotton sticks
i) Medical tape for IES electrode
j) ECG electrodes for the ground
k) Adhesive ground electrode
l) One IES-5 electrode in the package.
m) Cool box (figure 6)
n) Ice
o) Towel
p) Thermometer (mercury or digital)

Figure 6. Example of a coolbox used for CPT

E.CPT preparation

Time: >10 minutes before start of the experiment

1. Prepare the CPT bath for the experiment:
    a. For 1℃ bath:
        i. Add water cold water until 1/4 of the coolbox is full
        ii. Add 3-4 kgs of ice. Make sure water constantly has ice in it.
        iii. Measure the temperature with mercury thermometer to confirm the temperature. Add more ice if necessary.
        iv. Close the cap of the coolbox

    b. For 6℃ bath :
        i. Add water cold water until 1/2 of the coolbox is full
        ii. Calibrate water temperature by putting ice or warm water until desired temperature is achieved.
        iii. Close the cap of the coolbox. When closed the water will remain stable for 30 minutes

2. Place the towel on the floor opposite the electric chair. (under the feet of the participant)

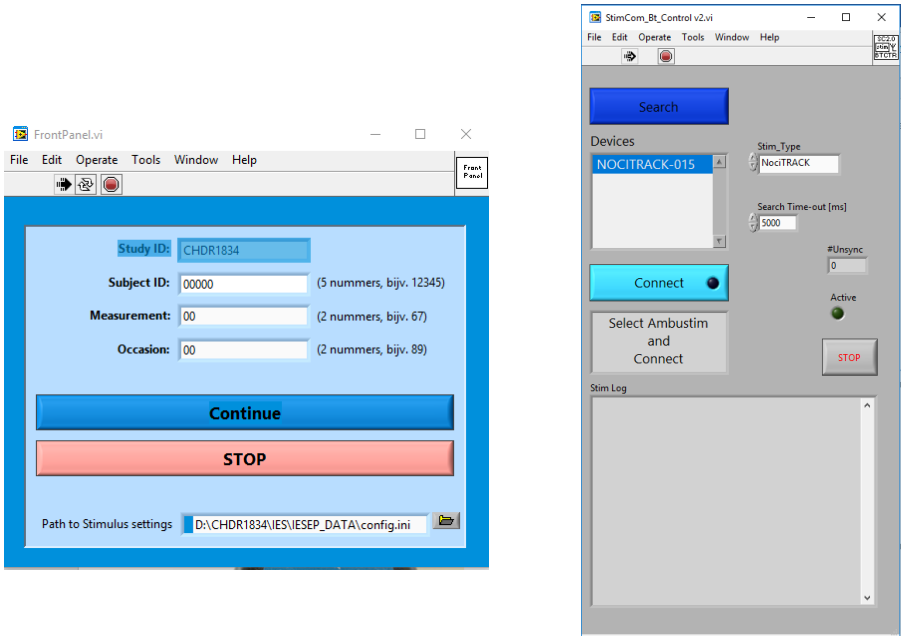


Figure 7. Subject data interface (left), Stimulus communication interface (right)

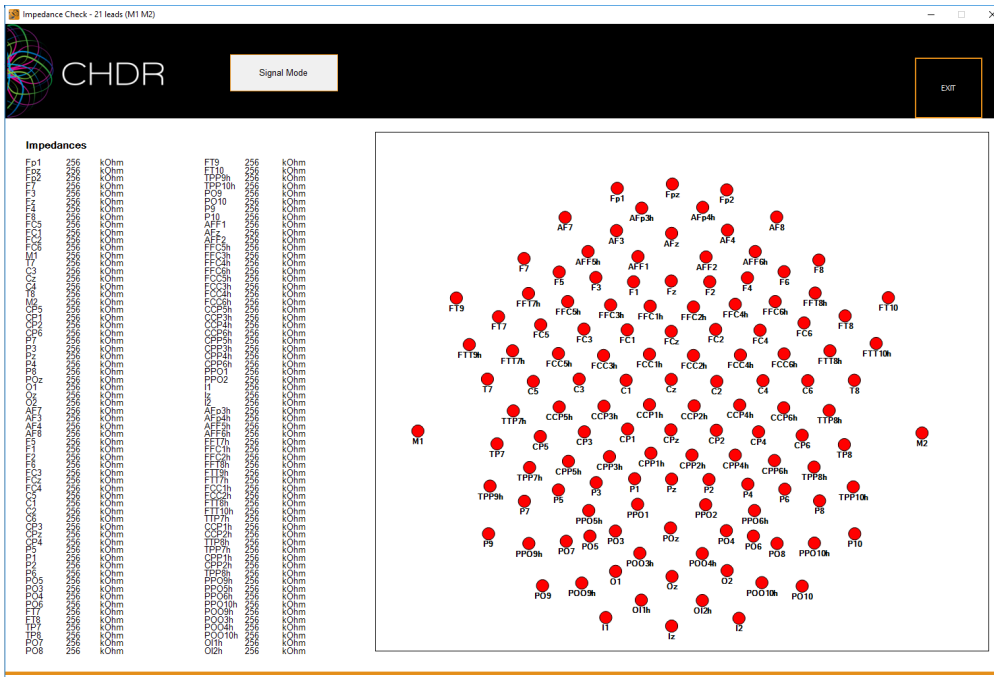


Figure 8. Impedance interface with high impedances

F. System Start-up

Time: > 5 minutes before session

1. Turn on the computer.
2. Turn on the EEG amplifier.
3. Turn on the NociTrack stimulator.
4. Start the program IESP_CHDR_shortcut on the computer
5. Enter the subject information (e.g. Subject code, measurement code etc.) and press continue. (figure 7, left)
6. Click search Stimulator communication interface will start up automatically. Press search button.
7. Select the stimulator on the device list and press connect. (figure 7, right)
8. Click OK to start impedance interface. (figure 8)

G. Subject reception and preparation

Time: start of the session

1. Meet with the subject at the entrance of the lab. To do so, be present at the entrance 10 minutes before the start of the session and leave the lab door open.
2. Give the subject a hard-copy of the information letter and the informed consent (preferably, the subject has already received and read the information letter in advance, via e-mail). Ask the subject:
   - **"Do you have any questions?"**
   - **"Would you like to participate in the study?"**
   - **"Do you want to sign the informed consent?"**
3. Explain to the subject what is going to happen. Ask the subject:
   - **"Please, set the mobile phone to airplane mode."**
   - **"Do you need to go to the toilet? The session will take approximately 2 hours."**
4. Instruct the subject:
   - **"Please take off your left shoe."**
   - **"Sit-down on the chair and set the chair to a comfortable position."**
   - **"Make sure there is sufficient space for the legs."**
   - **"The chair should be inclined slightly backwards to relieve the muscles around the neck."**
5. Use the colored measurement tape to measure which size of the cap is required.
6. Measure the distance between the nasion and the inion.
7. Measure the distance between the pre-auricular points.
8. Place the electrode cap on the head of the subject, by pulling over the forehead towards the back.
9. Ask the subject:
   - **"Please adjust the cap to fit as tightly as possible on the head."**
   - **"Please, attach the strap around the chin as tightly as possible, and pull the rope at the side of the cap for a better fit."**
10. Using the measured distances, make sure that the Cz electrode is exactly in the middle of those measured positions. If necessary, slightly adjust the position of the cap, and ask the subject for feedback on the fit.
11. Visually check if Fz is cantered as well, to make sure that the cap is not skewed.

12. Use medical abrasive gel and a cotton stick to clean the positions of the ground electrode. (e.g. forehead)
13. Use a tissue to clean the same location.
14. Make sure that the location of the ground electrode is dry and attach the ground electrode.
15. Explain the subject:
    - **"You will not feel anything from EEG measurement."**
    - **"Gel will be injected into the electrodes. To improve conduction, the skin will be scratched a bit with a blunt needle. This should not hurt, if it does, pleas say so."**
    - **"If you feel discomfort, you can indicate this at any time."**
16. Take the needle and the syringe. Show to the subject you take a new needle from the package and fill the needle and syringe with gel.
17. Fill the required electrodes in the cap (Fz, Fpz, Cz, Cpz, T7, T8, C3, C4, C5, C6, M1, M2) with gel by injecting gel while scratching the skin by turning the blunt needle with a circular motion. Use the back end of a cotton stick for extra scratching if necessary.
18. Use the impedance display on the screen to make sure impedances are below 5 kOhm. (figure 9)

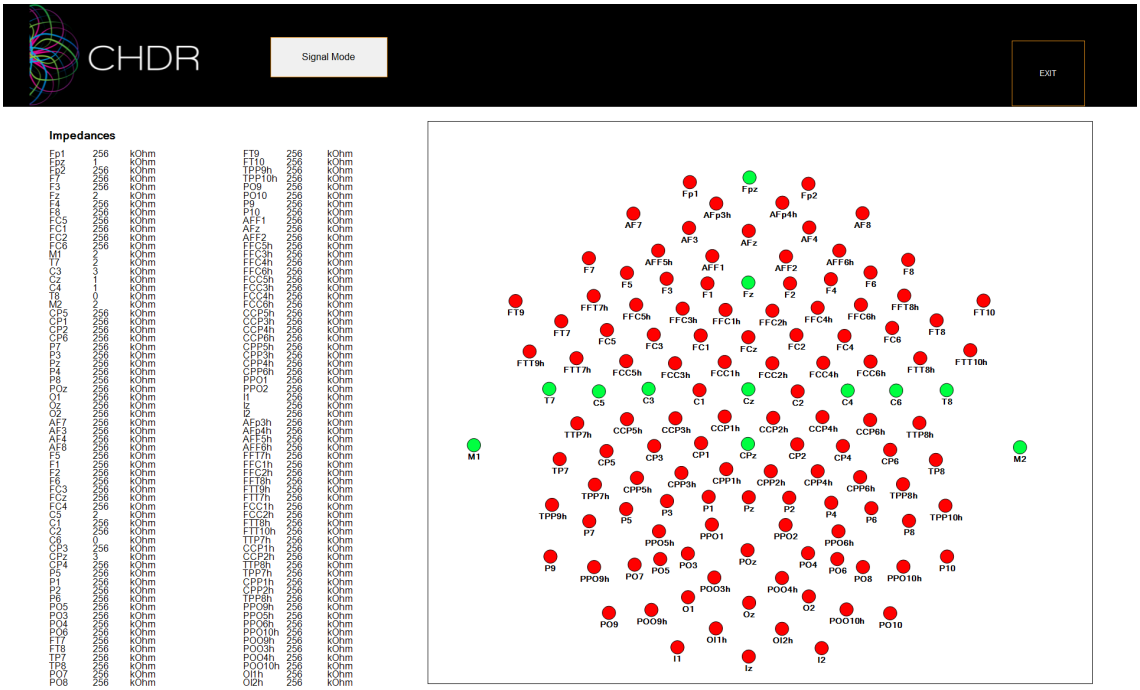


Figure 9: Impedance interface with channels connected

19. Explain to the subject that you will attach the electrodes for stimulation:
    - **"The first electrode is an electrode with small pins that will not penetrate the skin, but solely serve to stimulate the upper layer of skin."**
    - **"The second electrode is a sticky electrode which serves as a ground."**

20. Attach the electrodes as depicted in Figure 11. Ask the subject:
    - **"Please, hold the IES-5 electrode at the back of the right hand."**

    Attach the electrode with medical tape and ask the subject:

- **“Is the pressure on the electrode needles painless?”**

Glue the adhesive ground electrode right behind the IES-5 electrode on the wrist, as is depicted in Figure 12. Ask the subject:

- **“Can you press the electrode firmly onto the skin?”**

21. Attach the stimulator-to-electrode cable to the electrodes.

22. Place the coolbox on the towel, in a position where subject can easily place their left foot in.

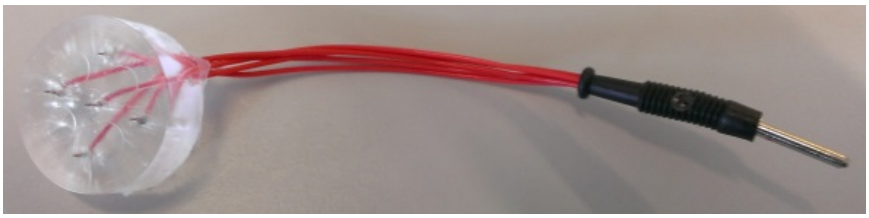


Figure 10: The IES-5 electrode.

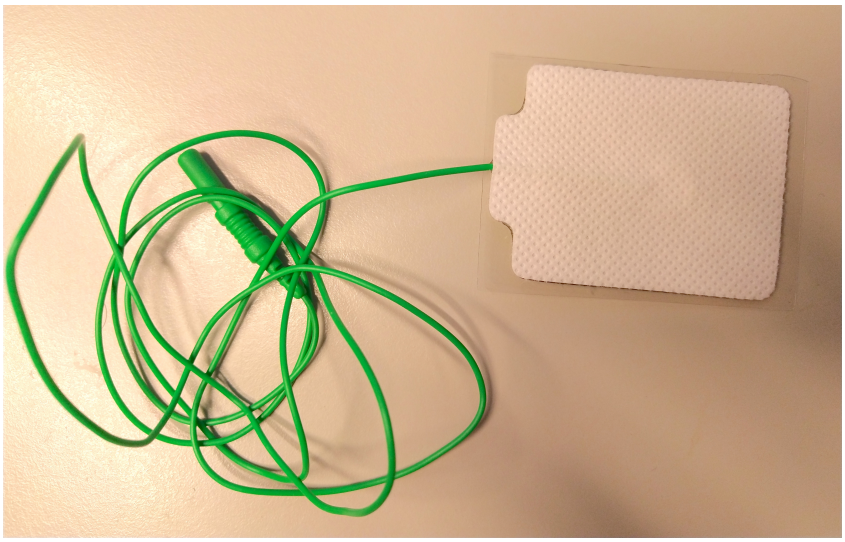


Figure 11: The adhesive ground electrode for stimulation.

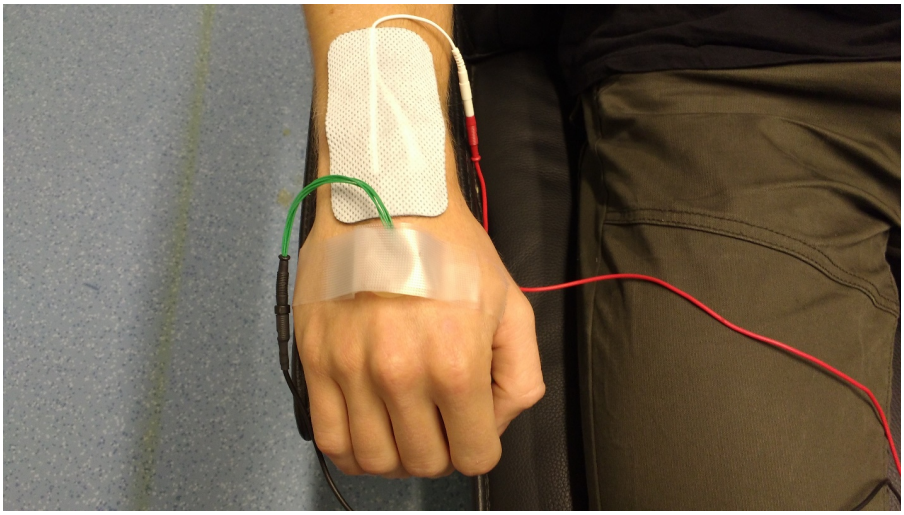


Figure 12: Example placement of the IES-5 and the ground electrode.

H. Familiarization

Time: 40 minutes after start of the session

1. Close the impedance interface.
2. A screen will open for familiarization.
3. Explain to the subject:
   - **"First, a measurement will be made for familiarization and initialization of the experiment."**
   - **"I will press on start in the application. However, the measurement will not start until you press the response button. You can pause or stop the experiment by releasing the response button."**
4. Ask the subject:
   - **"Please, hold the stimulator in the hand opposite to the side of stimulation."**
5. Press the start button on the interface.
6. Explain to the subject:
   - **"The first measurement will just serve to get acquainted with the stimuli. Therefore, you should hold the response button as long as possible and release the response button after you have clearly felt couple of stimuli. If the sequence reaches 1 mA, the measurement will stop automatically."**
7. After participant has released the button ask:
   - **Have you clearly felt the stimulus?**

8. When the subject is ready, start the second measurement via the LabView interface and tell the subject that he/she can start by pressing the response button. Explain that:
   - **"The second measurement will serve to determine the initial detection threshold. Therefore, you should release the response button as soon as you feel a sensation that you ascribe to the stimulus."**

9. After participant has released the button ask:

- **Did you release the button on the first stimulus that you felt?**

10. If necessary, the measurement can be repeated by starting the measurement a third time via the interface. If the second measurement was successful, press 'Continue' in the LabView interface.

11. A screen will open for measurement of the initial detection threshold for second stimulus type(Figure 13). When the subject is ready, start the second measurement via the LabView interface by pressing 'Start and tell the subject that he/she can start by pressing the response button.
12. Explain that:
    - **"Now the measurement will be repeated for another type of stimulus. Therefore, you should again release the response button as soon as you feel a sensation that you ascribe to the stimulus."**

13. After participant has released the button ask:
    - **Did you release the button on the first stimulus that you felt?**

14. If necessary, the third measurement can be repeated by starting the measurement a fourth time via the interface. If the third measurement was successful, press 'Continue' in the LabView interface.
15. A new screen will appear (Figure 14), which is the control interface for multiple threshold tracking.

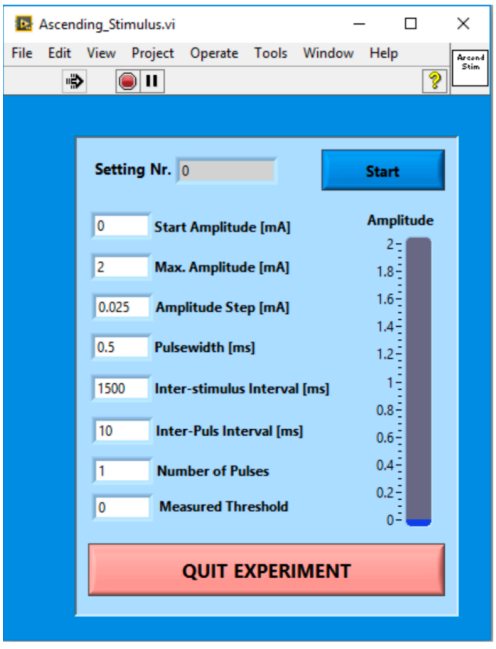


Figure 13: Interface for familiarization and measurement of the initial detection threshold.

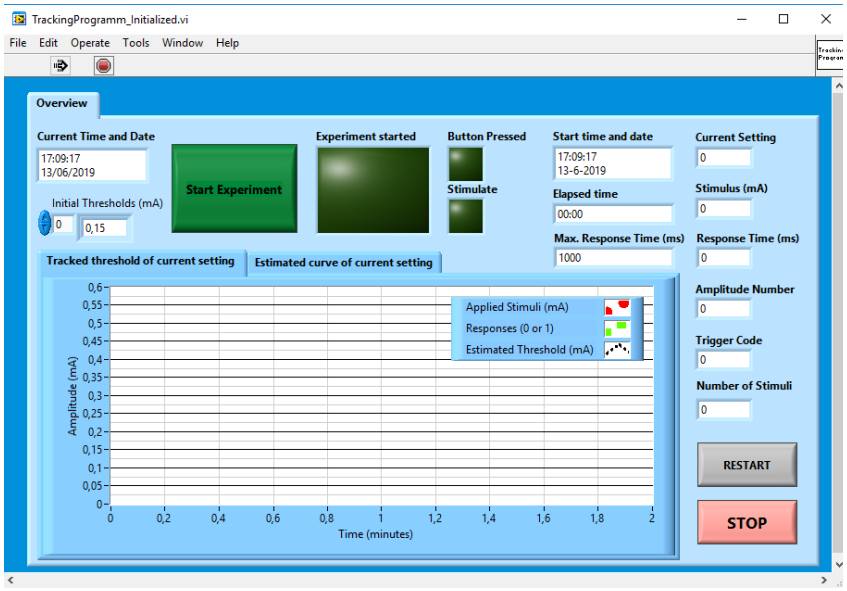


Figure 14: Interface for multiple threshold tracking.

I. Experiment

Time: 50 minutes after start of the experiment

1. Make sure once more that the water temperature is correct
2. Explain the experimental procedure to the subject:
   - **“To receive stimuli, you have to press the button.”**
   - **“You have to release the button immediately when you feel a sensation that you prescribe to the stimulus.”**
   - **“After releasing the button, you can re-press the button after approximately one second.”**
   - **“If you need a short break, you can wait longer before re-pressing the button.”**
   - **"I will ask you to immerse your foot in the water tank after around 10 minutes"**
   - **"Try to keep your foot in the water as long as you can tolerate. Once pain becomes intolerable remove your foot"**
   - **Once you remove your foot you can dry your foot with the towel if you want, but make sure you release response button.**
3. Ask the subject:
   - **“Please, blink as few times possible while holding the response button.”**
   - **“Keep looking towards the focus image on the wall while holding the response button.”**
   - **“Try to relax and not move while holding the response button.”**
   - **“Do not talk while holding the response button.”**
   - **“Keep your attention focused on the detection of stimuli.”**
   - **"Please try to follow these rules during immersion as well"**
   - **“Doing this will greatly enhance the signal quality.”**
4. Check the EEG interface to make sure that the all channels have converged.

5. Press 'Start Experiment' in the MTT interface to start recording.
6. Indicate the subject may now press the button and start the procedure
7. After 80 stimuli per type Instruct the subject:
   - **"Please release the response button"**
   - **"I will soon ask you to immerse your foot into the water tank "**
   - **"Keep your foot in the water as long as you can"**
   - **"Try to stay relaxed and not move during the immersion."**
   - **"Try not to move your facial muscles during the immersion"**
   - **"Keep looking towards the focus image on the wall"**
   - **"Do not press the response button before I instruct you"**
   - **"I will ask you to move your foot in the water several times during the immersion. Please stir water with your foot when I instruct you to do so."**
8. Open the cap of the tank.
9. Record the water temperature.
10. Record the immersion time:
    - **"Please immerse your foot into the water"**
    - **"Do not press response button yet"**
11. Keep an eye on EEG signal, once EMG activity settles down instruct the subject:
    - **"Press the response button now"**
    - **" Keep your attention focused on the detection of stimuli"**
    - Every 3 minutes instruct the participant: **"Please move your foot around in the water a bit"**
12. Once the subject removes his/her foot tell them:
    - **"If you want to dry your foot, release the response button first. Please put the towel aside after drying your foot"**
      Note: this is necessary to avoid the grounding problem, that can be caused by wet towel. This may corrupt the EEG data by 50 Hz noise.
13. Record the water temperature after immersion.
14. Continue the experiment without CPT.
15. After 230 Stimuli per type press the "Stop" button in LabView to stop the program.

J. Round-up

1. Inform the subject:
   - **"The experiment was completed successfully."**
2. Turn-off the stimulator and disconnect the subject from all cables.
3. Instruct the subject:
   - **"You can take off the EEG cap."**
   - **"You can wash your hair in the sink in the lab, or downstairs in the shower, (ZH-109, go down the stairs close to the red couches, in front of the stairs.)."**
4. Ask the subject to fill in participant questioner.
5. When the subject is ready to leave, tell the subject:
   - **"Thank you for your participation in the experiment."**
   - Give the participant the compensation for participation in the experiment. Ask him/her to sign compensation declaration.

- Ask the subject if he/she would like to be informed about the result of the experiment.
- Provide the subject with contact information in case he/she has any questions.

K. Clean-up

1. Turn-off the software, and the EEG amplifier.
2. Throw the needle, and ECG electrode to the bin.
3. Clean the cap electrodes directly after the experiment. Use the air floss to wash the 12 electrodes that were used during the experiment. (This step is important because dry gel may cause 50Hz noise during next experiment session)
4. Dry the cap on the ventilator.
5. Empty the coolbox into the sink in the lab.
6. Empty any left tank with ice.
7. Clean and dry the tank and coolbox if necessary.
8. Put all equipment back where it belongs.
9. Put all used towels into the basket to be washed later.

Figure 15: Focus image, to keep the eyes of the subject oriented in one direction, reducing EOG artefacts.