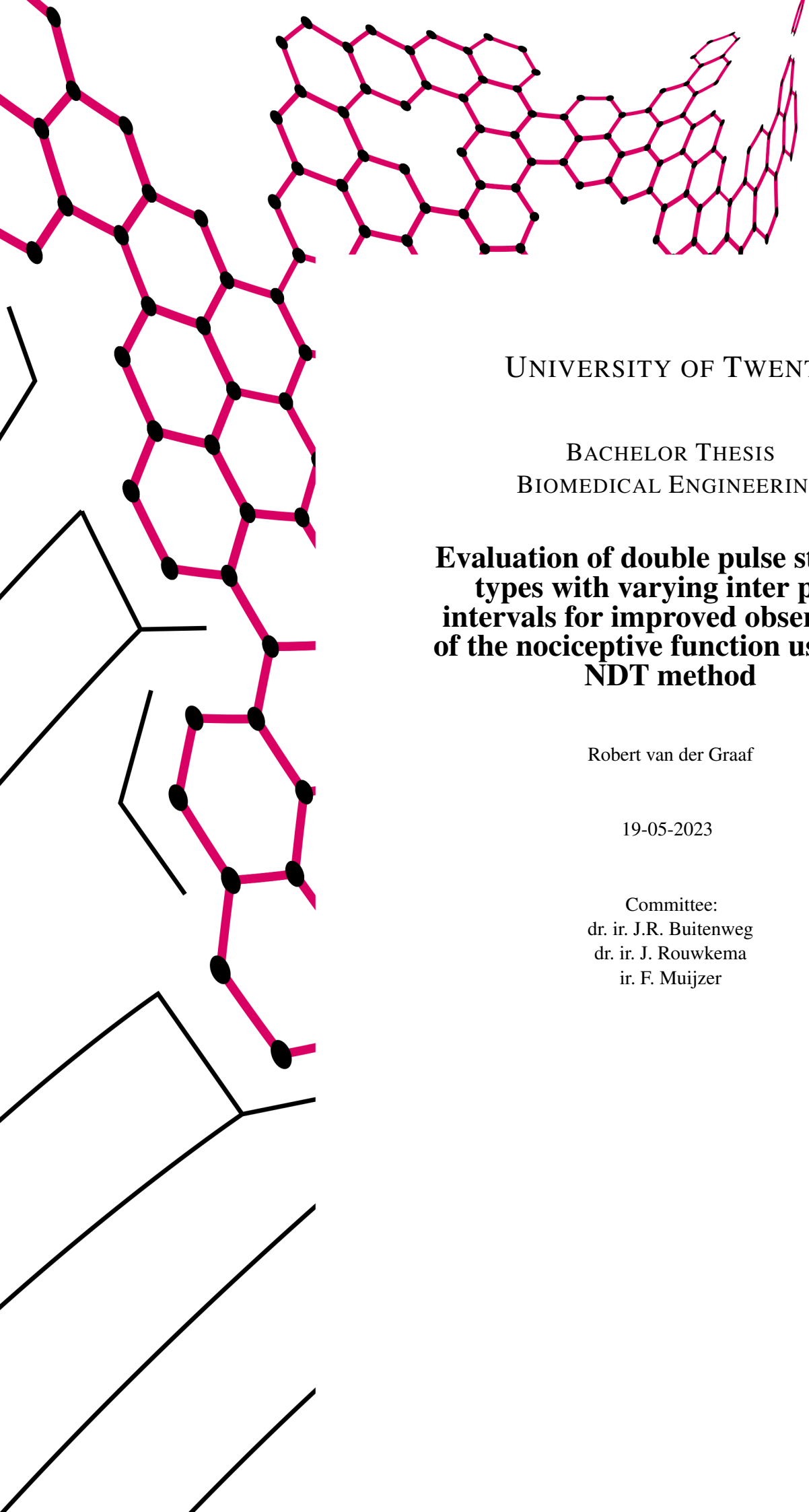

UNIVERSITY OF TWENTE

BACHELOR THESIS
BIOMEDICAL ENGINEERING

Evaluation of double pulse stimulus types with varying inter pulse intervals for improved observation of the nociceptive function using the NDT method

Robert van der Graaf

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Committee:
dr. ir. J.R. Buitenweg
dr. ir. J. Rouwkema
ir. F. Muijzer

Abstract

Objectives: Appropriate diagnostic tools that allow early detection and treatment of chronic pain are lacking. Recently, a new method was developed for observing the properties of nociceptive processing that are involved in the development and maintenance of chronic pain, through tracking the nociceptive detection thresholds (NDT's) using intra-epidermal electrical stimulation. Experiments with this method leaded to findings which suggest that the initial stimulus types that have been used in these experiments might be suboptimal for both estimation quality and information content on the nociceptive function. In this study the estimation quality of the psychophysical functions of a new stimulus set is explored, which consists only of double pulse stimuli and a larger range of inter pulse intervals.

Methods: A total of nine participants were enrolled in the study, which were healthy individuals. All participants performed the NDT method once, in which intra-epidermal electric stimulation is used to specifically stimulate nociceptive $A\delta$-fibres. Stimulation was given at the dorsal hand. Subsequently, obtained results were individually analysed using a generalized linear model.

Results: The obtained thresholds of all stimulus types have a relatively high overall stability in every measurement and show little noise. Every measurement contains a part where the stimulus type with the highest and the lowest inter pulse interval gives the highest and lowest threshold, respectively. Between stimulus type threshold differences vary per measurement, as well as the average threshold between measurements. Besides, the average response time increases with the inter pulse interval. The estimated regression parameters and their significance showed that there is a significant increase and decrease of detection probability with respect to stimulus amplitude and trial number respectively. Besides, it showed that in every measurement the double pulse stimuli with an inter pulse interval of 10 ms results in a significant increase in detection probability for every subject, and an IPI of 100 ms results in a significant increase for a part of the subjects, relative to the IPI of 200 ms.

Conclusion: The change in results with respect to the initial stimulus set show that the new stimulus set, consisting only of double pulse stimuli and a larger range of inter pulse intervals, has a higher estimation quality and contains more information about the facilitatory effect, such as temporal summation of post-synaptic potentials. It seems as if there is an increase in nociceptive detection threshold with an increase in inter pulse interval, which is caused by a facilitatory effect like temporal summation of post-synaptic potentials.

Keywords: Chronic pain, nociceptive detection threshold, nociceptive processing, inter pulse interval, intra-epidermal electric stimulation, psychophysical methods

Contents

1 Introduction

Early detection and therapeutic action with the development of chronic pain would results in a better treatment outcome and less clinical efforts per patient, but unfortunately appropriate diagnostic tools are lacking. An increased sensitivity to nociceptive stimuli, caused by maladaptive nociceptive processing mechanisms, is widely recognized as a key factor in the development and maintenance of chronic pain [1]. It is hard to diagnose chronic pain, select treatments and monitor effectiveness of these treatments, because it is difficult to observe underlying mechanisms that generate the pain and measure the effect of treatments on these mechanisms. In particular, it remains difficult to selectively activate the part of the nervous system involved in nociceptive processing and pain, and identify maladaptive nociceptive processing based on quantative outcome measures. These measures are often biased by temporary psychological states and therefore inadequate for observing the underlying psychological mechanisms of chronic pain. Development of new tools would enable better observation of nociceptive processing and support early diagnosis and effective treatment.

Recently, a new method was developed for observing the properties of nociceptive processing that are involved in the development and maintenance of chronic pain. This method consists of utilizing conscious subjective detection of intra epidermal electric stimulation of nociceptive nerve fibers (Nociceptive Detection Threshold, NDT). In this NDT method, preferentially nociceptive $A\delta$-afferents in the upper layer of the skin are activated by temporally defined current stimuli with varying number of pulses and varying inter pulse intervals (IPI's) [2]. By applying this technique below two times the detection threshold, activation of deeper non-nociceptive $A\beta$-nerve fibers is mostly prevented [3][4]. In the present method, an algorithm is implemented for automated collection of stimulus-response pairs with stimulus type specific strengths around the conscious perception threshold of the human subject. This algorithm allows tracking of multiple stimulus type specific detection thresholds during the time of the experiment (Multiple Threshold Tracking, MTT) [5, 6, 7]. The use of the MTT algorithm has revealed that the detection thresholds of single pulse stimuli are higher than the detection thresholds of double pulse stimuli. However, in all datasets measured until now, reliable estimation of thresholds from single pulse stimuli appears to be more difficult than for double pulse stimuli. This means that the single pulse stimulus has a lower estimation quality [6, 8, 9]. Furthermore, in these datasets, no significant differences are observed between thresholds from double pulse stimulus types with IPI's of 10ms and 40ms, respectively [8]. Because there is no difference, addition of this second IPI only confirms the reliability of the first IPI, just as a second IPI that is the same as the first. However, it does not provide additional information about the system that is being measured. These findings suggest that the initial stimulus types that have been used in these experiments might be suboptimal for both estimation quality and information content on the nociceptive function.

From previous studies we know that probability summation plays a big role in lower nociceptive thresholds and better estimation quality of the psychophysical function of double pulse stimuli [10]. However, the effect of the facilitatory mechanisms, such as the temporal summation of postsynaptic potentials, leading to an even lower threshold for double pulse stimuli remains relatively unknown [6]. This effect is expected to be activated by pulse train stimulation. To be able to explore this effect, it has to be isolated. However, the initial stimulus set has two changing parameters, namely the presence of probability summation by having single and double pulses, and the amount of effect of temporal summation by having different IPI's. In short, this makes that there are three problems with the initial stimulus set: the low estimation quality of the single pulse stimulus, the lack of information content on the nociceptive system because of identical results for the double pulse stimuli, and the change in two parameters while trying to observe the effect of one parameter. Through replacing the single pulse stimulus with a double pulse stimulus the instability of the felt stimuli amplitudes is expected to reduce, and the big difference between the thresholds of the single and double pulse stimuli is expected to disappear. Besides, the effect of probability summation is equalized for every stimulus type, leaving only one changing parameter. Lastly, increasing the range of IPI's is expected to result in a difference in thresholds of the different stimulus types. The aim of this study is to explore the estimation quality of the psychophysical functions of a new stimulus set with IPI's of 10, 100 and 200 ms, and compare it with the initial stimulus set. To be able to compare the obtained results with those of the initial stimulus set, the rest of the method was deliberately kept the same as in previous studies.

2 Background

Pain is experienced as unpleasant, however it has an important function in our daily life. The International Association for the Study of Pain (IASP) defines pain as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage" [11]. Pain can be divided in three classes: nociceptive, neuropathic and nociplastic pain. The first class, nociceptive pain, plays a crucial role in protecting the body, as it indicates potential or actual tissue damage. A lack of pain, is even considered dangerous, because tissue damage or disease goes unnoticed, ultimately making the situation worse [12]. The other two types, neuropathic and nociplastic pain, seem to have no functional relevance. When the nociceptive or somatosensory system is damaged this results in neuropathic pain, which is a projected sensation of pain in the body part innervated by the effected nerves [13]. In the case of nociplastic pain there is altered nociception, but no evidence of actual or potential tissue damage [14].

2.1 Chronic pain

When pain becomes persistent it outlasts the healing process and loses its function as a protecting mechanism [15]. When this persisting pain is present for longer than 3 to 6 months it is considered chronic [16, 11]. As soon as chronic pain is established treatment becomes relatively ineffective [17]. The best outcome is a 50% pain reduction in 25 to 30% of patients with chronic pain [18]. Chronic pain can be caused by all kind of injuries and diseases with a source that is mainly neuropathic or nociplastic [19]. Another common cause is surgery, which leads to persisting post surgical pain (PPSP) in 80% of the patients that got surgery [17, 20, 21, 22]. It is estimated that at least one out of ten people develops chronic pain every year [23] and approximately 20% of the adult population are affected by chronic pain [11]. The amount of pain that people experience on a daily basis can be so extreme that it limits them in their daily activities, which has a big impact on the quality of life and their ability to work [24]. Chronic pain even is one of the most large scale and difficult problems that the medical community has had to face [25], often seen together with other symptoms like depression, anxiety, physical dysfunction and social isolation [26]. Alongside the big social and emotional impact it also has a big economic impact. A Danish study estimated that about one million working days are lost every year in Denmark because of chronic pain [27] and a US study found that $61 billion a year is lost in the US because of common pain conditions that cause a loss of productivity [28, 29].

2.2 Neurophysiology

Before a noxious stimuli results in a painful sensation, a process is set in motion. First, a noxious stimulus is translated into neural activity by peripheral nociceptive nerve fibers, which is then transmitted and modulated on several locations along the central ascending pathway, through the dorsal horn in the spinal cord into the brain. Descending pathways from the brain to the dorsal horn modulate the neural activity of the ascending pathway. The strength and quality of a perceived stimulus depends on modulation of the neural activity by peripheral mechanisms [30] and central ascending and descending mechanisms [31, 32, 33]. When in a healthy condition, the ascending and descending pathways are in balance, which means the sensitizing effect of the ascending mechanisms is opposed by the inhibiting effect of the descending mechanisms [34]. When these pathways are out of balance this can lead to modifications in nociceptive processing like hyperalgesia. This condition is an increase in ascending sensitization or a decrease or even failure in descending inhibition, which results in an increased amount of pain. When modifications in nociceptive processing stay for a long period of time, this can lead to chronic pain disorders.

2.2.1 Peripheral processing

When a noxious stimulation is strong enough to be a likely cause of injury, nociceptors can be activated. Nociceptors are sensory receptors for pain and are formed by dense networks of free nerve endings of peripheral nociceptive fibers that translate the noxious stimuli to neural activity [35]. Peripheral nociceptive fibers exist of C- and Aδ-fibers. The C-fibers are unmyelinated and respond to thermal, mechanical and chemical stimuli. When a nerve fiber responds to these different stimuli types they are called polymodal. The Aδ-fibers are myelinated fibers and consist of two types: polymodal fibers (Type I) and fibers that are only sensitive to thermal and chemical stimuli (Type II) [36]. These nociceptive fibers are found mostly in the epidermis of the skin, which means they are closer to the skin surface compared to other sensory receptors in the skin. Pain excited by these fibers can be described as sharp pricking first pain and a slow burning second pain [37]. Activation of Aδ-fibers, which have a relatively larger diameter (1-6 μm), and therefore a higher conduction velocity (Type I: 25-55 m/s, Type II: around 15 m/s), results in a sharp pricking first pain. Activation

of C-fibers, which are umyelinated and smaller (0.2-1.5 μm) with a slower conduction velocity (0.5-2 m/s), results in the slow burning second pain. First the Aδ-fibers will be activated, and when the stimulus intensity increases, eventually C-fibers will be activated. A third type of nerve fiber that is located deeper in the skin is the Aβ-fiber, which carries information related to touch. It is larger (6-12 μm) and thus faster (33-75 m/s) than C- and Aδ-fibers [38]. When these fibers are activated by a non-noxious stimulus, this results in a tactile sensation and can lead to inhibition of surrounding Aδ-fibers, which is known as the gate-control theory [39]. According to this theory pain is modulated in the dorsal horn by input from non-noxious stimuli. Tactile activation of Aβ-fibers closes the gate, inhibiting nociceptive input by restricting it from travelling up to the brain [39]. However, in case of inflammation or injury the activation threshold for noxious stimuli is decreased and is known as peripheral sensitization. With peripheral sensitization noxious stimuli lead to even more pain (hyperalgesia) and normal non-noxious stimuli can also become painful (allodynia) [40].

2.2.2 Central processing

The cell bodies of the nociceptive fibers are located in the dorsal root ganglia, close to the spinal cord. Here, the neural signals from the peripheral nerves are being transmitted to the dorsal horn in the spinal cord, after which it is send up the spinal cord via the spinothalamic tract to the thalamus. The thalamus works as a relay station and will direct the flow of information that it receives from the spinal cord to the brain areas that are designated for processing certain neural activity [41]. Activation of these brain regions results in a conscious pain experience [11]. In the various parts of the grey matter of the dorsal horn, called laminae, nociceptive fibers synapse with dorsal horn neurons. There are three types of dorsal horn neurons: nociceptive specific (NS) neurons, wide-dynamic-range (WDR) neurons or interneurons [42, 35]. Interneurons are located in and between all laminae to receive input from nociceptive fibers via the ascending patway, as well as from descending pathways. They have an important role in processing tactile and nociceptive neural activity [43].

In the ascending pathway, nociceptive information can be processed by several mechanisms. The first and most important one is the gate control theory, which was already discussed in the previous section. Other examples are wind-up effects and short and long term plasticity. Wind-up effects are caused by repeated low-frequent C-fiber stimulation, leading to an increase in perceived pain intensity over time [44]. Short- and long-term plasticity are inhibiting or facilitating effects on the postsynaptic potentials after repeated stimulation, resulting in a effective decrease or increase in postsynaptic neural activity, respectively [45]. Central sensitization is a common cause of chronic pain and expresses itself in a higher postsynaptic neural activity, with the same peripheral neural activity [1].

The brain does not passively receive pain information from the body, but also actively regulates it with multiple central descending pathways via the medulla in the spinal dorsal horn [34]. It provides a survival function, because pain experience will be altered according to the situation, enabling your body to function properly in life threatening situations [46]. There are two systems that regulate descending inhibition and facilitation of noxious stimuli. The first is diffuse noxious inhibitory control (DNIC), which is also described as the "pain inhibits pain" phenomenon [47, 48], as the intensity of a painful stimulus is reduced by the application of a second painful stimulus. The second is top-down control modulation via the pariaqueductal gray (PAG) and the rostral ventromedial medulla (RVM). The nociceptive processing is then either amplified or inhibited in the dorsal horn. It remains unclear via which system descending modulation takes place, and because of this it is described with the general term 'conditioned pain modulation' [49].

2.3 Observation of Nociceptive Processing

Identification of the underlying mechanisms could result in better diagnosis and is crucial for development and selection of effective treatments for chronic pain, because it identifies potential targets for treatment [50, 51]. To understand and identify the underlying mechanisms that generate a certain type of chronic pain, it is important to objectively quantify the nociceptive function. However, because pain is a complex, subjective phenomenon, this is a difficult task. Development of a solution method has to consist of an selective stimulation for activation of the nociceptive system, and techniques to observe and quantify behavioral and neurophysiological response to stimulation.

2.3.1 Nociceptive Stimulation

To be able to observe the nociceptive system, stimulation has to selectively activate Aδ and C-fibers in a safe, reproducible and quantifiable way [52]. Researchers already invented a lot of different methods for this. Mechanical pressure stimulation can create a lot of different pain intensities and stimulus durations, but because of activation of tactile Aβ-fibers it is non-selective. Besides, it is difficult to apply fast and precisely controlled stimuli. Contact thermal stimulation

can selectively activate nociceptive Aδ and C-fibers, but the change in temperature is to slow for accurate measurement of responses on the stimulation. However, thermal stimulation using a laser allows quick and selective stimulation [52]. Therefore, laser stimulation is considered as one of the best options for nociceptive stimulation. However, the equipment that is needed for this method is expensive [53] and because of heat build up and peripheral sensitization, the inter stimulus interval is relatively large with a stimulus every 5 to 20 seconds [3]. A cheaper method for quick stimulation is electrical stimulation. However, transcutaneous electrical stimulation also activates tactile Aβ-fibers because of their lower electrical threshold. Nonetheless, using a special electrode with small needles that protrude the epidermis of the skin [53, 54], in combination with a stimulus intensity that is less than twice the detection threshold [3, 4], will selectively activate Aδ and C-fibers. The big advantage of electrical stimulation is the precise controllability of the temporal stimulus pattern, that is determined by stimulus properties such as the pulse shape, pulse amplitude, pulse width (PW), number of pulses (NoP) and inter pulse interval (IPI). By studying the effect of these different properties given responses, the behavior of the nociceptive system can be observed and give a better understanding of how the several mechanism of the system work. For example, by increasing the amplitude more fibers are being activated [55], and by changing the number of pulses and the inter pulse interval, inhibition and facilitation of repeated stimulation can be studied [6] [56].

2.3.2 Observation Methods

Because pain is a subjective experience, observation is often based on the indicated pain perception of patients. Questionnaires such as the Numeric Rating Scale (NRS) and Visual Analogue Scale (VAS) are often used to give a single measure to depict the experienced pain intensity. There are also more detailed questionnaires, such as the McGill Pain Questionnaire, which specifically estimate multiple sensory and emotional aspects of perceived pain [57]. Besides, there are questionnaires targeting specific patient groups, for example the painDETECT questionnaire for patients with neuropthic pain [58]. Questionnaires give a good overall idea of pain perception and the effect it has on patients, but they are easily biased by temporary psychological states. Besides, they provide little quantification, and therefore little information of the different mechanisms of nociceptive processing.

Psychophysical methods are used to describe the relation between physical stimuli and corresponding subjectively reported responses [59]. Methods that are often used are Quantative Sensory Testing (QST) methods, from which a relation between the presented stimuli and the given responses is presented in a psychometric curve. This curve relates the detection probability and the stimulus amplitude in a sigmoidal function, and is described by a threshold and a slope. The threshold is the amplitude with a 0.5 detection probability and the slope represents the reliability of stimulus detection by a subject [60]. The pain threshold is currently used to determine gain or loss of sensory function. A lower nociceptive threshold, for example, means an increase in sensory function, leading to an increased perception of pain, which is known as hyperalgesia [61]. The nociceptive threshold is thought to be a useful tool in the prediction of chronic pain disorders [62] [63].

Because a stimulus is processed by a lot of different peripheral and central mechanisms, a single threshold cannot be used to distinguish these mechanisms. Through presenting stimuli with different properties randomly, the obtained thresholds can be used to determine the contributions of different nociceptive mechanisms to stimulus processing. However, these QST methods are still influenced by type of stimulation instrument, room temperature, site of stimulation, size of stimulation area and inter stimulus interval (ISI) [64], as well as psychological factors such as patient motivation, vigilance and attention [65].

2.4 Challenge and Goals

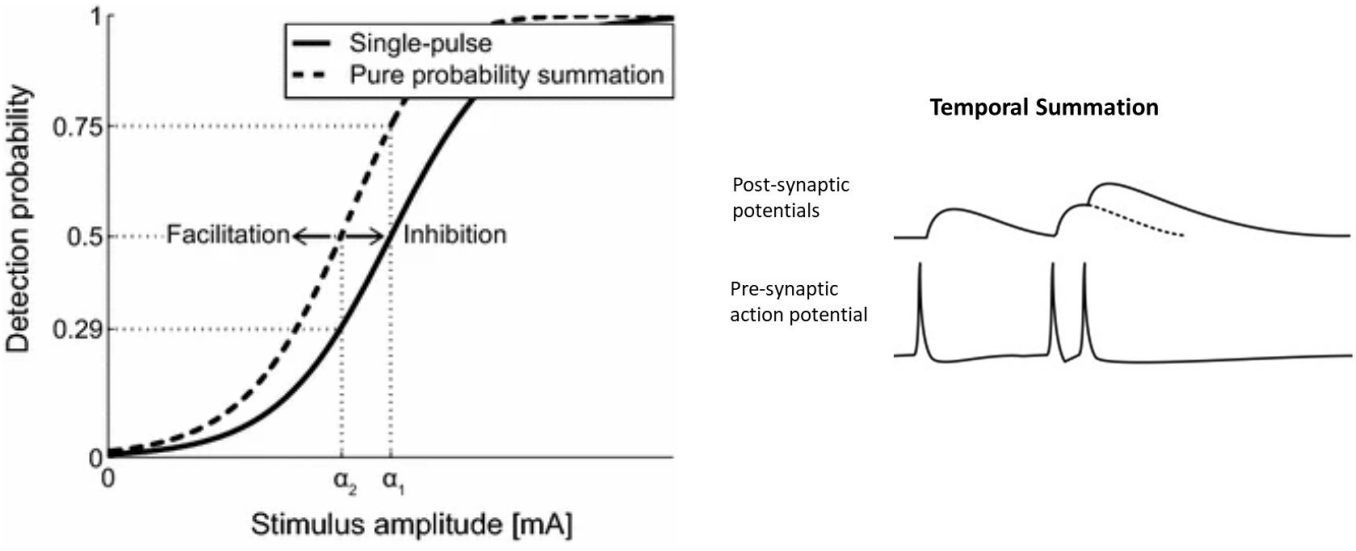
2.4.1 Previous Study Results

Using data sets from measurements performed with the currently used stimulus set in the NDT method [8], raw stimulus amplitude, corresponding responses and obtained thresholds were plotted against the trial number, which can be seen in Figure 2, to be able to compare these results with the results obtained with the new stimulus set. Previous studies found that estimation quality of the psychophysical function of a single pulse stimulus is low, because it is very unstable [6, 8, 9]. Addition of a pulse lowers the NDT significantly, and resulted in a steeper slope due to probability summation and facilitatory mechanisms such as the temporal summation of double pulse stimuli [6]. One of these studies also showed that there is no difference in thresholds and slopes between double pulse stimuli with an IPI of 10 ms and 40 ms

[8].

As mentioned in the previous paragraph, addition of a pulse involves probability summation. According to this theory the detection probability of a double pulse stimuli is determined by the detection probability of each of the individual pulses [10]. The detection probability of a double pulse stimuli is given by the following formula: $p_d = 1-(1-p_1)(1-p_2)$. In case of pure probability summation the probabilities of the individual pulses are independent and equal, and this formula can be reduced to $p_d = 1-(1-p_1)^2$. In Figure 1a the effect of probability summation can be seen as the decrease in threshold and increase in slope of the psychophysical curve. Here it is showed that the threshold of a double pulse stimulus equals a 0.29 detection probability for a single pulse with the same amplitude. The other way around, the threshold of a single pulse equals a 0.75 detection probability for a double pulse with the same amplitude. However, when the probabilities of the individual pulses are not independent and equal, the probability of the second pulse will be influenced by the the first pulse through inhibitory or facilitatory mechanisms, which results in a different psychophysical curve than the pure probability summation curve [6].

In addition to probability summation, it was found that the threshold of a double pulse stimulus was significantly lower than the expected threshold that was based on pure probability summation, which means that first pulse has a facilitating effect on the the detection probability of the second pulse [6]. In a later study peripheral super- and sub-excitability were found to be canceled out by a stronger central mechanism, like temporal summation [8]. This effect can be seen in Figure 1b and occurs when two stimuli are given with an IPI that is shorter than the duration of the post-synaptic signal. The second post-synaptic potential will summate with the current amplitude of the first potential, resulting in a larger potential. This leads to an increase in detection probability, and thus a decrease in nociceptive detection threshold [66].



(a) [6] **(b)**

Figure 1: Effect of double pulse stimulation in terms of (a) probability summation and (b) central temporal summation of post-synaptic potentials. The effect of probability summation can be seen as the decrease in threshold and increase in slope of the psychophysical curve. The fascilitatory effect of temporal summation can be seen as the second post-synaptic potential will summate with the current amplitude of the first potential, resulting in a larger potential. This leads to an increase in detection probability, and thus a decrease in nociceptive detection threshold.

As expected, when analysing the results obtained with the initial stimulus set both Figures 2a and 2b show a big difference in threshold between the single and double pulse stimuli, with the single pulse stimuli having a much higher threshold, where the thresholds of the double pulse stimuli are almost identical. Besides, it can be seen that the single pulse stimulus results in much more unstable data than the double pulse stimulus, which is displayed best by the individual raw stimulus points showing sudden increase or decrease, indicating the low estimation quality of the psychophysical function of a single pulse stimulus. On the contrary, the double pulse stimuli result in much more stable data.

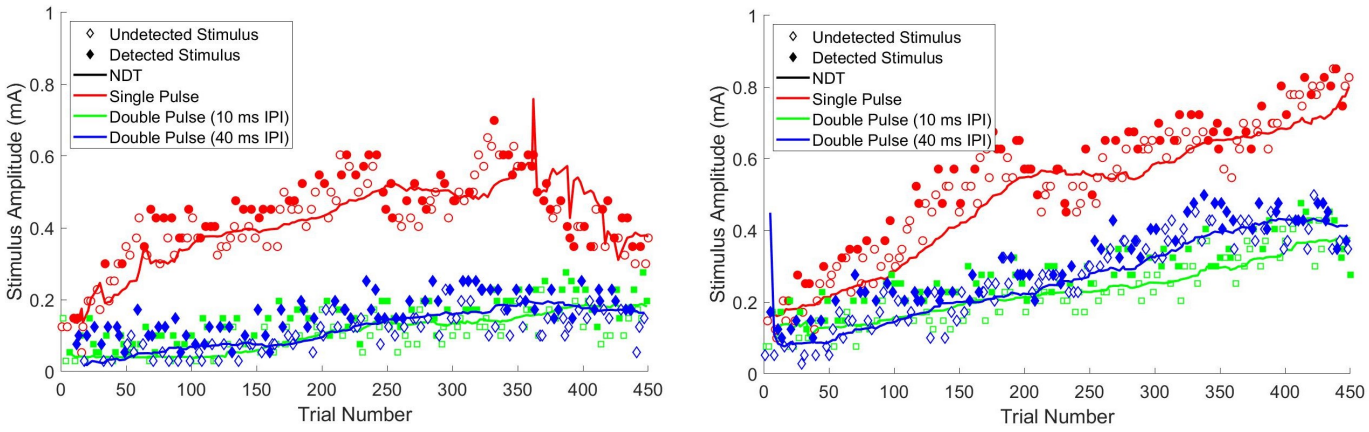


Figure 2: Typical examples of measurements with the initial stimulus set using the NDT method. Raw stimulus amplitude, corresponding responses and obtained thresholds are plotted against the trial number. It stands out that the single pulse stimulus has a much higher threshold than the double pulse stimuli, which are almost identical. The threshold of the single pulse stimulus also seems to result in relatively unstable data.

In the last section of the introduction the problems and their possible solutions were discussed. Through changing the single pulse to a double pulse stimuli, and using a larger range of IPI's, a better estimation quality and more information content on the nociceptive system is expected, with the facilitatory mechanisms in particular. The new stimulus set consists of a stimulus with a small IPI at which the effect of temporal summation should be the largest, and a stimulus with a large IPI at which the effect of temporal summation is expected to be a lot smaller. Another stimulus was added with an IPI between these two limit IPI's to give a more detailed view of the possible change in the nociceptive threshold. As smallest IPI a value of 10 ms was taken, because we want to stay above the time that is required for nerve repolarization. As largest IPI a value of 200 ms was taken, because this is thought to be close to the maximum amount of time it takes for a post-synaptic signal in dorsal horn neurons to decay, and temporal summation does not occur anymore [67]. Lastly, an IPI of 100 ms was added in between.

3 Methods

3.1 Participants

A total of nine healthy participants were enrolled in the study. The participants were recruited through a canvas page of the University of Twente. To be included the participants had to be healthy individuals. Exclusion criteria were skin abnormalities, abnormal blood pressure, heart problems, diabetes, chronic pain, implanted stimulation devices, pregnancy, usage of stimulants or narcotics (e.g. alcohol, drugs or painkillers) within 24 hours before the experiment and pain complaints at the time of the experiment. All participants got remuneration for their participation in the study in the form of a gift voucher and could withdraw from the experiment at any time.

3.2 Stimuli

In the NDT method intra-epidermal electric stimulation is used to specifically stimulate nociceptive $A\delta$-fibres that lie mostly in the epidermis and thus closest to the skin surface [3]. This is done by placing a custom-made cathodic electrode with five 0,2 mm interconnected microneedles, which slightly protrude into the epidermis, at the dorsal hand. Perceived stimulation will result in a pinprick-like sensation [54]. Another (rectangular 9 x 5 cm TENS) electrode, placed proximal just behind the needle electrode, works as an anode. Stimuli are applied in the form of square wave pulses by a 1-channel constant current cathode stimulator (NociTRACK AmbuStim, University of Twente, Enschede, The Netherlands). The stimulus set consists of three stimulus types:

- A double $210\mu s$ pulse with a inter pulse interval of 10 ms (DP10)
- A double $210\mu s$ pulse with a inter pulse interval of 100 ms (DP100)
- A double $210\mu s$ pulse with a inter pulse interval of 200 ms (DP200)

3.3 Procedure

The participant was seated in a comfortable chair. Next, two experiments were done where a custom computer program (written in LabVIEW 2011) controlled all stimulation procedures as well as the registration of stimulus amplitudes in mA, stimulation times in ms and responses to stimuli. The participant was instructed to hold the button until stimulation was felt and release it immediately after a perceived stimulus. After approximately one second the button had to be repressed. The first experiment was for the participant to familiarize with the electric stimulation and to make a rough initial estimate of the detection threshold. This was done by using a standard staircase procedure with a step size of 0,025 mA. During the second experiment an algorithm was used to simultaneously track the detection thresholds of the three different stimulus types (Multiple Threshold Tracking, MTT). It applied stimulus types in a random order and used an adaptive staircase procedure with a vector of five equidistant amplitudes that was centered around the detection threshold. The algorithm chose a random amplitude from this vector as next stimulus for a certain stimulus type. All the amplitudes in this vector were increased or decreased with a step size of 0,025 mA, when a stimulus was detected or non-detected, respectively. After this, the next amplitude was randomly selected from the updated vector. This procedure was repeated until all three stimulus types were applied 100 times. When this was done the experiment was ended.

3.4 Results Analysis

The analysis of this experiment was done separately for each measurement to exclude incomplete and poor quality data. Data selection was done as seen in Figure 3. The data was checked for incompleteness and screened through calculating detection rate and visually inspecting the raw data plots. Low detection rate (below 20%) and high drifting stimulus amplitudes without reaching a stable phase resulted in exclusion of the data for further analysis. Analysis of the obtained stimulus-response pairs was performed in Matlab (The MathWorks Inc., version 2020b). Raw stimulus amplitude, corresponding responses and obtained thresholds were plotted against the trial number to get a rough expression of the data this stimulus set produces. In addition, a generalized linear model (GLM) was used in combination with a logit link function to estimate the detection probability for different stimulus amplitudes of the stimulus types on an individual level. The used model is shown in Wilkinson notation in Equation 1, including the effects of stimulus amplitude (AMP, in mA), IPI of 10 ms (IPI_{10}, boolean), IPI of 100 ms (IPI_{100}, boolean), and trial number (TRL). With this model the effect of the different parameters on the detection probability can be found. It takes the stimulus type with an IPI of 200

ms as reference, which means the effect of IPI_{10} and IPI_{100} are relative to this stimulus type. The threshold and slope estimates were obtained from the estimated model regression parameters. The model also return the p-values of all the different regression parameters, which show if certain regression parameters have a significant effect on the detection probability. The lower this value, the higher the significance, and when it is smaller than 0.05 the effect of a certain parameter is considered significant.

$$ln\left(\frac{P_d}{1-P_d}\right) \sim 1 + AMP + IPI_{10} + IPI_{100} + TRL \tag{1}$$

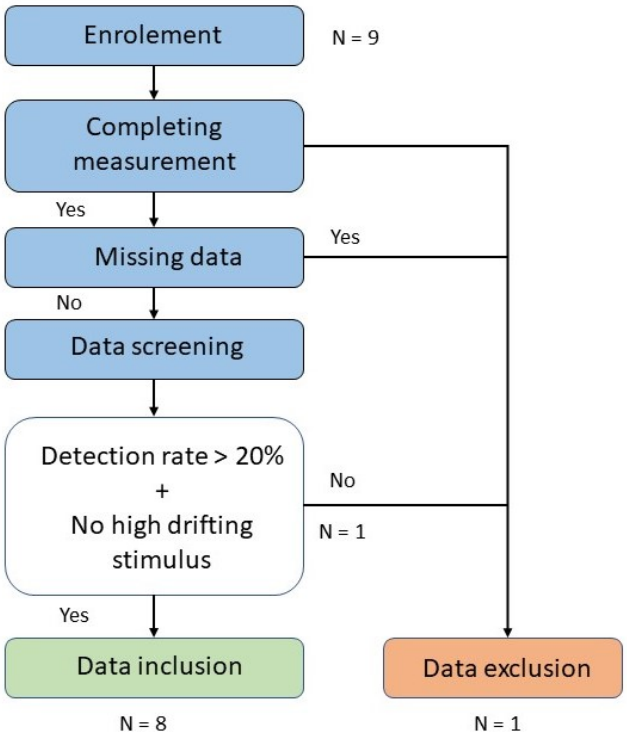


Figure 3: Flowchart that was used for data selection. A total of nine participants enrolled in the study, which all completed a single measurement with the NDT method. Subsequently, one measurement was excluded due to a detection rate below 20% and a high drifting stimulus.

4 Results

In total nine healthy controls participated in this study, which all completed the NDT method measurements. However, data of one subject was excluded because of a detection rate below 20% and a high drifting stimulus without reaching a stable state. This resulted in eight subjects that were selected for further analysis.

4.1 Excluded Data

The raw data that was excluded of further analysis can be seen in Figure 4. Notice the difference in y-axis with the included data in Figure 6. The stimulus amplitude keeps on increasing, because the subject does not feel the stimulation most of the time. However, when the stimulus reaches an amplitude of around 1.5 mA it will remain in a steady state without being detected. It also stands out that the DP10 stimulus has a higher detection rate than the other stimulus types.

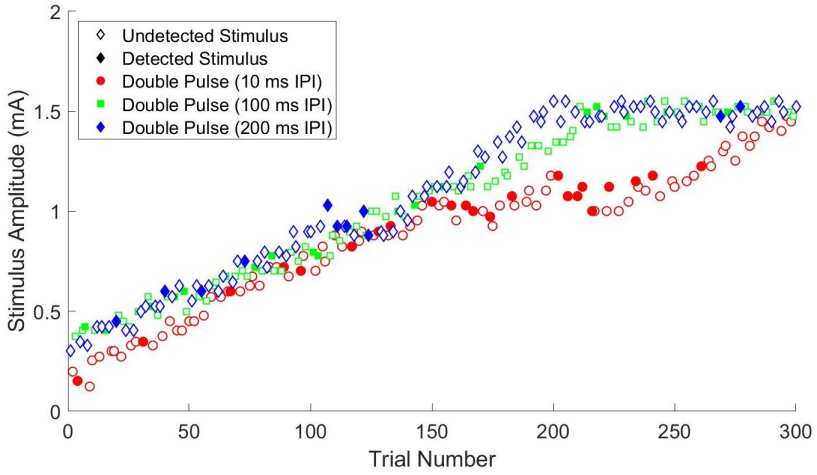
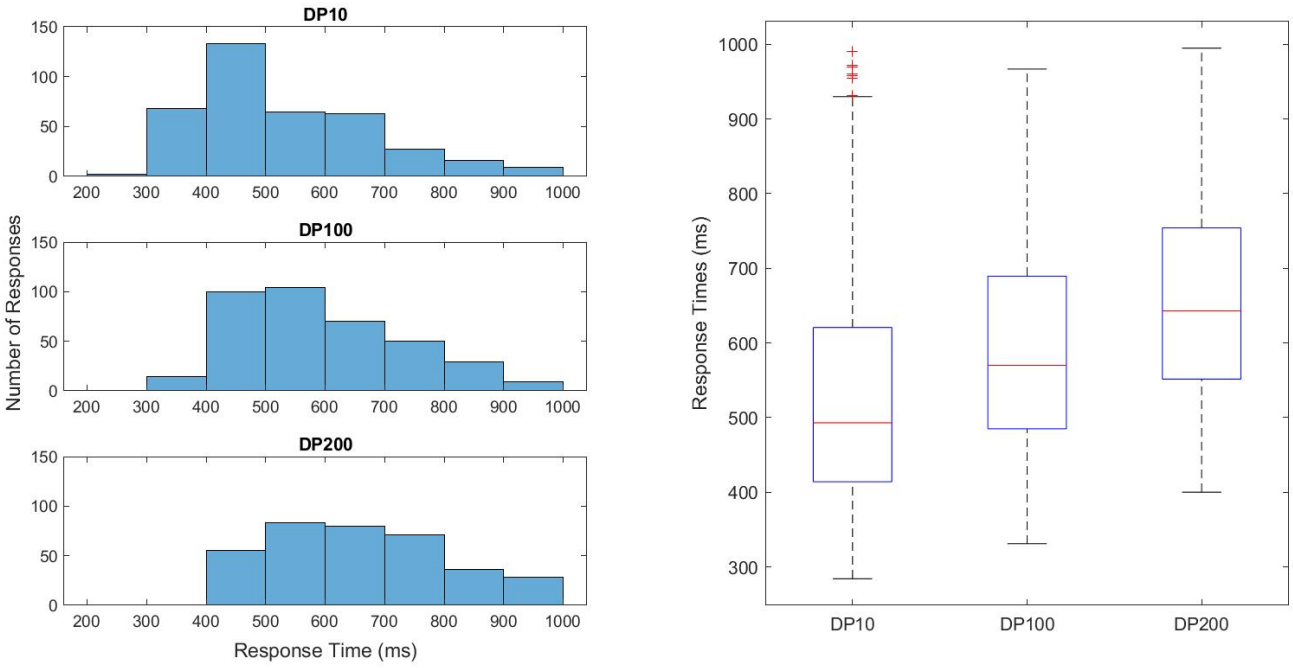


Figure 4: Raw stimulus amplitudes and corresponding responses of excluded data from subject 4. The large amount of undetected stimuli indicate the low detection rate. Besides, the stimulus amplitude keeps on increasing. This means this data is unsuitable for analysis.

4.2 Response Times

To indicate the type of nerve fibers that were stimulated and whether the first or second pulse of the double pulse stimulus was detected, the reaction times of all subject where merged into one data set and visualised in a boxplot seen in Figure 5b and histograms seen in Figure 5a. Here the reaction times of the different stimulus types can be compared with each other. Figure 5a shows a relatively sharp peak at response times between 400 and 500 ms for stimulus type DP10. This peak seams to have flattened out to a broader peak for DP100 between 400 to 600 ms and even further for DP200, where a slight peak between 500 and 700 ms is seen. Besides the peak flattening out, it also moves to the right on the x-axis, which means it changes from a slight positive skew to a more normal distribution. It seems that the average response time increases with inter pulse interval, which can also be seen in Figure 5b. The median response time is 493 ms for DP10 and increases to 570 ms and 643 ms for DP100 and DP200, respectively. The whole distribution shifts to higher response time values, as the minimum, maximum and interquartile range also shift upwards in the boxplot.



(a) (b)

Figure 5: Visualization of response times per stimulus type in a (a) histogram and a (b) boxplot. The peak shifts up the x-axis in the histogram, and the median response time increases from 493 ms for DP10 to 570 ms for DP100, and ultimately 643 ms for DP200. This shows an increase of response time with IPI.

4.3 Nociceptive Detection Threshold

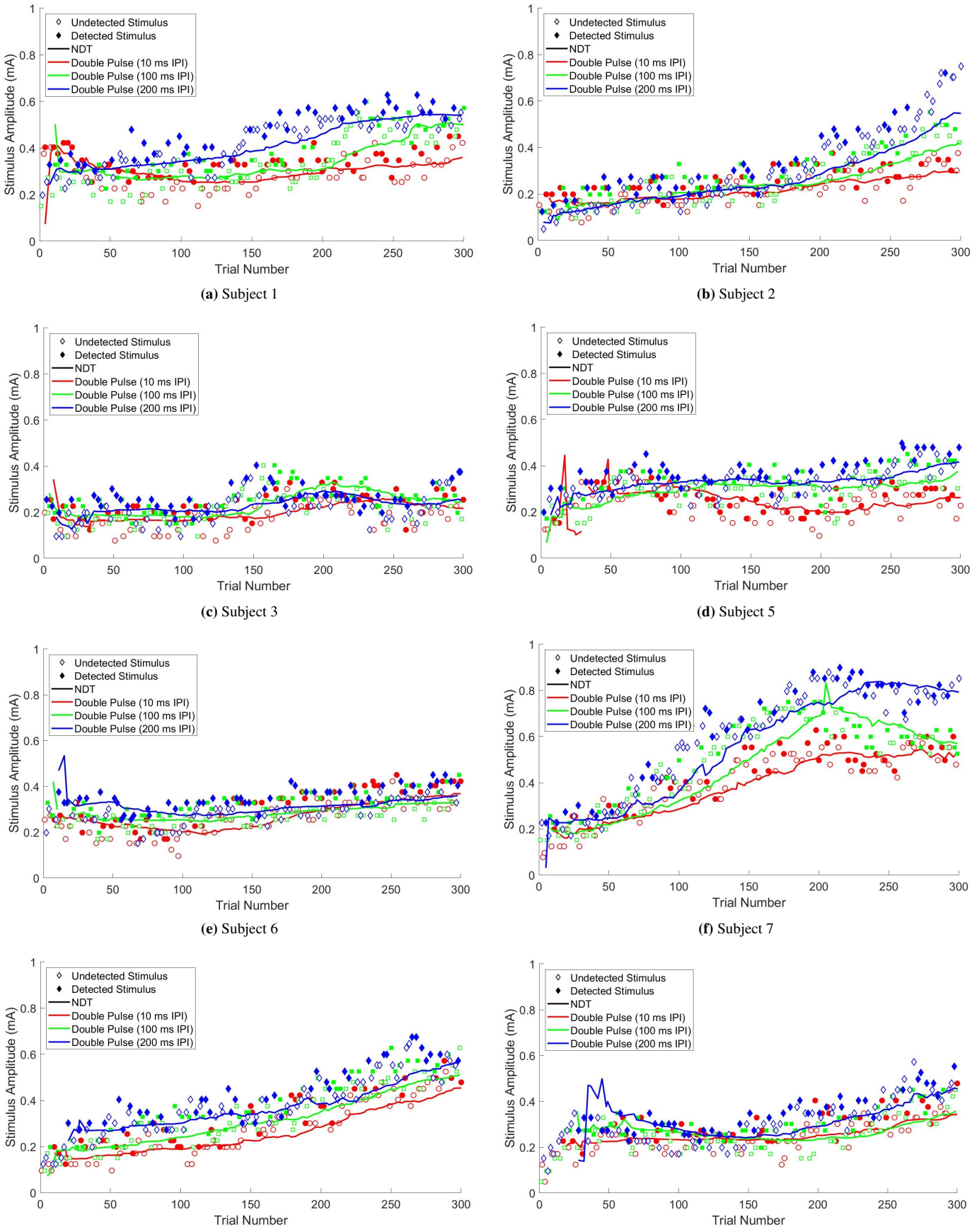
4.3.1 Raw Data

From the included data, raw stimulus amplitude, corresponding responses and obtained thresholds were plotted against the trial number to get a rough expression of the data this stimulus set produces. The data plots can be seen in Figure 6.

In Figure 6a the obtained threshold of DP10 starts at a higher amplitude than the other stimulus types, which start at almost the same amplitude, and keeps gradually decreasing until a trial number of ± 100 at which it is lower than those of the other stimulus types. The rest of the measurement it gradually increases again, together with the other stimulus types. From then on DP200 has the highest threshold, followed by DP100 and DP10. It also stands out that the threshold of DP100 begins close to the threshold of DP10 but from a trial number of ± 200, drifts to DP200 and ends closer to this stimulus type. Figure 6b shows all the different thresholds starting and staying almost the same as each other, until a trial number of ± 200, after which they diverge to DP200, DP100 and DP10 with the highest, middle, and lowest threshold, respectively. The thresholds seen in Figure 6c and 6e show almost no difference between the different stimulus types, however their threshold drifts are very small. Besides, Figure 6e, stays very stable through the whole measurement. Stimulus type DP10 shows some noise in the beginning of the measurement, which can be seen in Figure 6d until a trial number of ± 50, after which it stabilizes. The thresholds of the different stimulus types have almost no difference until a trial number of ± 100, where they start to diverge to DP200, DP100 and DP10 having the highest, middle, and lowest threshold, respectively. Where DP200 and DP100 stay at approximately the same value, DP10 starts to decrease from a trial number of ± 100 until ± 200. From there, they all very gradually increase until the end of the measurement. The thresholds in Figure 6f are less stable than the other measurements, because of the high threshold drift from a trial number of ± 100. Then, from a trial number of ± 200 the thresholds seem to stabilize, where the threshold of DP100 even starts decreasing. The threshold of this stimulus type started closer to the threshold of DP200, but ends closer to the threshold of DP10. Unlike the other figures, Figure 6g shows the thresholds of the different stimulus types starting and ending with DP200, DP100 and DP10 with the highest, middle, and lowest threshold, respectively. Furthermore, they also increase at the same rate and show little noise. Just as in Figure 6a, Figure 6h shows a threshold which starts high at the start after which it converges to the thresholds of the other stimulus types. At a a trial number of ± 150 all the thresholds almost lie on top of each other. From then on they all start gradually increasing and the threshold of DP200

diverges from the thresholds of the other two stimulus types, which stay close to each other.

Most of the measurements more or less show an increase in threshold over trial number, clearly seen best in Figure 6b and 6g. Also, the obtained thresholds of all stimulus types appear relatively stable in every measurement and have little noise. As mentioned earlier, every measurement contains a part where DP200, DP100 and DP10 have the highest, middle, and lowest threshold, respectively. The difference between the thresholds of the three stimulus types varies per measurement. Besides, the average threshold values for each type also differs between the various measurements.



(a) Subject 1

(b) Subject 2

(c) Subject 3

(d) Subject 5

(e) Subject 6

(f) Subject 7

(g) Subject 8

(h) Subject 9

Figure 6: Raw data plots of all included measurements with raw stimulus amplitude, corresponding responses and obtained thresholds plotted against the trial number.

4.3.2 General Linear Model

As mentioned in the Results Analysis section, a generalized linear model (GLM) was used in combination with a logit link function to estimate the detection probability for different stimulus amplitudes of the stimulus types on an individual level. With this model the effect of the different parameters, such as stimulus stimulus amplitude (*AMP*, in mA), IPI of 10 ms (IPI_{10}, boolean), IPI of 100 ms (IPI_{100}, boolean), and trial number (*TRL*), on the detection probability can be found. The estimated model regression parameters and their significance is showed for every GLM in Table 1. The estimated regression parameters where used to obtain the threshold estimates, which are then plotted in the same figure with the tracked NDT's from the raw data. A complete overview of these plots for every subject can be seen in Figure A.1, of which one example can be seen in Figure 7. This figure shows that the GLM's have a relatively good fit for all subjects, except for subject 7, which can be seen in Figure A.1f. However, the GLM NDT's seem to be estimated a little higher than the tracked NDT's, which can also be seen more or less for all the other subjects in Figure A.1. This raises some questions about the reliability of the GLM.

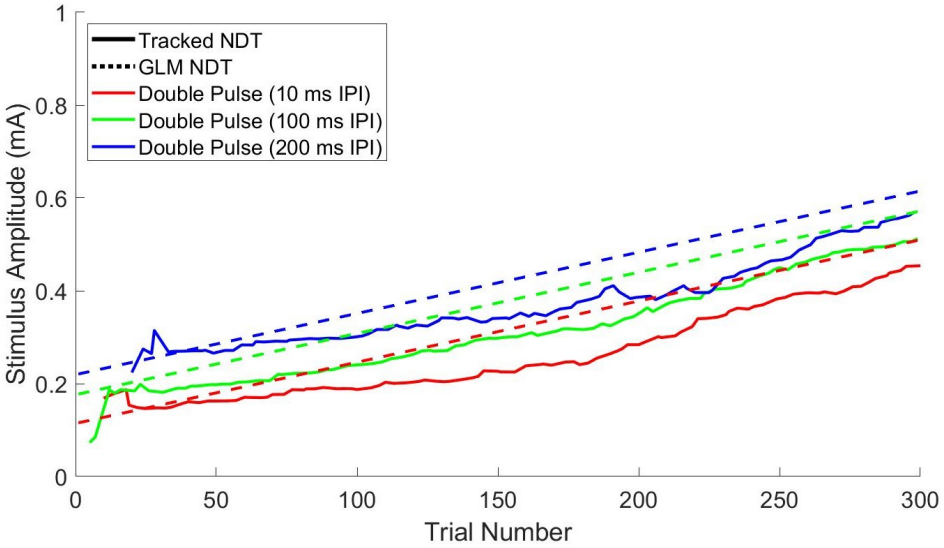


Figure 7: Typical example of estimated GLM NDT in comparison with a the tracked NDT. The GLM leads to a relatively good fit. However, the GLM NDT's are estimated a little higher than the tracked NDT's.

Table 1 shows all the estimated regression parameters and their significance. For every GLM it can be seen that there is a significant increase and decrease of detection probability with respect to stimulus amplitude and trial number respectively. However, the GLM of subject 7 shows a much lower significance for both of these parameters, with the effect of the trial number even being far from significant (p = 0.706). In the previous paragraph it was showed that this GLM has a very bad fit on the obtained data of this subject, confirming the relatively high p-values. Because of this bad fit the results of this glm will not be discussed more extensively. The two other regression parameters of the GLM are IPI10 and IPI100. Because the model takes the stimulus type with an IPI of 200 ms as reference, these parameters show the effect of the stimulus types with an IPI of 10 ms and 100 ms relative to the stimulus type with an IPI of 200. Table 1 shows that an IPI of 10 ms results in a significant increase in detection probability for every subject. An IPI of 100 ms results in a significant increase for a part of the subjects, namely subjects 1, 2, 6, 8, and 9. The difference in estimates of IPI10 and IPI100 between subjects, shows a difference in effect of IPI on detection probability, which was also seen in Figure 6.

Table 1: Regression parameter estimates and corresponding significance values, obtained from the GLM. The lower the significance value, the higher the significance, and when it is smaller than 0.05 the effect of a certain parameter is considered significant. There is a significant increase and decrease of detection probability with respect to stimulus amplitude and trial number respectively. Furthermore, DP10 results in a significant increase in detection probability for every subject. DP100 results in a significant increase for a part of the subjects. As the model uses DP200 as reference, parameters IPI10 and IPI100 show the effect of DP10 and DP100 relative to DP200.

	Subject 1		Subject 2		Subject 3		Subject 5	
Parameter	**Estimate**	**p**	**Estimate**	**p**	**Estimate**	**p**	**Estimate**	**p**
(intercept)	-8.65	<0.001	-3.70	<0.001	-4.72	<0.001	-5.56	<0.001
Trial number (TRL)	-1.43	<0.001	-1.15	<0.001	-0.48	0.001	-0.33	0.021
Stimulus amplitude (AMP)	18.97	<0.001	9.83	<0.001	19.63	<0.001	15.47	<0.001
IPI10	2.96	<0.001	1.23	<0.001	0.70	0.034	1.72	<0.001
IPI100	1.72	<0.001	0.68	0.031	0.10	0.745	0.56	0.078

	Subject 6		Subject 7		Subject 8		Subject 9	
Parameter	**Estimate**	**p**	**Estimate**	**p**	**Estimate**	**p**	**Estimate**	**p**
(intercept)	-12.73	<0.001	-2.22	0.005	-9.77	<0.001	-5.68	<0.001
Trial number (TRL)	-1.68	<0.001	-0.082	0.706	-2.68	<0.001	-0.92	<0.001
Stimulus amplitude (AMP)	39.71	<0.001	2.73	0.026	23.42	<0.001	16.62	<0.001
IPI10	1.22	0.003	0.72	0.063	2.46	<0.001	1.01	0.004
IPI100	0.99	0.011	0.55	0.092	1.01	0.005	0.86	0.012

5 Discussion

In this study a new stimulus set was explored, consisting of only double pulse stimuli with varying inter pulse intervals for improved observation of the nociceptive function using the NDT method. Research was conducted in nine healthy control subjects to explore the estimation quality of the psychophysical functions of this new stimulus set and possible differences in thresholds because of varying inter pulse intervals in this set.

5.1 Excluded Data

As mentioned in the results section, one of nine data sets was excluded from further analysis because of a detection rate far below 20% and a high drifting stimulus amplitude without reaching a stable state. The raw data points showed two interesting things. First, the stimulus amplitude stopped increasing when a stimulus amplitude of $\pm 1.5mA$ was reached, and stayed like this, while the subject still did not feel the applied stimuli. This is something that cannot be directly changed in the settings of the experiment. It could be that the cause of this upper boundary lies deeper in the settings of the custom computer program that was made for this experiment. Another option would be that the impedance of the electrode was too high, resulting in the voltage limit being reached. The actual current that is being applied then remains the same. However, to find the cause of this problem further research is needed. Furthermore, the detection rate of DP10 was higher than that of the other stimulus types. This can be explained by the effect of temporal summation of postsynaptic potentials which has a larger influence when the inter pulse interval is shorter [68].

5.2 Response Times

The increase in median, as well as the other results mentioned about Figure 5 can be explained by the the fact that on average, through probability summation and temporal summation, the second pulse of the double pulse stimulus is felt. With an increase in IPI the second pulse is further away from the start of the stimulus, which results in a longer response time.

5.3 Nociceptive Detection Threshold

5.3.1 Raw Data

The obtained threshold of DP10 that starts higher then the other stimulus types, and ultimately decreases, resulting in a lower threshold as mentioned about 6a, can be explained by a bad initial threshold estimation. The initial threshold amplitude is then estimated higher than it actually is, which consequently has to be corrected in the experiment, resulting in the stimulus amplitude decreasing and converging to its real value. As the results of Figure 6h contain the same phenomenon, this will probably be because of the same reason. In Figure 6b and 6d it was found that the obtained thresholds of the different stimulus types start at the same amplitude and increase with the same rate in the beginning of the measurement, after which they diverge in the second part. Because the observed difference between the thresholds of the different stimulus is so small and not even proven to be significant, it could be that this difference is not always present. Another option is that the NDT method as used in this experiment is not yet good and precise enough to consistently show these small differences. Because this method still relies on participation quality of the subject, bad participation in the form of for example distraction or inattention can also be a cause. The results stated that in Figure 6d it can be seen that the obtained threshold of DP10 is somewhat noisy in the beginning of the measurement. When the initial threshold is a little off, this can lead to a lot of undetected stimuli, resulting in a bad or even no threshold estimation, making it noisy. As soon as this inequality is gone and stimulus amplitude gets to a relatively stable state, the threshold estimation also has a higher quality, resulting in less noise. The results of Figure 6f showed that the thresholds in this figure are relatively unstable, because of the high threshold drift from a trial number of ± 100. However eventually it seems to stabilize from a trial number of ± 200, where the threshold of DP100 even starts decreasing. This high threshold drift followed by a sudden stabilization will probably be a change in attention. In the results of Figure 6g it was stated that the thresholds of the different stimulus types start and end with DP200, DP100 and DP10 with the highest, middle, and lowest threshold, respectively. This is a perfect example that this method is capable of displaying these small differences.

Most of the measurements more or less show an increase in threshold over trial number, which is called habituation. It is known that nociceptive processing adapts to repeated stimulus application leading to a habituation of neurophysiological

[69] and psychophysical [70] responses. The exact cause of this phenomenon is still unknown, but probable causes are for example a decrease in attention of the subject to the applied stimuli or a changing criterion for stimulus detection [9]. Altered habituation seems to be a important factor in several types of chronic pain syndromes. Therefore, further research in this phenomenon is important [71, 72, 73]. In all measurements a distribution can be seen where DP200, DP100 and DP10 have the highest, middle, and lowest threshold respectively. These small differences indicate that the threshold increases with the inter pulse interval. This can be explained by the effect of temporal summation of postsynaptic potentials, which has a larger influence when the inter pulse interval is shorter [68, 8]. The difference between the thresholds of the different stimulus types is not equal in every measurement, as well as the average threshold values for every type. This can be due to between subject differences. To exclude this effect a generalized linear mixed model has to made, where group analysis of the obtained data sets is made possible. Due to insufficient time this was not done, but because this can better display the differences it is recommended for further research. Overall, the obtained thresholds of all stimulus types seem pretty stable with little noise in every measurement. This is a good sign and opens a path to a larger study including more participants, where also measurement of evoked potentials could provide additional information on the effect of inter pulse interval on the nociceptive detection threshold.

5.3.2 General Linear Model

In the results it was discussed that the estimated thresholds using the GLM, were a little higher than the tracked NDT's. It seems that the more habituation occurs in a measurement, the higher the thresholds estimations of the GLM. The reason of this remains unknown, and further research would be needed to determine this. The GLM fit on the data of subject 7 was found to be bad in the results of the raw data, as well as from the results of the GLM data. The bad GLM fit on the data of this subject, will probably be caused by the shifts in attention leading to unstable data. In the results from Table 1 it was found that an IPI of 10 ms results in a significant increase in detection probability for every subject. An IPI of 100 ms results in a significant increase for a part of the subjects. This indicates a possible relation between IPI and NDT. An increase in IPI will then result in a higher NDT, through the decrease of effect of temporal summation. However, because the size of this study was relatively small, validation of this hypothesis is still needed.

5.4 Previous Study Results

When comparing the measurements of this study depicted in Figure 6 and the measurements of previous studies depicted in Figure 2 it can be seen that both show habituation, and the stimulus amplitudes and thresholds are in the same range. As expected, the big difference between the thresholds of the single and double pulse stimuli has disappeared and all stimulus types show relatively stable data without the noise and instability that was seen in data from the single pulse stimuli. Besides, a small difference appeared between the different stimulus types, implying the facilitatory effects of mechanisms such as central temporal summation.

5.5 Limitations and Recommendations

In choosing the different IPI's for the three stimulus types, the largest IPI has the most uncertainties, because this range exceeds the range of IPI's reported in literature. It could be that the actual time of decay of a post-synapic potential is even longer, which could lead to an even larger range of IPI's, with a higher potential of finding relevant information. To validate this range of IPI's, further research is needed. Furthermore, the GLM does not result in a perfect fit on the subject data, which could lead to unreliable results. Further research in finding a better model could give more reliable results. To be able to validate the significant effect of IPI on the NDT, which was implicated by this study, a study of a larger scale is needed. Besides, using a general linear mixed model (GLMM) for group analysis could lead to a better fit.

6 Conclusion

This study explored the estimation quality of the psychophysical curve of the new stimulus set consisting of only double pulse stimuli with a larger range of inter pulse intervals using the NDT method, and was conducted in a healthy control group. The study showed that the new stimulus set has a higher estimation quality of the psychophysical curve than the initial stimulus set. Besides, it seems as if there is a correlation between an increase in nociceptive detection threshold and an increase in inter pulse interval, which is caused by a facilitatory effect such as temporal summation of post-synaptic potentials. This difference in threshold between the three stimulus types indicates that the new stimulus set has more information content about the facilitatory mechanisms influencing the nociceptive threshold. Therefore, this study suggests that the new stimulus set can be a valuable tool in future research on maladaptive mechanisms of the nociceptive system, with the facilitatory mechanisms in particular.

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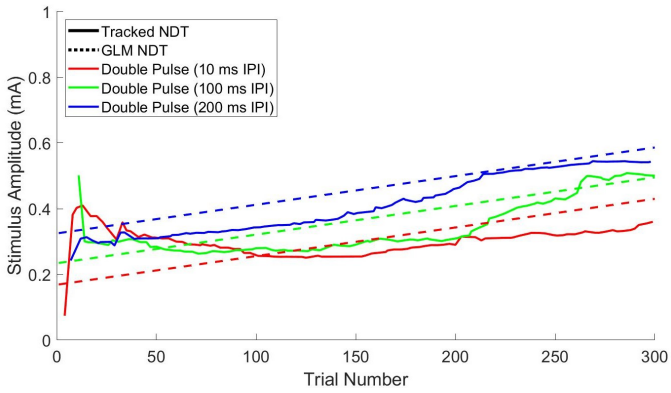
A Appendix



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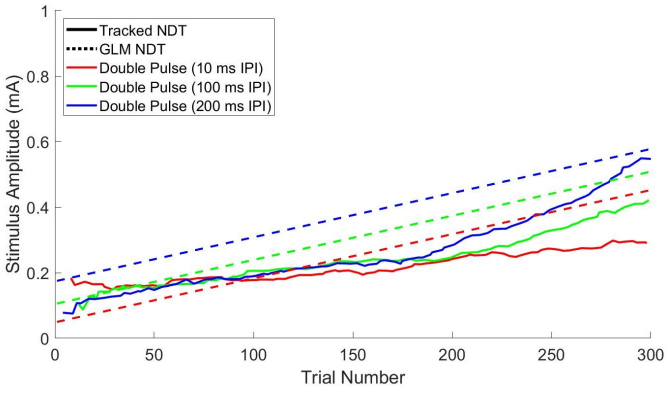

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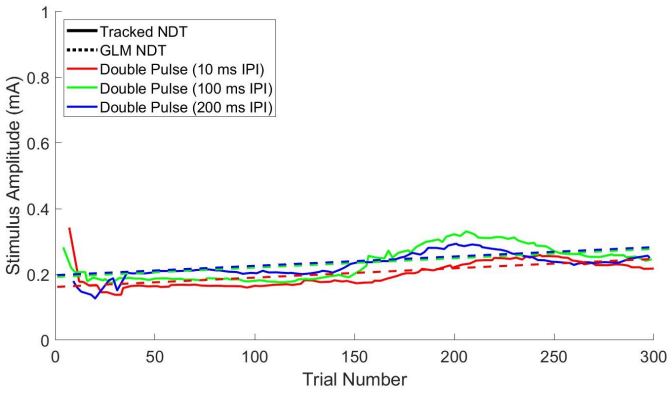

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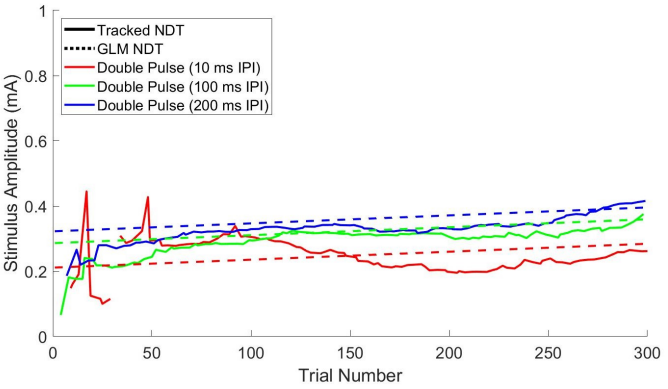

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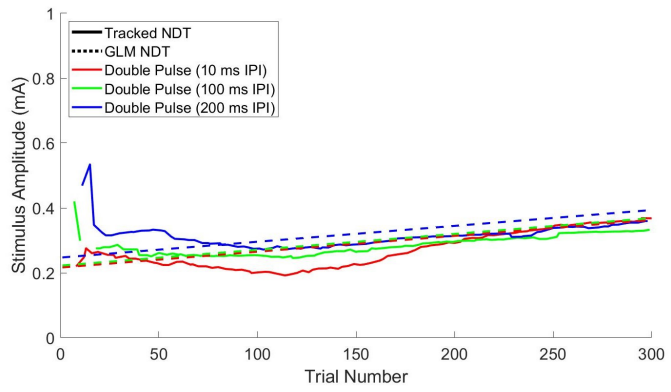

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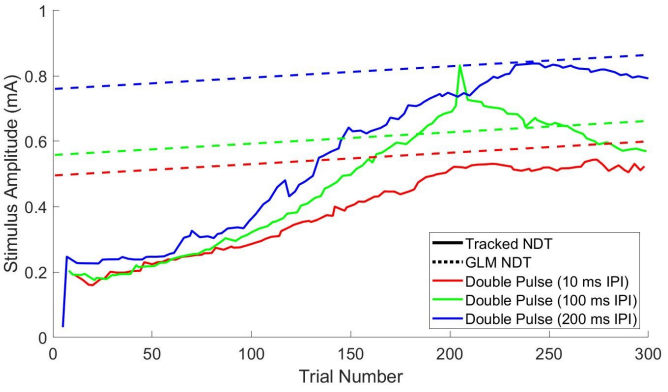

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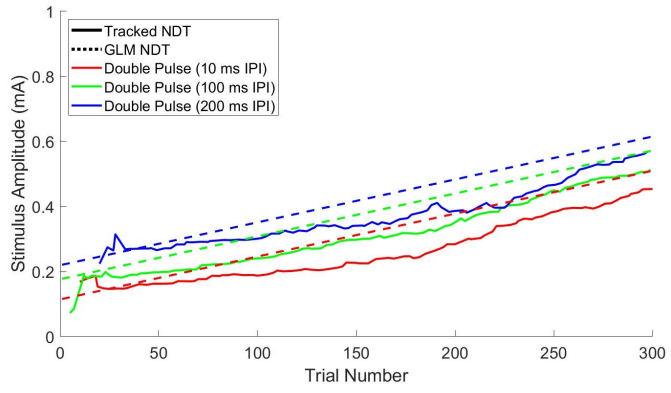

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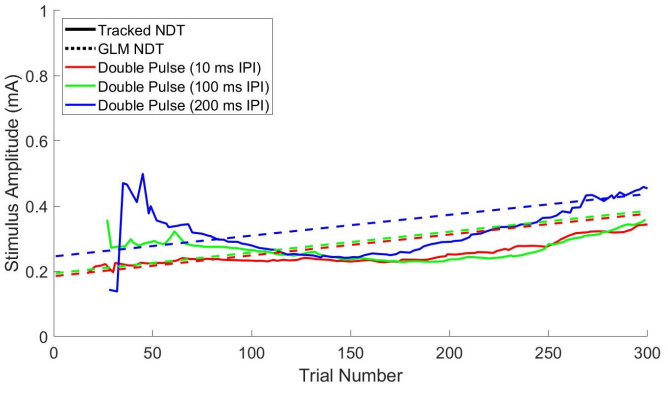

(h) Subject 9

Figure A.1: Plots of estimated GLM NDT's with the tracked NDT's plotted against the trial number.