FORSØGSPROTOKOL

Projekttitel
Vurdering af kutane elektriske stimulationsprotokoller til undersøgelse af excitabilitet i nervefibre

Engelsk titel: Evaluating Cutaneous Electrical Stimulation Protocols for Studying Excitability Properties of Nerve fibers

Forsøgsansvarlig
Jenny Tigerholm, Ph.d., Postdoc
Center for Neuroplasticity and Pain (CNAP)
Integrative Neuroscience
Institut for Medicin og Sundhedsteknologi
Aalborg Universitet
Fredrik Bajers Vej 7D3
9220 Aalborg
Tlf.: 99403451
E-mail: jvt@hst.aau.dk

Projektgruppe

Jenny Tigerholm, Ph.d., Center for Neuroplasticity and Pain (CNAP), Integrative Neuroscience, Institut for Medicin og Sundhedsteknologi, Aalborg Universitet

Carsten Dahl Mørch, Ph.d., Center for Neuroplasticity and Pain (CNAP), Integrative Neuroscience, Institut for Medicin og Sundhedsteknologi, Aalborg Universitet

Aida Hejlskov Poulsen, M.Sc., Center for Neuroplasticity and Pain (CNAP), Integrative Neuroscience, Institut for Medicin og Sundhedsteknologi, Aalborg Universitet

Silvia Lo Vecchio, Ph.d., Translational Pain Biomarkers, Institut for Medicin og Sundhedsteknologi, Aalborg Universitet

Strahinja Dosen, Ph.d., Integrative Neuroscience, Institut for Medicin og Sundhedsteknologi, Aalborg Universitet

Steffen Frahm, Ph.d., Integrative Neuroscience, Institut for Medicin og Sundhedsteknologi, Aalborg Universitet

Erika G. Spaich, Ph.d., Integrative Neuroscience, Institut for Medicin og Sundhedsteknologi, Aalborg Universitet

Professor Ole Kæseler Andersen, Ph.d., Dr. Scient. Center for Neuroplasticity and Pain (CNAP), Integrative Neuroscience, Institut for Medicin og Sundhedsteknologi, Aalborg Universitet

Experimental Protocol

Background

Peripheral sensory nerve fibers convey information about our environment to the central nervous system. Distortion of these signals may lead to severe pathological conditions such as small fiber neuropathy, lack of sensation, or even chronic itch (Serra et. al., 2012, Sittl et. al., 2012; Momose et. al., 2019). Small diameter fibers (Aδ- and C-fibers) mediate pain and itch sensations. The main obstacle when studying peripheral neuropathies is to measure the excitability of small fibers, e.g. how easily these fibers become activated. In large fibers, the excitability can be assessed indirectly by standard nerve conduction tests, using electrical stimulation through conventional patch electrodes (large surface area electrodes). However, the excitability of small nerve fibers can traditionally only be recorded by a needle which is inserted directly into a large nerve bundle. This technique is technically difficult as well as time-consuming, and it is not suitable as a diagnostic tool for small fiber neuropathies in clinical practice. Therefore, our research group has developed a new technique called the Perception Threshold Tracking technique (PTT) to indirectly measure the excitability of small nerve fibers (Hennings et. al. 2017; Hoberg et. al., 2019, Tigerholm et. al., 2019, Hugosdottir et. al., 2019a; Hugosdottir et. al., 2019b; Hugosdottir et. al., 2019c). The PTT technique is an inexpensive, non-invasive, and time-efficient method that can be used in a clinical setting. During PTT, a pin electrode is placed on the skin surface to activate the small fibers preferentially. When a small current is applied through the electrode, a high electrical density is generated in the superficial layers of the skin where the small fibers terminate, leading the electrode to preferentially activate these fibers (as opposed to non-pain fibers which terminate in deeper skin layers). By replacing the pin electrode with a standard patch electrode, the excitability of large fibers can also be studied easily by the PTT technique. By using different shapes of the electrical current, specific aspects of the excitability of nerve fibers can be studied. In the current project, four different nerve excitability protocols will be evaluated, each protocol consisting of a set of electrical currents with different pulse shapes. The protocols can be used to detect excitability abnormalities which may occur during different pathological conditions. Recently, our research group has developed a computational model to study nerve fiber activation by cutaneous electrical stimulation. The computational model is a combination of a finite element model of the skin and a compartmental model of a nerve fiber (Tigerholm et. al., 2019). Preliminary electrical pulse shapes for the four protocols have already been derived by the computational model and from previous results from PTT experiments. The hypothesis is that altering the cutaneous electrical current shape during a PTT experiment will depolarize the fiber's cell membrane differently whereby different aspects of excitability can be studied. In this project, all four nerve fiber excitability protocols will be evaluated in healthy subjects. The background to the four protocols which will be evaluated during this project is presented below.

Subproject 1: Nerve Excitability Protocol to Identify Abnormal Ion Channel Alterations

Diabetes is a global health problem. In Denmark alone, over 250,000 people have been diagnosed with diabetes, and the number is predicted to increase to 430,000 by the year 2030 (Statens Institut for Folkesundhed, 2017). Furthermore, 30% of diabetic patients develop diabetic peripheral neuropathy with symptoms ranging from numbness to burning

chronic pain (Abbott and Malik, 2011). Available diagnostic tools for diabetic peripheral neuropathy mainly include clinical examination, skin biopsy and quantitative sensory testing of small fiber functionality (Smith et. al., 2017). Existing diagnostic tools for diabetic peripheral neuropathy are insufficient (Smith et. al., 2017). Voltage-gated ion channels have been speculated to play a pivotal role in the development of diabetic peripheral neuropathy. Voltage-gated ion channels are small pore-formed channels protruding through the cell membrane where electrically charged ions can flow. They enable the generation and propagation of nerve signals, e.g., from the skin through the spinal cord up to the brain. For small fibers, there are around ten subtypes of voltage-gated ion channels, which are relevant for normal nerve functionality (Woolf and Ma, 2007; Gold et. al.,1996; Gao et. al., 2012). It is particularly useful to use electrical stimulation to activate nerve fibers when studying voltage-gated ion channels since it alters the voltage across the cell membrane without any sensory conduction occurring. There are different subtypes of voltage-gated ion channels which have been altered in animal models of diabetic peripheral neuropathy. However, the underlying subtypes of voltage-gated ion channels related to such abnormalities in humans are essentially unknown (Bierhaus et. al., 2012; Blesneac et. al., 2018; Tsantoulas et. al., 2017; Craner et. al., 2002). It would be of great interest to identify which ion channels are altered in diabetic patients both for the understanding of the pain mechanisms in diabetic neuropathy but also for the development of novel diagnostic tools.

Subproject 2: Nerve Excitability Protocol to Identify Bursting Activity

Oxaliplatin is part of adjuvant chemotherapy treatment of, e.g., colorectal cancer. One of its side effects is peripheral neuropathy, which is characterized by pain from non-painful cold stimuli (Kanat and Ertas, 2017). While all patients develop some degree of a short-lasting neuropathy, some of the patients develop chronic neuropathy (Kanat and Ertas, 2017). The risk of developing chronic neuropathy is increasing with higher cumulative dosages of Oxaliplatin and the dosage is reduced if symptoms of neuropathy are reported by the patient. Therefore, neuropathy is the main dose-limiting side effect of Oxaliplatin (Kanat and Ertas, 2017). An animal study has shown that large non-pain fibers become activated multiple times in spite of being stimulated only once (Sittl et. al., 2012). This behavior is called bursting and may also occur in small fibers. If this is the case, it may explain the increased pain experienced by Oxaliplatin treated patients. However, it is challenging to study bursting with the PTT technique since it can only detect the perception threshold, and it is impossible for the subject to perceive whether the fibers are activated multiple times or only once. By computational modeling, a novel electrical stimulation protocol has been derived from targeting the underlying mechanisms which generate the bursting activity, the so-called resurgent sodium current (Sittl et. al., 2012; Cannon and Bean, 2010). If a successful protocol for detecting bursting could be developed, this would lead to an increased understanding of the mechanisms which generate the pathological pain experienced by the Oxaliplatin treated patients. A burst detection protocol could also be useful in other neuropathies since mutations of genes coding for sodium channels found in neuropathic patients have been shown to increase resurgent sodium currents (Cannon and Bean, 2010).

Subproject 3: Nerve Excitability Protocol to Increase Preferential Activation of Large Non-Pain Fibers

Large conventional patch electrodes preferentially activate large non-pain fibers. During certain circumstances, there is a need to increase the preferential activation of the large fibers, or more specifically, to increase the pain threshold and/or decrease the detection threshold. A successful protocol that increases the preferential activation could potentially improve transcutaneous electrical nerve stimulation, which is an effective therapy to mask discomfort resulting from, e.g., painful diabetic neuropathy (Stein et. al., 2013).

Additionally, this protocol could be used to improve electrical stimulation-based sensory feedback in amputee patients. Existing prostheses are advanced mechatronic systems that can be used to restore motor functions lost due to an amputation. However, they do not provide somatosensory feedback to the users, and therefore the amputees do not “feel” their bionic limbs. As reported in a recent review of user needs (Cordella et. al., 2016), many of the patients would like to have tactile feedback incorporated into their prosthetic hands. Electrical stimulation could potentially mediate sensory feedback (Schofield et. al., 2014), but unfortunately electrical stimulation can also be associated with discomfort and occasional pain to the user of the prostheses (Antfolk et. al., 2013). This is partly due to the spontaneous discharge of hyperexcitable peripheral small fibers related to both morphological changes as well as the upregulation of sodium channels (Kuffler, 2018). Furthermore, previous studies have shown that a long hyperpolarizing sub-threshold prepulse increases the threshold for pain fibers significantly, compared to large non-pain fibers (Hennings et. al., 2017). This indicates that different shapes of the electrical stimulation can increase preferential activation of the large non-pain fibers.

An electrical stimulation protocol that increases the preferential activation of non-pain fibers may improve the sensory feedback in amputee patients. Increasing/decreasing the pain/detection threshold would increase the range of stimulation parameters that elicit comfortable tactile sensations, allowing thereby better control of the quantity and quality of sensations produced by the feedback.

Subproject 4: Nerve Excitability Testing Protocol to Efficiently Induce Itch

Itch is a sensation that causes the desire to scratch, which during healthy conditions has a protective function. During pathological conditions, the itch sensation is enhanced, and the patients cannot stop scratching. Excessive scratching does not only lead to skin injury but also to severe psychological stress (Verhoeven et. al., 2008). The research field of itch is small, and there is a strong need to understand itch fibers' basic properties. In this subproject, we aim at testing a new nerve excitability protocol to induce itch in healthy controls by electrical stimulation. Itch has many similarities with pain, and the nerve fibers which mediate the two sensations probably have the same morphological as well as excitability properties (Jurcakova et. al., 2018). The method used in this project will be the same as our research group uses for studying nociceptor fibers (Hennings et. al. 2017; Hoberg et. al., 2019, Hugosdottir et. al., 2019a; Hugosdottir et. al., 2019b; Hugosdottir et. al., 2019c), but we will focus on the itch sensation. Since the itch fibers also terminate in the epidermis, our PTT technique should also be able to activate itch fibers, similar to pain fibers. If itch could be effectively induced by electrical stimulation, this would generate a useful tool for studying itch in healthy controls.

Strategy for Literature Search

The basis of the study has been found among peer-reviewed articles, publicly available material and Aalborg University's own research. 62803 articles were found and 36 references used (please see the section List of References) for this study, which form the basis for the research within cutaneous electrical stimulation. The literature has been found by reviewing of the search engines: PubMed and Google scholar. The following search words were used in combinations for review of the literature: Nav1.7, Nav1.8, Nav1.9, HCN, diabetic neuropathy, itch, cutaneous electrical stimulation, perception threshold technique, sensory-feedback, microneurography, small fiber neuropathy, phantom limb patients, excitation threshold, electrophysiology, nerve fiber excitability, cutaneous afferent fibers, nociceptors, neuropathy and quality of life.
Only studies with interest within human research have been used and the studies that have been produced within the last 25 years.

Purpose

The purpose of this project is to evaluate four nerve excitability protocols in healthy human subjects. These protocols can be developed into diagnostic tools or research methods to gain information about fibers for basic research. The general hypothesis is that altering the cutaneous electrical current shape during a PTT experiment will depolarize the nerve fibers' cell membrane differently whereby different aspects of cell membrane excitability may be studied.

Purpose of Subproject 1: Nerve Excitability Protocol to Identify Abnormal Ion Channel Alterations

The purpose of subproject 1 is to evaluate the accuracy of a nerve excitability protocol in healthy subjects before testing the protocol in diabetic neuropathy patients. The nerve excitability protocol has been derived by computational modeling to identify abnormalities of four subtypes of ion voltage-gated channels (Nav 1.7, the Nav 18, the Nav 1.9, and the HCN channel). These four ion channels are all altered in animal models of diabetic neuropathy and, therefore, are likely candidates for generating the altered excitability in diabetic neuropathy patients. The hypothesis for subproject 1 is that due to the unique dynamics of each subtype of voltage-gated ion channels, it is possible to identify abnormal voltage-gated ion channel alterations by altering the shape of the electrical stimulation.

Purpose of Subproject 2: Nerve Excitability Protocol to Identify Bursting Activity

The purpose of subproject 2 is to evaluate a nerve excitability protocol which could detect repetitive activity of fibers. The resurgent sodium current plays a major role in generating repetitive activation in nerve fibers. The hypothesis is that by targeting the dynamic properties of sodium resurgent currents, it is possible to generate a nerve excitability protocol that can efficiently measure burst activity. The purpose of subproject 2, is to evaluate if the resurgent current could be detected in healthy subjects.

Purpose of Subproject 3: Nerve Excitability Protocol to Increase Preferential Activation of Large Non-Pain Fibers
The purpose of subproject 3 is to investigate if different pulse shapes of electrical stimulation can increase the preferential activation of large fibers. The hypothesis is that due to different excitability properties of small and large fibers, it is possible to generate a nerve excitability protocol which may increase preferential activation of large fibers. A successful protocol development could improve not only electrical stimulation-based sensory feedback in limb prostheses for amputees but also the application of transcutaneous electrical nerve stimulation, which is an effective therapy to mask discomfort resulting from e.g. diabetic neuropathic pain.

Purpose of Sub-project 4: Nerve Excitability Protocol to Efficiently Induce Itch
The purpose of subproject 4 is to test how different shapes of electrical stimulation can induce itch in healthy subjects. The hypothesis is that due to itch fibers' similarities with nociceptive fibers, the same electrode developed for preferential activation of nociceptive fibers as well as the electrical simulation applied could be utilized to generate a nerve fiber excitability protocol for inducing itch. If itch could be effectively induced by electrical stimulation, this would generate a useful tool for studying itch in healthy controls.

Subjects

For this study we wish to recruit 90 (see sample size estimations for each subproject below) healthy volunteers through notices at Aalborg University, on www.forsog.dk, on Facebook and in the press (notice is attached). The following inclusion and exclusion criteria will be used:

Inclusion

Healthy men and women in the age 18-80 years who speak and understand English.

Exclusion

- Pregnancy or breast feeding
- Drug addiction defined as the use of cannabis, opioids or other drugs
- Previous and present neurologic, musculoskeletal or mental illnesses (e.g., epilepsy, neuropathy, fibromyalgia and depression)
- Skin diseases
- Past history of conditions possibly leading to neuropathy
- Inability to cooperate
- Current use of medications that may affect the study, e.g., analgesics
- Previous traumatic experience of an electrical accident
- Consumption of alcohol or painkillers within the last 24 hours
- Participation in other pain studies throughout the study period
- Patients with cardiac diseases (e.g., pacemaker).

Design and Methods

Common for all subprojects is the use of electrical stimulation. The electrical stimulation is used as a means to artificially activate nerve fibers. In the present project, it will be used to activate cutaneous nerve fibers using a non-invasive electrode placed on the surface of the skin. A short-lasting (50μs – 300ms) rectangular or non-rectangular current is passed through the electrodes and into the skin. This current, provided the intensity is sufficient, has the effect of depolarizing the nerve fibers that may trigger the generation of action potentials. The intensity at which this electrical stimulation activates enough cutaneous afferents for the subjects to perceive the stimulus is termed the perception threshold of the electrical stimulation. The threshold of an electrical stimulation depends on the state of the nerve fiber and can be perturbed by the shape of the electrical stimulation and previous activation or conditioning by other electrical stimulation (Klein *et al.*, 2004; Hennings et. al. 2017; Hugosdottir et. al., 2019a; Hugosdottir et. al., 2019c; Hoberg et. al., 2019). The electrical stimulation will be applied either through a patch electrode or a collation of small area cathode electrodes, which preferentially activate small fibers. The electrodes developed for small fiber activation elicit a pricking or burning sensation. The small-area cathodes that may be used are:

- A planar concentric electrode (Kaube et al., 2000)
- Small concentric pin electrode (Inui et al., 2002)
- Pin electrode (Klein et al., 2004; Lelic et al., 2012)
- Micropatterned surface electrode (Leandri et. al., 2018)
- Planar pin-array electrode (Hugosdottir et. al., 2019b)
- Aau electrode (developed at CNAP, Aalborg University)

In all four subprojects, the perception threshold and/or the pain threshold will be measured. The perception and pain thresholds are determined by the following procedure:

An electrical stimulation is applied through the electrode. The intensity of the stimulation is stepped up until the subject indicates, by pressing a button, that he/she feels the stimulation. Subsequently, other electrical stimulation intensities will be evaluated, and the subject will indicate if perceiving the electrical stimulation. Different stimulation intensities will be tested until the perception threshold is accurately estimated.

The procedure for measuring the pain threshold will be the same as detecting the perception threshold, except that the subject will be instructed to press the button when the sensation becomes painful.

Subproject 1: Nerve Excitability Protocol to Identify Abnormal Ion Channel Alterations

Subproject 1 consists of one experimental session, lasting approximately 1 hour. During the session, the subjects will receive weak electrical stimulation on the skin, 5-10 cm proximal to the lateral aspect of the ankle, using the patch electrode and subsequently one of the electrodes designed for preferential activation of small fibers. The electrical stimulation protocol consists of 5-10 different pulse shapes (see figure 1 for preliminary

pulse shapes). Each of the pulse shapes consists of square and ramp pulses. The perception threshold will be estimated for each shape of the electrical stimulations. The order of the electrical stimulation pulse shapes and the electrode will be randomized.

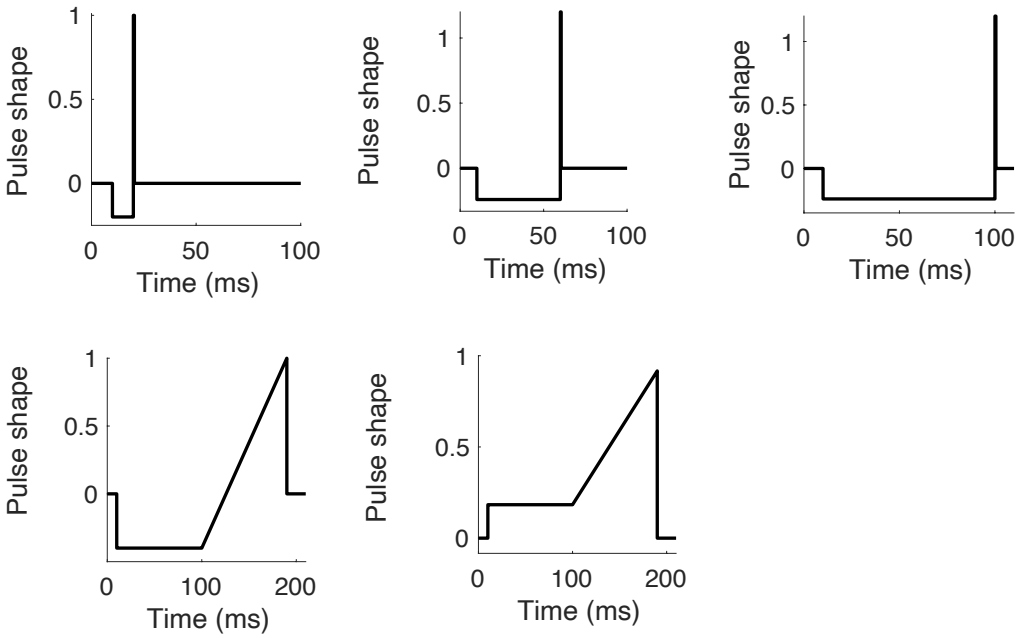

Figure 1. The preliminary pulse shapes of electrical stimulation which will be used in subproject 1. Minor adjustments of the pulse shapes and the number of pulses might occur in the final protocol.

Subproject 2: Nerve Excitability Protocol to Identify Bursting Activity

Subproject 2 consists of one experimental session, lasting approximately 2 hours. During the session, the subjects will receive weak electrical stimulation on their skin, 5-10 cm proximal to the lateral aspect of the ankle, using the patch electrode and one of the electrodes designed for preferential activation of small fibers. The electrical stimulation protocol consists of 5-10 different pulse shapes. Each of the pulse shapes consists of a slowly increasing prepulse followed by a square pulse (see figure 2 for the general shape of the electrical stimulation). The perception threshold for the protocol will be evaluated for different temperature conditions (20°C-32°C). The temperature will be altered by using a 3 × 3 cm thermode (Pathway, Medoc Ltd., Ramat Yishai, IL) which will be placed on top of the electrode and fastened with a velcro band. The order of the electrical stimulation pulse shapes, the temperature conditions and the electrode will all be randomized.

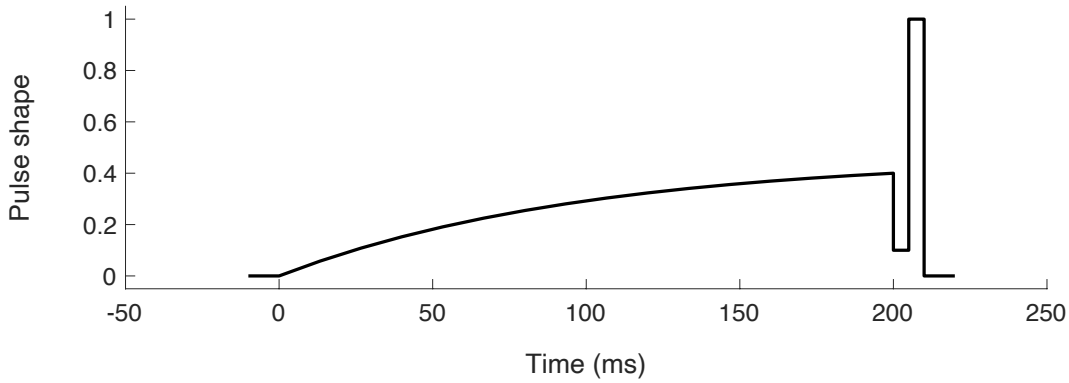

Figure 2. The general form of the electrical pulse shapes which will be used in subproject 2. A slowly increasing long prepulse followed by a short square pulse. The different pulse shapes in the protocol are derived by small alterations of the general pulse shape, such as the duration between the prepulse and the square pulse. Minor adjustments of the pulse shapes might occur in the final protocol.

Subproject 3: Nerve Excitability Protocol to Increase Preferential Activation of Large Non-pain Fibers

Subproject 3 consists of one experimental session, lasting approximately 2 hours.
The perception and pain thresholds will be estimated. The electrical stimulation protocol consists of 5-10 different pulse shapes. Each of the pulse shapes consists of a long prepulse followed by short square pulses or only short square pulses (see figure 3 for preliminary pulse shapes). During the session, the subjects will receive weak electrical stimulation on their volar forearm with the standard patch electrode to determine the perception thresholds and the pain thresholds. Different distances between the cathode and the anode will be used. The order of the electrical stimulation pulse shapes and the distance between the anode and the cathode will be randomized.

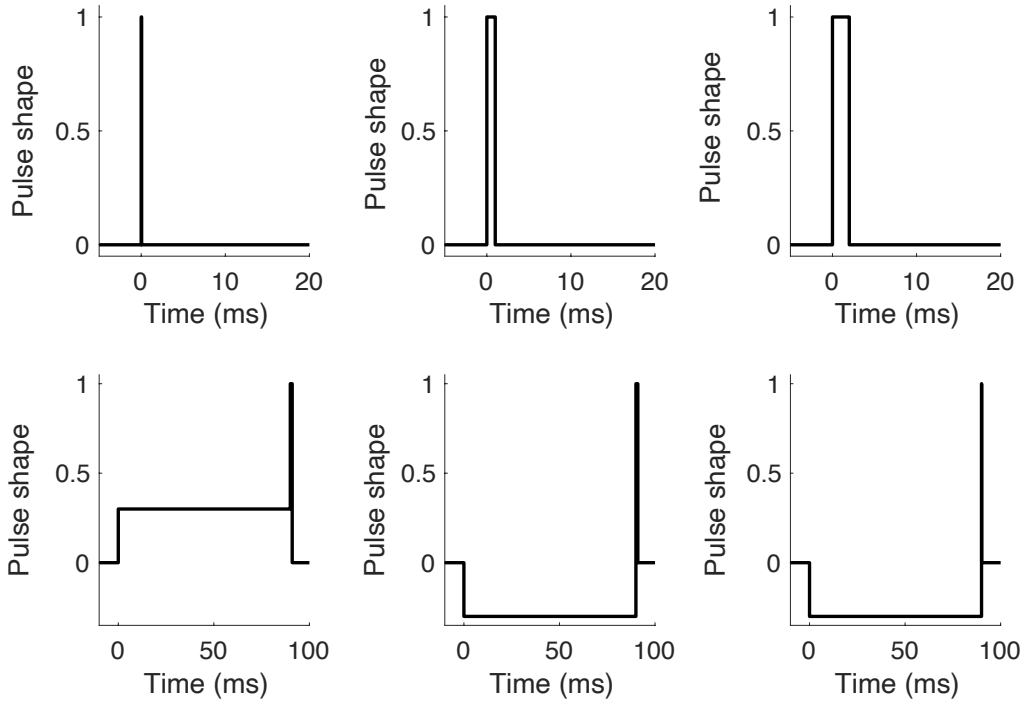

Figure 3. A preliminary protocol of the pulse shapes of electrical stimulation which will be used in subproject 3. Minor adjustments of the pulse shapes and the number of pulses might occur in the final protocol.

Subproject 4: Nerve Excitability Protocol to Efficiently Induce Itch

Subproject four consists of one experimental session, lasting approximately 2 hours. During the session, the subjects will receive weak electrical stimulation on their volar forearm, using the patch electrode and one of the electrodes designed for preferential activation of small fibers. A train of 10-50 pulses will be applied with different frequencies (1Hz-10Hz) and pulse durations (0.05ms-100ms). For a preliminary shape of the electrical input, please see figure 4. Additionally, as control stimulation, single electrical pulses (no pulse train) with different durations will be applied. The perception threshold as well as the pain threshold will be estimated for each shape of the electrical stimulation. The subject will be asked to describe and rate the sensation of the electrical input. The experiment will be performed with and without charge balancing of the electrical stimulation. The order of the electrical stimulation pulse shapes and the electrode will be randomized.

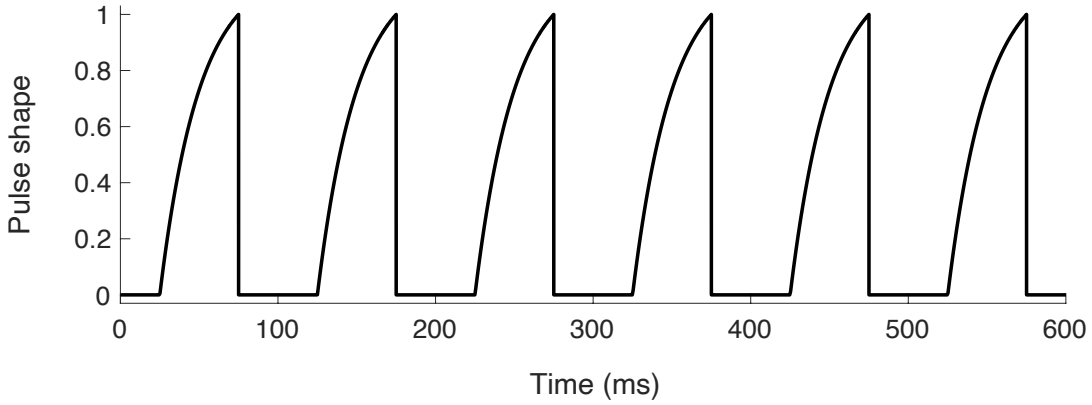

Figure 4. The preliminary pulse shapes of the electrical stimulation which will be used in subproject 4. A series of slowly increasing pulses will be applied. The length of the ramp stimulation will be varied from 0.05ms to 100ms.

Risks, Side Effects and Disadvantages

The methods applied in this project have been routinely used in both research and in clinical studies. There are no reports neither from our own nor from other institutions on any long-term side effects of the methods. The applied electrical stimulation can be interrupted at any time. The subjects will be under observation during the trial.

The electrical stimulation may trigger a phenomenon known as the axon flare reflex that creates a redness/irritation of the skin at the site of the electrical stimulation. This redness/irritation of the skin possesses no harmful effect and usually disappears after a maximum of one hour. Furthermore, subjects who have never experienced this type of stimulation or who may have experienced a harmful electrical shock in an accident, may feel anxious. It is our experience that the novelty of the situation is alleviated by allowing the subjects to experience the electrical stimuli at their own pace and with slowly increasing intensities at the beginning of the experimental session before the real experimental procedures start. At that time, it is also possible for the subjects to evaluate whether or not they can get used to the stimuli. If not, they can then leave the experiment. In this way undue emotional stress is avoided. The subjects will also be screened for any previous traumatic experiences of electrical accidents prior to inclusion in the experiment in order to avoid accepting subjects that may be prone to emotional stress due to electrical stimulation.

In subproject 2, the skin temperature will be lowered to 20°C for less than 60 min. There are no reports from neither our own nor from other institutions on any long-term side effects of reducing the temperature to 20°C within this timeframe. If the subject perceives the reduced temperature as painful, the experiment will be interrupted.

Statistics

The output measurements of all 4 subprojects are the perception thresholds and/or pain thresholds. Previous studies have shown that the standard deviation of perception and pain thresholds is approximately 0.2mA (σ) (Hennings et. al. 2017; Hoberg et. al., 2019; Hugosdottir et. al. 2019a; Hugosdottir et. al. 2019b; Hugosdottir et. al. 2019c; Doll, et al. 2014).

The average perception thresholds and/or pain thresholds of the subjects will be compared between electrodes through a repeated-measures ANOVA. Additionally, for subproject 2, the temperature conditions will also be compared with repeated-measures ANOVA.

The support for sufficient margin of error (d) is different for each subproject.

Subproject 1

The outcome measurements for subproject 1 are the perception thresholds and these measurements may in future studies be compared with the perception thresholds measured in diabetic neuropathy patients. A margin of error of 0.075mA (d) is required to identify abnormal ion channel alteration occurring within the biological range estimated in animal models of diabetic neuropathy (Craner et. al., 2002). We want to have a significant level (p-value) lower than 0.05 (1.96 = Z) and hence we get the following;

$$n = \left(\frac{Z * \sigma}{d}\right)^2 = \frac{(1.96 * 0.2)^2}{(0.075)^2} \approx 27{,}32$$

As this is an estimate, we expect to recruit 30 volunteers for this subproject to counteract a variance larger than the expected variation.

Subproject 2

The outcome measurements for subproject 2 are perception thresholds and these measurements may in future studies be compared with the perception thresholds measured in neuropathy patients. A margin of error of 0.1mA (d) is required to identify abnormal resurgent currents with the size measured in animal models of pathological bursting fibers (Sittl et. al., 2012; Cannon and Bean, 2010). We want to have a significant level (p-value) lower than 0.05 (1.96 = Z) and hence we get the following;

$$n = \left(\frac{Z * \sigma}{d}\right)^2 = \frac{(1.96 * 0.2)^2}{(0.1)^2} \approx 15{,}37$$

As this is an estimate, we expect to recruit 20 volunteers for this subproject to counteract a variance larger than the expected variation.

Subproject 3

The outcome measurements for subproject 3 are perception and pain thresholds. In this subproject we aim at increasing preferential activation of large fibers and therefore a margin error below 0.1mA is sufficient. We want to have a significant level (p-value) lower than 0.05 (1.96 = Z) and hence we get the following;

$$n = \left(\frac{Z * \sigma}{d}\right)^2 = \frac{(1.96 * 0.2)^2}{(0.1)^2} \approx 15{,}37$$

As this is an estimate, we expect to recruit 20 volunteers for this subproject to counteract a variance larger than the expected variation.

Subproject 4

The outcome measurements for subproject 4 are perception thresholds, pain thresholds and sensation measurements. One previous study, with similar electrical stimulation protocols, supports that a margin error below 0.1mA is sufficient (Ikoma et. al., 2005). We want to have a significant level (p-value) lower than 0.05 (1.96 = Z) and hence we get the following;

$$n = \left(\frac{Z * \sigma}{d}\right)^2 = \frac{(1.96 * 0.2)^2}{(0.1)^2} \approx 15{,}37$$

As this is an estimate, we expect to recruit 20 volunteers for these subprojects to counteract a variance larger than the expected variation.

Ethical Considerations

The methods proposed in the present research project are similar to methods that are extensively used in other research projects. The electrical stimuli may be mildly painful. The subjects are participating voluntarily without any personal gain. The subjects are free and able to withdraw from the study at any time during the experiments.

The knowledge generated by the project will increase the understanding of nerve fiber excitability properties and support the development of new diagnostic tools for small fiber neuropathies. Such diagnostic tools may enable the clinicians to observe small fiber dysfunctions and apply relevant treatment at an early stage of neuropathy, possibly slowing or completely preventing further pathological progression. In addition, the methods developed in the present project might enable better somatosensory feedback to the users of myoelectric prostheses. This in turn can improve the utility as well as the feeling of

embodiment of an artificial limb. In this respect, the results may also prove to be cost saving for society. Although there are limited risk factors and discomforts involved for the healthy subjects, we believe that the potential benefits of the studies outweigh the potential risks involved.

Insurance

The subjects are covered by the Danish Patient Compensation Association (Patient-erstatningen).

Personal Data

Data will be stored after termination of the project. These data can only be used for the interpretation of this project and will therefore not be of interest to third party.

Data are stored in accordance with the stipulations in the data protection rules and other relevant legislation.

The project is registered through internal registration in the Article 30 Register of AAU.

Project Economy

The project has been initiated by Postdoc Jenny Tigerholm and Associate Professor Carsten Dahl Mørch, both affiliated with Center for Neuroplasticity and Pain (CNAP) at Aalborg University.

The project is funded by CNAP with DKK 1,000,000. CNAP is supported by the Danish National Research Foundation (DNRF121). The amount is administered by Department of Health Science and Technology, Aalborg University.

None of the researchers involved have financial interest in the study.

Compensation to Subjects

The subjects will receive DKK 150 per hour as compensation for their participation in the experiment. The amount is liable to tax and will therefore be reported to the Danish tax authorities as B-income.

Subjects who do not complete the experiment will receive proportional compensation for the time spent.

Publishing of Results

All results of the project will be published regardless of the outcome of the project.

Time Schedule

The project is expected to start immediately after approval in March 2020 and will be completed in March 2023.

Guidelines for Oral Information and Informed Consent

Summoning Potential Subjects

When potential subjects address the contact person, the following should be stated:

- That it is a request for participation in a scientific research project
- The purpose of the project
- That participation is voluntary and that the subject can withdraw from the project at any time without consequences
- That the potential subject has time to consider his/her participation before giving consent to participation in the project and that the potential subject is welcome to bring a family member or a friend to the information meeting. The potential volunteer will receive the leaflet “The Rights of a Trial Subject in a Health Scientific Research Project”/ "Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt" which includes information on confidentiality, right of access to documents and right to complain.
- That the material “Information for Participants”/”Deltagerinformation” will be forwarded by mail/e-mail to the potential subject in order for him/her to know more about the project before the information meeting.
- Finally, time for the information meeting is arranged

The Information Meeting

The information meeting is held in a quiet room where it is possible to have an uninterrupted conversation. Coffee/tea/soft drink may be served. The information meeting is held by the person responsible for the project or a senior researcher who has been authorized to provide the information.

The meeting is to include the following information/questions:

- Participation is voluntary and the subject can withdraw from the project at any time without consequences
- The subject has time to consider his/her participation before giving consent to participation in the project, and the subject is welcome to bring a family member or a friend to the information meeting.
- The subject is asked whether he/she wants a family member/friend to be present at the meeting.
- The purpose of the experiment is presented, and it is explained how the experiment is performed. The “Information for Participants”/”Deltagerinformation”, which has been sent to the potential subject in advance, is the starting point for the information meeting.

- The subject is asked if he/she is healthy or whether he/she has an infectious disease.
- The subject is asked whether he/she is a Danish citizen. If the answer is no, he/she is asked if he/she has a valid work permit.
- The leaflet “The Rights of a Trial Subject in a Health Scientific Research Project”/ "Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt" is handed over. It is explained that it includes information on confidentiality, right of access to documents and right to complain.
- The subject is asked whether he/she has read “Information for Participants”/ ”Deltagerinformation”. If this is not the case, we will ask the subject to read it.
- When it has been ensured that the subject has read the “Information for Participants”/”Deltagerinformation”, he/she is asked whether he/she has questions about the experiment.
- After this a demonstration is given in the lab; measuring equipment and its use is presented to the subject.
- It is underlined that participation is voluntary, and that the subject has time to consider his/her participation (please note that The National Committee on Health Research Ethics recommends 24 hours of deliberation time)
- Again it is underlined that participation is voluntary and that the subject can withdraw his/her consent at any time without consequences.
- The subject is informed that if he/she does not need time to consider the participation, the consent can be given at the information meeting.
- Time/place for the experiment is agreed.
- Finally, information about the contact person of the experiment is given (it is shown to the subject that the name and contact details appear from the “Information for Participants”/”Deltagerinformation”) and it is informed that this person can be contacted at any time if further questions should arise.

List of References

Abbott C.A., Malik R.A., van Ross E.R., Kulkarni J., Boulton A.J. 2011, "*Prevalence and characteristics of painful diabetic neuropathy in a large community-based diabetic population in the U.K"*, Diabetes Care 34, 2220–2224.

Antfolk C, D'Alonzo M, Rosén B, Lundborg G, Sebelius F, Cipriani C. *"Sensory feedback in upper limb prosthetics"*. Expert Rev Med Devices. 2013;10:45-54.

Bierhaus A., Fleming T., Stoyanov S., Leffler A., Babes A., Neacsu C., Sauer S.K., Eberhardt M., Schnölzer M., Lasitschka F., Neuhuber W.L., Kichko T., Konrade I., Elvert R., Mier W., Pirags V., Lukic I.K., Morcos M., Dehmer T., Rabbani N., Thornalley P.J., Edelstein D., Nau C., Forbes J., Humpert P.M., Schwaninger M., Ziegler D., Stern D.M., Cooper M.E., Haberkorn U., Brownlee M., Reeh P.W., Nawroth P.P.; "*Methylglyoxal modification of Nav1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy.* Nature Med."; 2012,18:926-33.

Blesneac I., Themistocleous A.C., Fratter C., Conrad L.J., Ramirez J.D., Cox J.J., Tesfaye S., Shillo P.R., Rice A.S.C., Tucker S.J., Bennett D.L.H., *"Rare NaV1.7 variants associated with painful diabetic peripheral neuropathy"*, Pain; 2018,159:469-480.

Cannon SC, Bean BP. "*Sodium channels gone wild: resurgent current from neuronal and muscle channelopathies."* The Journal of clinical investigation; 2010,120:80-83.

Cordella F, Ciancio AL, Sacchetti R, Davalli A, Cutti AG, Guglielmelli E, Zollo L., 2016*,"Literature Review on Needs of Upper Limb Prosthesis Users",* Front. Neurosci., vol. 10, May 2016.

Craner MJ, Klein JP, Renganathan M, Black JA, Waxman SG, "*Changes of sodium channel expression in experimental painful diabetic neuropathy";* Ann Neurol. 2002, 52:786-92.

Doll R, Buitenweg J, Meijer H, Veltink P.; "*Tracking of nociceptive thresholds using adaptive psychophysical methods*", Behavior Research Methods; 2014, 46:55–66.

Gao L, McMullan S., Djouhri L., Acosta C., Harper A.A, Lawson S.N; "*Expression and properties of hyperpolarization-activated current in rat dorsal root ganglion neurons with known sensory function"*, 2012, PLoS One, 7

Gold M.S, Shuster M.J, Levine D.J; "*Characterization of six voltage-gated K+ currents in adult rat sensory neurons"*; J Neurophysiol.; 1996, 75: 2629-46.

Hennings K., Frahm K.S., Petrini L., Andersen O.K., Arendt-Nielsen L., Mørch C.D.;, *"Membrane properties in small cutaneous nerve fibers in humans"*; Muscle Nerve; 2017, 55:195-201.

Hoberg, T. N., Frahm, S., Hennings, K., Arendt-Nielsen, L. & Mørch, C. D., "*Assessing the modulation of cutaneous sensory fiber excitability using a fast perception threshold tracking technique"*, 2019, Muscle & Nerve. 60: 367-375

Hugosdottir R, Mørch CD, Andersen OK, Helgason T, Arendt-Nielsen L., "*Preferential activation of small cutaneous fibers through small pin electrode also depends on the shape of a long duration electrical current."*, BMC Neurosci. 2019a; 20:48.

Hugosdottir R, Mørch CD, Jørgensen CK, Nielsen CW, Olsen MV, Pedersen MJ, Tigerholm J., "*Altered excitability of small cutaneous nerve fibers during cooling assessed with the perception threshold tracking technique"* BMC Neurosci. 2019b:20:47

Hugosdottir R, Mørch CD, Andersen OK, Arendt-Nielsen L. "*Investigating stimulation parameters for preferential small-fiber activation using exponentially rising electrical currents".* J Neurophysiol. 2019c; 122:1745-1752

Inui K, Tran TD, Hoshiyama M, Kakigi R., 'Preferential stimulation of Aδ fibers by intra-epidermal needle electrode in humans', 2002, *Pain*, 96: 247–252.

Ikoma A, Handwerker H, Miyachi Y, Schmelz M, "*Electrically evoked itch in humans"*, Pain, 2005, 113:148-154

Jurcakova D, Ru F, Kollarik M, Sun H, Krajewski J, Undem B, "*Voltage-Gated Sodium Channels Regulating Action Potential Generation in Itch-, Nociceptive-, and Low-Threshold Mechanosensitive Cutaneous C-Fiber"*, Molecular Pharmacology, 2

Kanat O, Ertas H, Caner B. "*Platinum-induced neurotoxicity: A review of possible mechanisms."* World J Clin Oncol, 2017;8:329-335.

Kaube H1, Katsarava Z, Käufer T, Diener H, Ellrich J., '*A new method to increase nociception specificity of the human blink reflex*', Clinical Neurophysiology, 2000, 111:413–416.

Klein JP, Renganathan M, Black JA, Waxman SG., "*Changes of sodium channel expression in experimental painful diabetic neuropathy"*. Craner MJ, Ann Neurol. 2002, 52:786-92.

Klein T, Magerl W., Hopf H., Sandkuhler J., Treede. R; "*Perceptual Correlates of Nociceptive Long-Term Potentiation and Long-Term Depression in Humans"*, The Journal of Neuroscience; 2004, 24: 964 –971;

Kuffler DP, Origins of Phantom Limb Pain, Mol Neurobiol. 2018; 55:60-69.

Leandri M, Marinelli L, Siri A, Pellegrino L. "*Micropatterned surface electrode for massive selective stimulation of intraepidermal nociceptive fibres".* J Neurosci Methods. 2018; 293:17-26.

Lelic D., Mørch C., Hennings K., Andersen O., Drewes A.; "*Differences in perception and brain activation following stimulation by large versus small area cutaneous surface electrodes"*; European journal of pain; 2012;16: 827-37.

Momose A, Yabe M, Chiba S, Kumakawa K, Shiraiwa Y, Mizukami H.
"*Role of Dysregulated Ion Channels in Sensory Neurons in Chronic Kidney Disease-Associated Pruritus"*. Medicines. 2019; 6.

Schofield JS, Evans K. R., Carey J. P., and Hebert J. S., *"Applications of sensory feedback in motorized upper extremity prosthesis: a review,"* Expert Rev. Med. Devices, 2014., 5:499–511,

Serra J., Bostock H., Solà R., Aleu J., García E., Cokic B., Navarro X., Quiles C.; "*Microneurographic identification of spontaneous activity in C-nociceptors in neuropathic pain states in humans and rats*."; 2012, Pain, 153:42-55

Smith S.M., Dworkin R.H., Turk D.C., Baron R., Polydefkis M., Tracey I., Borsook D., Edwards R.R., Harris R.E., Wager T.D., Arendt-Nielsen L., Burke L.B., Carr D.B., Chappell A., Farrar J.T., Freeman R., Gilron I., Goli V., Haeussler J., Jensen T., Katz N.P.; "*The Potential Role of Sensory Testing, Skin Biopsy, and Functional Brain Imaging as Biomarkers in Chronic Pain Clinical Trials: IMMPACT Considerations"*; J Pain., 2017, 18:757-777

Sittl R, Lampert A, Huth T, Schuy ET, Link AS, Fleckenstein J, et al. "*Anticancer drug oxaliplatin induces acute cooling-aggravated neuropathy via sodium channel subtype Na(V)1.6-resurgent and persistent current".* Proceedings of the National Academy of Sciences of the United States of America 2012;109:6704-6709.

Stein C, Eibel B, Sbruzzi G, Lago PD, Plentz RD. "*Electrical stimulation and electromagnetic field use in patients with diabetic neuropathy: systematic review and meta-analysis".* Braz J Phys Ther. 2013;17:93-104

Sygdomsudviklingen i Danmark fremskrevet til 2030. Statens Institut for Folkesundhed, 2017.

Tsantoulas C., Laínez S., Wong S., Mehta I., Vilar B., McNaughton P.A.; "*Hyperpolarization-activated cyclic nucleotide-gated 2 (HCN2) ion channels drive pain in mouse models of diabetic neuropathy",* 2017, Sci Transl Med; 9

Tigerholm J, Poulsen AH, Andersen OK, Mørch CD. "*From Perception Threshold to Ion Channels-A Computational Study".* Biophys J. 2019;117:281-295.

Verhoeven EW, de Klerk S, Kraaimaat FW, van de Kerkhof PC, de Jong EM, Evers AW., "*Biopsychosocial mechanisms of chronic itch in patients with skin diseases: a review"*. Acta Derm Venereol. 2008;88:211-8

Woolf C.J., Ma Q.; 2007; "*Nociceptors--noxious stimulus detectors"*; Neuron; 55:353-64.