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PREFERENTIAL ELECTRICAL STIMULATION OF SMALL NOCICEPTIVE FIBERS AND INDUCTION OF SECONDARY HYPERALGESIA

BY
ROSA HUGOSDOTTIR

DISSERTATION SUBMITTED 2021

AALBORG UNIVERSITY
DENMARK

PREFERENTIAL ELECTRICAL STIMULATION OF SMALL NOCICEPTIVE FIBERS AND INDUCTION OF SECONDARY HYPERALGESIA

PHD-THESIS

by

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AALBORG UNIVERSITY
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Dissertation submitted

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CV

Rosa obtained a B.Sc. degree in Biomedical Engineering from Reykjavik University in 2011. With an interest in research, Rosa worked the following year as a research assistant at Landspitali University Hospital in Iceland on electrical stimulation for rehabilitation of finger muscles of patients with spinal cord injury. Rosa obtained her master's degree from Aalborg University in 2015 and throughout the studies became inspired from different research projects, which all had the same focus towards increasing the quality of life in different patient groups.

Following that she obtained a PhD-stipend from the Center for Neuroplasticity and Pain (CNAP) and has since then worked on studies related to this dissertation. During this time, she also took on the role of being mom to two tiny research assistants, Hugo in 2015 and Julian in 2019. During the PhD period Rosa has supervised bachelor and master students from Aalborg University and an exchange student from Delft University and has co-authored scientific articles together with students. Her research is focused on preferential electrical stimulation of small nociceptive fibers and methods for inducing secondary hyperalgesia in humans. Rosa has been active in CNAP and the Integrative Neuroscience research group and participated in various pain conferences, IASP, EFIC, SASP, with presentations and discussions related to the PhD studies.

After moving back to Iceland with the family in the end of 2019, Rosa completed this PhD thesis on the basis of two published journal articles, one accepted conference article, and one recently submitted article.

PREFACE

The present PhD thesis provides a summary of work performed in the period from 2015-2020 at the Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University, Denmark. The work was financially supported by the Danish National Research Foundation (DNRF121). The thesis is organized as an overview of the background and a discussion of the applied methods and the findings from four scientific papers. The topic of the four papers are related as results on preferential small fiber activation from paper 1 and 2 were used to modify the high frequency electrical stimulation pain model in paper 3. However the main content of the thesis is divided into chapters depending on the content of the papers. In chapter 2, the topic of 'preferential activation of small nociceptive fibers' will be discussed, which was the focus of paper 1 and 2. Chapter 3 contains discussion on the high frequency electrical stimulation pain model, which was investigated in papers 3 and 4. The results are documented in more details in the papers.

Throughout the thesis, the articles are referred to as:
Paper 1: Hugosdottir, R[1]., Mørch, C. D[1]., Andersen, O. K[1]., Helgason, T[2]., & Arendt-Nielsen, L[1]. (2019). Preferential activation of small cutaneous fibers through small pin electrode also depends on the shape of a long duration electrical current. *BMC Neuroscience. 20(1), 48.*
Paper 2: Hugosdottir, R[1]., Mørch, C. D[1]., Andersen, O. K[1]., & Arendt-Nielsen, L[1]. (2019). Investigating stimulation parameters for preferential small fiber activation using exponentially rising electrical currents. *Journal of neurophysiology*, 122(4), 1745-1752.
Paper 3: Hugosdottir, R[1]., Kasting, M. J. M[3]., Mørch, C. D[1]., Andersen, O. K[1]., & Arendt-Nielsen, L[1]. A modified human model of secondary hyperalgesia: high frequency electrical stimulation with an exponential prepulse to increase preferential activation of nociceptive fibers. Accepted for publication in IFMBE conference proceeding.
Paper 4: Hugosdottir, R[1]., Kasting, M. J. M[3]., Mørch, C. D[1]., Andersen, O. K[1]., & Arendt-Nielsen, L[1]. Priming with capsaicin facilitates the effect of high frequency electrical stimulation in humans. Submitted to Journal of Neurophysiology.

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ENGLISH SUMMARY

Chronic pain is a major global burden, which lowers quality of patients' lives due to e.g. distress, impairments and economic burden. Pain is an important warning signal and in a normal nociceptive system, a balance between pain facilitation and inhibition is crucial, but this balance can be disturbed causing facilitation of pain. If the system does not adapt and rebalance, the purpose of pain as a warning signal may be lost and it may instead develop into a chronic pain state. Better understanding of the specific underlying mechanisms of chronic pain is needed to improve diagnosis and development of appropriate and mechanisms-based therapeutic strategies.

Experimental models can be used to reveal pain mechanisms in humans. Secondary hyperalgesia is a phenomenon of increased sensitivity, observed in experimental and chronic pain conditions. Experimentally induced secondary hyperalgesia has been used as a model of neuropathic pain.

Heat/capsaicin application and high frequency electrical stimulation (HFS) are well known methods to induce secondary hyperalgesia. One mechanism believed to be involved in secondary hyperalgesia is heterosynaptic long term potentiation (LTP) of synaptic plasticity in the spinal cord.

When using electrical stimulation in a pain model, it is essential to activate nociceptive fibers rather selectively, which is difficult as their activation threshold is higher than for large non-nociceptive fibers. Patch electrodes with large cathodes (3 cm^2) therefore activate mainly large diameter non-nociceptive fibers. Pin electrodes with small diameter cathodes provide high current density in the skin epidermis where primarily nociceptive fibers terminate. Moreover, slowly rising electrical pulses have been shown to cause accommodation of large non-nociceptive fibers, which leads to increased activation threshold of these fibers. This may be utilized to reverse the activation of large and small fibers with electrical stimulation. Accommodation is likely generated by the voltage gated ion channels and distribution of ion channel subtypes differs between small nociceptive and larger fibers. The optimal electrical stimulation parameters, such as pulse shape and duration, for maximizing the effect of accommodation in large fibers have not yet been established.

The present thesis was set out to further investigate the human experimental HFS model and increase its efficiency and physiological relevance by utilizing preferential electrical stimulation. Therefore, the first two studies examined the electrical stimulation parameters to increase preferential activation of small nociceptive fibers. In both studies electrical pulses were applied on the volar forearm of 25 healthy subjects with pin- and patch electrodes. Main outcomes were based on perception thresholds that were obtained for different pulse shapes in paper 1 and for different durations of a special bounded exponential (B.EXP) pulse shape in paper 2.

In papers 1 and 2 results showed that perception thresholds of the patch electrodes, that mainly activate large fibers, were increased for long duration B.EXP pulses. The threshold increase for the patch electrodes is likely due to accommodation of large sensory nerve fibers. For same pulse shapes delivered with the pin electrode, such

threshold increase was not observed, which indicates absence of accommodation in small nociceptive fibers. In paper 2, largest accommodation was found for the 100 ms B.EXP pulse, where perception threshold reached a current of twice the rheobase.
In paper 3 and 4, a modified HFS model was investigated. In paper 3, HFS was applied following a preconditioning 100 ms B.EXP pulse (HFS+B.EXP) through the pin electrodes. Secondary hyperalgesia was assessed with mechanical pinprick stimulation in the area around the HFS stimulation and in a distal control area at baseline and for 50 minutes after HFS. Results indicated that secondary hyperalgesia could be facilitated when HFS was applied following a prepulse for generating accommodation in large fibers, compared to the standard HFS paradigm. This was however provided that it was assessed with 128 mN pinprick stimuli as no differences were observed with 256 mN pinprick stimuli. In paper 4, the effect of HFS delivered subsequent to a heat/capsaicin application (HFS+HEAT/CAP) was investigated. The heat/capsaicin was applied to prime the system by generating a state of hyperexcitability. Similar to results in paper 3, the HFS+HEAT/CAP paradigm caused increased secondary hyperalgesia, again provided that it was assessed with 128 mN pinprick stimuli (paper 4).
When taken together, this work has clarified certain aspects regarding stimulation parameters for preferential electrical stimulation of small nociceptive fibers. The findings from the present work suggest that a B.EXP pulse shape causes optimal rate of current increase to generate accommodation and increase the perception threshold of large sensory fibers. The optimal duration of the pulse is 100 ms, but for the sake of practicalities, durations from above 20 ms may also be sufficient to accommodate large fibers to some extent. This may be utilized to increase preferential activation of small nociceptive fibers as attempted by using the B.EXP pulse to precondition the HFS paradigm, assuming it would generate large fiber accommodation prior to the conditioning of the small nociceptive fibers. This method is believed to increase the specificity of the HFS model by limiting confounding mechanisms related to activation of large Aβ fibers. The priming with heat/capsaicin also increased the amount of hyperalgesia, but unlike the HFS+B.EXP paradigm, the increased sensitivity may be due to synergistic mechanisms of heat/capsaicin and HFS and future work is required to explore potential utilities of the combined method.

DANSK RESUME

Kroniske smerter er et stort globalt problem, som forårsager en forringelse af patienternes livskvalitet, fx på grund af bekymringer og svækkelse, ligesom kroniske smerter giver anledning til en stor økonomisk byrde. Smerte udgør et vigtigt advarselssignal og i et normalt nociceptivt system er en balance mellem smertefacilitering og smertehæmning afgørende, men denne balance kan forstyrres og skabe facilitering af smerte. Hvis systemet ikke tilpasser og rebalancerer sig, kan smertens rolle som advarselsfunktion forsvinde og i stedet kan der udvikles en kronisk smertetilstand. En bedre forståelse af de specifikke mekanismer, der ligger til grund for kroniske smerter er nødvendig for at forbedre diagnosen samt udvikle passende og mekanismebaserede behandlingsstrategier. Eksperimentelle modeller kan anvendes til at afsløre smertemekanismer hos mennesker. Sekundær hyperalgesi er et fænomen, der forårsager forøget sensibilisering, hvilket er observeret i både eksperimentelle og kroniske smertetilstande. Eksperimentelt induceret sekundær hyperalgesi har været anvendt i en model for neuropatisk smerte.

Opvarmning af huden, påføring af capsaicin og elektrisk stimulation med høj frekvens (højfrekvent elektrisk stimulation, HFS) er velkendte metoder til at fremprovokere sekundær hyperalgesi. En af de mekanismer, der menes at være involveret i sekundær hyperalgesi, er heterosynaptisk langvarig potensering (long term potentiation, LTP) af synaptisk plasticitet i rygmarven.

Når der anvendes elektrisk stimulation i en smertemodel, er det essentielt at aktivere de nociceptive fibre meget selektivt, hvilket er svært, idet aktiveringstærsklen er højere for disse end for store ikke-nociceptive fibre. Nåleelektroder med en lille katode diameter genererer en høj strømtæthed i overhuden, hvor de nociceptive fibre primært ender. Standardelektroder med større katode diameter (2 cm^2) aktiverer primært store ikke-nocieptive fibre. Endvidere har elektriske impulser med stigende styrke vist at skabe akkommodation af store ikke-nocieptive fibre, hvilket fører til en forøget aktiveringstærskel i disse. Dette kan udnyttes til at reversere aktiveringsrækkefølgen af store og små fibre med elektrisk stimulation. Akkommodation genereres sandsynligvis af de spændingsstyrede ionkanaler og fordelingen af undertyper af ionkanaler er forskellig hos små nociceptive fibre og større fibre. De optimale parametre for elektrisk stimulation, fx pulsform og -varighed, til maksimering af effekten af akkommodation i store fibre er endnu ikke kendt.

Formålet med denne afhandling var at foretage yderligere undersøgelser af den humane eksperimentelle HFS-model og forøge dens effektivitet og fysiologiske relevans ved at benytte selektiv elektrisk stimulation. Derfor undersøgte de to første studier hvilke elektriske stimulationsparametre, der kunne forøge den selektive aktivering af små nociceptive fibre. I begge studier gives elektriske impulser på indersiden af underarmen af 25 raske forsøgspersoner med små og store katoder.

Resultaterne i artikel 1 var baseret på de perceptionstærskler, der blev målt for forskellige pulsformer, og i artikel 2 var det for forskellige varigheder af en speciel afgrænset ekspotentiel (bounded exponential, B.EXP) pulsform.

I begge disse studier viste resultaterne, at perceptionstærsklerne af store fibre, blev forøget ved langvarige B.EXP-pulser. Tærskelforøgelsen for store fibre skyldes sandsynligvis akkommodation i store nervefibre. For de samme pulsformer udført med nåleelektroden blev der ikke observeret tærskelforøgelser, hvilket indikerer at de små nociceptive fibre ikke akkommoderer. I artikel 2 blev den største akkommodation fundet ved B.EXP-pulsen på 100 ms, hvor perceptionstærsklen nåede en styrke på to gange rheobasen.

Studierne, der ligger til grund for artikel 3 og 4, undersøgte en modificeret HFS-model. I artikel 3 blev HFS givet gennem nåleelektroder med en 100 ms B.EXP pre-puls (HFS+B.EXP). Sekundær hyperalgesi måles med prik-stimulationer i området omkring HFS-stimulationerne og i et distalt kontrolområde ved baseline og i 50 minutter efter HFS. Resultaterne indikerede, at sekundær hyperalgesi var faciliteret, når HFS udføres med en præ-puls til akkommodation af store fibre sammenlignet med standard HFS-paradigmet. Det forudsættes dog at stimulationen blev givet med en 128 mN prik-stimulationer, da der ikke blev observeret forskelle med 256 mN prik-stimulationer. Forsøgene i artikel 4 undersøgte effekten af HFS givet efter varme og capsaicin (HFS+HEAT/CAP). Varme og capsaicin blev anvendt for at generere en tilstand af hyperexcitabilitet. Svarende til resultaterne fra forsøgene i artikel 3 forårsagede HFS+HEAT/CAP-paradigmet sekundær hyperalgesi, igen forudsat at det var med 128 mN nåleprik-stimulationer.

Alt i alt har disse studier klarlagt aspekter omkring stimulationsparametre for selektiv elektrisk stimulation af små nociceptive fibre. Resultaterne indikerer at en B.EXP-puls giver den optimale rate af strømforøgelse til at akkommodere og forøge perceptionstærsklen i store sensoriske fibre. Den optimale varighed af pulsen er 100 ms, men af praktiske årsager kan varigheder over 20ms være tilstrækkelige til at akkommodere store fibre i et vist omfang. Dette kan anvendes til at forøge selektiv aktivering af små nociceptive fibre som forsøgt ved anvendelse af B.EXP-prepulsen i HFS-paradigmet, idet det antages, at dette vil generere tilpasning af store fibre inden konditioneringen af de små nociceptive fibre. Denne metode menes at forøge specifiteten af HFS-modellen ved at begrænse modstridende mekanismer, der er relateret til aktivering af store Aβ-fibre. Forbehandlingen med varme og capsaicin forøgede også mængden af hyperalgesi. Men i modsætning til HFS+B.EXP-paradigmet kan den forøgede sensibilitet skyldes synergistiske mekanismer mellem varme/capsaicin og HFS. Fremtidige studier er nødvendige for at undersøge potentielle anvendelsesmuligheder for disse kombinerede metoder.

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Special thanks go to my family and friends, who have always been there with me and supported me in so many ways. Last but not least, I would like to thank my husband Benedikt and my wonderful little boys, Hugo and Julian, for all the support and lovely distractions throughout the journey. Personally, it has been very important for me to learn how to engage in a balanced home-work life by appreciating and enjoying the moments with my lovely family and making the most of the working hours at the University.

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CHAPTER 1. INTRODUCTION

1.1. CHRONIC PAIN

Short lasting, acute pain is normal and necessary to provide a protection against potential danger. Pain is a complex process involving both the peripheral- and central nervous system and in a normal system pain is initiated by activation of peripheral nociceptive fibers, which provide input about potential harm to the spinal cord dorsal horn neurons. Following complicated processing (including possible sensitization) in the spinal cord and higher centers pain is experienced (1). The balance between pain facilitation and inhibition is a key towards a nociceptive system in balance, but this can be disturbed causing unnecessary increased pain sensation. If the system doesn't adapt and re-balance, it is considered maladaptive and pain may be facilitated (2,3). The purpose of pain as a warning signal can at this state be lost and may instead develop into a chronic pain stage. In chronic pain states, activation of few nociceptive pulses may be amplified centrally (4) and modulation of primary afferents may contribute (5). In fact, chronic pain is a major global burden and comes with major suffering due to distress, functional impairments and economic burden (6). The prevalence of approximately 20% and 30% has been estimated in large-scale studies in Europe (6) and in the United States (7), respectively. Despite this, chronic pain is often underprioritized by healthcare professionals due to its complicated nature, lack of pain management training, partly unknown mechanisms, and sometimes unknown origin. Adequate diagnose and development of appropriate and mechanism-based therapeutic strategies, still require better understanding of the specific underlying mechanisms related to pain and pain chronification. An important aspect of revealing the underlying mechanisms, both peripheral and central, is to be able to assess properties of primary afferent nociceptive fibers and modulate their actions.

1.2. HUMAN EXPERIMENTAL PAIN MODELS

Human experimental pain models consist of different methods and modalities used to provoke pain and probe the state of the human pain system to increase our understanding of the complicated pain mechanisms in humans. Unfortunately, direct measurements from the human central nervous system are not possible. Animal models, have therefore been used as proxies to provide specific details regarding the underlying mechanisms, although they may differ from the human system (8) and translatabilities are unfortunately not optimal (9). Experimental pain models usually consist of modality specific stimuli that activate the pain system in a standardized way and simulate specific mechanism, which can be assessed with either psychophysical or neurophysiological measures (e.g. fMRI, EMG, or EEG). An important feature of an experimental model is that the stimuli is reproducible, easily controlled and mostly selective for activating the intended fiber types (10).

Natural experimental pain can be provoked using thermal-, chemical- and mechanical stimuli, and pain system can also be provoked with artificial electrical stimuli. Major short comings often relate to the non-specificity towards the nociceptive fibers and the rapidity of pain onset (11). Laser stimuli can deliver selective stimuli towards nociceptive fibers (12,13) and provide responses with well characterized latencies and laser-evoked potentials have been widely used for assessing the integrity of both the central and peripheral nociceptive pathways (14). Main problems relate to technical difficulties and advanced equipment (11,15). Electrical stimuli can be used to elicit time-locked responses, stimuli are brief and safe if administered under controlled conditions, equipment is rather cheap, and stimulation parameters are easy to control. Its main disadvantage relates to the fiber recruitment order as the stimuli activates large-diameter mechanoreceptive fibers at lower intensity than the small nociceptive fibers (16) and hence notoriously have been challenged for the use as a selective nociceptive activator.

1.3. MODELS OF SECONDARY HYPERALGESIA

Experimentally induced secondary hyperalgesia has been considered as a model of neuropathic pain (17,18). Secondary hyperalgesia is a phenomenon often observed in experimental and chronic pain conditions including neuropathic pain and is centrally mediated via dorsal horn facilitatory neuroplastic changes (19). Secondary hyperalgesia is defined by the IASP as “An increased response to a stimulus which normally is painful” (20).

Long-term potentiation (LTP) of synapses in spinal cord dorsal horn is believed to play a role in secondary hyperalgesia and when “not under control” it likely contributes to the manifestations of chronic pain (21). Extensive research has been performed on LTP in spinal synapses in rats, but most focus has been on homotopic LTP, i.e. potentiation of the homosynaptic pathway (22,23). Recent research however indicates that LTP of the heterosynaptic pathway also exists in the spinal cord dorsal horn (24), further supporting its role in development of secondary hyperalgesia.

A well-studied human experimental pain model uses cutaneous high frequency electrical stimulation (HFS) through special pin electrodes to induce secondary hyperalgesia, which has been used as a model to gain understanding on central pain neuroplasticity. In this model, secondary hyperalgesia is usually assessed by psychophysical measures showing increased pain sensitivity in areas around HFS (25–28). Other methods have been used to induce secondary hyperalgesia such as capsaicin injection (29,30), heat-burn stimulation (31,32) and combined heat/capsaicin stimulation (33). The heat/capsaicin sensitization model causes a robust secondary hyperalgesia, but the combined method does not cause facilitated secondary hyperalgesia and the effect of heat and capsaicin is therefore not considered synergistic (33). “HFS and the heat/capsaicin sensitization models act through somewhat similar mechanisms, but some differences are also evident. The heat/capsaicin application acts selectively on the TRPV1-positive A- and C nociceptive fibers (17,34) and causes primary heat hyperalgesia (peripheral

sensitization) and secondary mechanical hyperalgesia (33). The HFS model acts on all epidermal primary afferent fibers, i.e. both TRPV1-postive and TRPV1-negative Aδ- and C-fibers (18)" (paper 4). Whether secondary hyperalgesia can be facilitated through synergistic effects of HFS and the heat/capsaicin sensitization models is unknown.

The induction of secondary hyperalgesia depends on the degree on intense activation of C-fibers with little or no contribution from A-fibers (18,35,36). The HFS model however cannot activate the nociceptive fibers selectively with the pin electrode as for high intensity coactivation of the large Aβ-fibers occurs (37) adding complexity to the selectivity of the activation. Improving the selectivity towards the small nociceptive fibers in the HFS model would provide a valuable mechanism-based tool to investigate secondary hyperalgesia and neuroplasticity in general in humans.

1.4. PREFERENTIAL ELECTRICAL STIMULATION

Electrical stimuli can be used to artificially activate sensory and/or nociceptive fibers. Instead of direct, specific receptor activation with subsequent neurotransmitters release as occurs during natural stimuli, electrical stimulation can cause changes in transmembrane potential, and directly activate voltage gated ion channels leading to action potential with no delay in activation due to transduction mechanisms.

The excitability of the nerve fibers determines the threshold for action potential, which depends on the nerve type and size, the electrode geometry, electrical properties of the extracellular tissue, electrode to fiber distance, and the stimulation parameters (16,38,39). The nerve fiber excitability also depends on the ion channel expression and their activation- and inactivation kinetics (40–42). Lower electrical current is usually needed to cause action potential in large- compared to small diameter nerve fibers (16). Due to this standard recruitment order, selective activation of small diameter fibers with electrical stimulation is difficult. This is problematic when using electrical stimulation in a human experimental pain model in an attempt to activate the small diameter nociceptive fibers.

The small diameter pin electrodes used in the HFS experimental model seem to provide the majority of the applied current (high current density) in the epidermal layer where the nociceptive fibers terminate (43–45). But, high intensity (as needed to induce secondary hyperalgesia) rectangular current pulses may cause the current to spread to deeper layers and co-activate large non-nociceptive fibers (37).

Promising animal studies have aimed at blocking large fibers and activating small fibers selectively by utilizing the different excitability properties of the small and large nerve fibers of a nerve branch (46–48). In these studies, tripolar cuff electrodes were used to deliver the blocking current directly on the same nerve as the recording electrode, which allows for reliable interpretation of the results, but is limited to such invasive techniques. Also using invasive cuff techniques, a study (49) suggested that the duration of a sine-wave current was a determining factor for initiating C-fiber response and eliminating A-fiber response. The invasive cuff electrodes limit the usability in experimental human pain research. Depolarizing pre-pulses have been

introduced to activate small fibers at lower current intensity than larger fibers and by utilizing computational models results could be explained by the activation and inactivation gates of the sodium channels (39).
Accommodation is a mechanism of decreased nerve fiber excitability due to depolarization. Results from Hennings and colleagues indicate that accommodation can be utilized to reverse activation of large and small motor neurons when applying linearly increasing currents in humans non-invasively (50). The rate of current increase however has been shown to affect the degree of accommodation (51,52). A computational study furthermore showed a potential for using exponentially increasing currents to reverse the activation order of motor neurons (53). A recent study suggested that slow sine wave of 4 Hz could selectively activate C-fiber nociceptors (54). The use of slowly rising pulses in combination with high current density stimulation through the small diameter pin electrode has not been investigated in details as a human experimental pain stimulation model. Furthermore, the optimal stimulation parameters for activating the nociceptive fibers preferentially including shape, duration and intensity are yet to be discovered.
Therefore, the aim of the first two studies in this PhD study was to examine parameters of slowly rising currents for preferential activation of small nociceptive fibers and accommodation of large sensory fibers. As the intensity should be relatively high to induce secondary hyperalgesia, slowly rising pulses themselves may not be considered feasible. Therefore in the third study, the slowly increasing current parameters identified in the first two studies, were used to precondition the standard HFS model and increase the selectivity of small fibers in the model.

1.5. AIMS AND EXPERIMENTAL STUDIES

The ovreall aim of this PhD project was to improve the physiological relevance and effect of the human HFS pain model by utilizing selective electrical stimulation and optimal stimulation parameters to increase selectivity of nociceptive fiber activation in the HFS model. Furthermore, the effect of HFS was examined in relation to an experimental condition with already established hightened central hyperexcitability provoked by priming the system with heat/capsaicin sensitization. An overview of the studies carried out in this PhD project is shown in figure 1-1. The aim of the PhD can be divided into four sub-aims (see below) that were adressed in the four papers listed in the preface of this thesis.

1) Investigate stimulation pulse shape for improving selective activation of small primary afferent nociceptive fibers (Paper 1)
2) Investigate in details the duration of the optimal slowly rising pulse for improving selective activation of small primary afferent nociceptive fibers (Paper 2)
3) Investigate the effectiveness of HFS following a slowly rising pre-pulse for targeting the small nociceptive fibers preferentially (Paper 3)
4) Investigate the effectiveness of HFS after priming of the central and peripheral nervous system with a heat/capsaicin application (Paper 4).

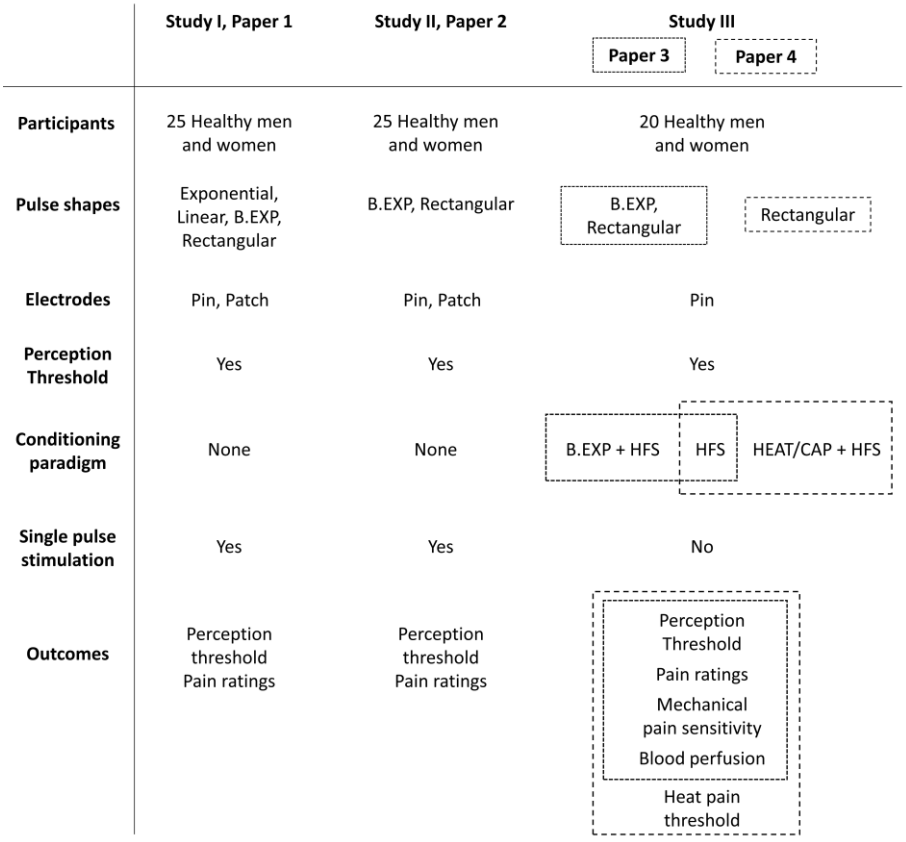

Figure 1-1. Flow chart for the three studies and papers included in the thesis. Dashed boxes in study III illustrate differences between paper 3 and paper 4. B.EXP = Bounded exponential, HFS = High frequency electrical stimulation, HEAT/CAP = heat and capsaicin.

CHAPTER 2. PREFERENTIAL ELECTRICAL STIMULATION OF SMALL NOCICEPTIVE FIBERS

Electrical stimulation has been widely used as a method for activating both sensory and motor neurons. In rehabilitation, functional electrical stimulation is used to restore motor control. In clinical neurophysiology, nerve function can be measured by use of electrical stimulation and recording along the nerve for measuring nerve conduction velocity (55). Electrical stimulation can furthermore be used to relief pain through gate-control mechanism by activation of large diameter Aβ fibers, which are believed to deliver inhibiting signals against pain facilitation occurring in the spinal cord (56,57). This is likely utilized in spinal cord stimulation, which is a clinically applied method to relief pain, primarily influenced by large Aβ fibers (58) but the underlying mechanisms are poorly understood. Opposite to the pain relief obtained by stimulation of Aβ fibers, assessment of small nociceptive fibers and experimental pain paradigms require selective- or at least as preferential activation of the small nociceptive fibers (10).

Selective small fiber activation with electrical stimulation is challenging and physiologically difficult due to the activation order of peripheral nerve fibers, as large fibers are recruited using lower intensities than the small fibers with conventional transcutaneous electrical stimulation (15,16). This is however not entirely exclusive as there are other factors related to fiber excitability, which do not directly depend to the fiber diameter.

As mentioned in the introduction, animal studies using invasive blocking techniques have been proposed to reverse activation order of small and large fibers with electrical stimulation (46,48,49). In early days, before it was understood how the action potential was generated, the idea of accommodation to slowly rising stimuli was investigated (52). The concept was based on inactivation of the nerves when slowly rising electrical pulses were applied without providing any mechanistic explanations. Pulse shapes ranging from linearly increasing to pulse shapes with more steep current increase approaching rectangular pulses were examined and results from these studies indicated that the amount of accommodation caused by a slowly increasing pulse depended on the shape of current increase (51,52,59). More than 50 years later, extensive work was done by Hennings and colleagues where they revisited the accommodation phenomenon using both computational modeling studies and experiments based on motor neuron activation (50,60). Their studies relied on linear and exponentially increasing current and the results indicated that more pronounced accommodation could be achieved in larger motor neurons compared to smaller motor neurons, which could be utilized to reverse their activation order (50,53). This could

possibly enable functional electrical stimulation to recruit motor neurons according to the physiological recruitment order (61).
Paradigms based on electrical stimulation to probe human pain mechanisms and sensory nerves would benefit greatly from methods to increase selectivity towards small fibers. In series of experimental studies, the threshold tracking technique has been investigated for assessing functions of large sensory nerve fibers in humans (62,63). Recently, the method of perception threshold tracking was proposed as a way to investigate the properties of small sensory nerve fibers with electrical stimulation (42,64). The perception threshold tracking utilizes small diameter pin electrodes to achieve spatial selectivity in the epidermis where the small nociceptive fibers terminate (15,43,64).
For stimulating small fibers in humans, physiological relevance of both stimulation electrodes and stimulation parameters has to be considered and invasiveness should be limited. Above evidence indicate that small fiber selectivity can be increased with slowly rising currents and also by narrowing the electrical field through small diameter electrodes. Selectivity towards different fiber diameters can therefore ideally be improved by the type of stimulation electrode and the applied stimulation parameters, such as pulse form, duration, and intensity. In this chapter, background on nerve fiber excitability will be provided (section 2.1) and methods applied (section 2.2-2.4) and findings obtained (2.5-2.7) in this PhD project on preferential small fiber activation in humans will be discussed and put into context according to previous research. Finally, a summary of the chapter is provided.

2.1. EXCITATION PROPERTIES OF NERVE FIBERS

For being able to develop methods for selective activation of small nociceptive fibers, understanding the biophysical basis for electrical stimulation and the excitability properties of the peripheral nerve fibers is essential. When electrical stimulation is applied there are several factors believed to affect the nerve fiber excitability and the order of nerve fiber activation. The diameter of the fiber and distance from the electrode to the fiber mainly determine the excitability (16), but the structure of the cell membrane, i.e. different proteins, also affect the excitability of the fibers (65). All cell activity includes protein processes, which passively or actively transfer ions through the cell membrane causing different intra- and extracellular ion concentration (65). An equivalent electrical circuit can be used to model the nerve fiber excitation during electrical stimulation, which can be understood as a current source and the membrane can be modeled by electrical resistors, accounting for the ion channels, and conductors, accounting for the membrane capacitance, in parallel (66–68). The ion concentration difference inside and outside the membrane generates the reversal potential, which is the basis for the action potential as shown theoretically by Hodgkin and Huxley in 1952 (66). It is now known that the gating factors described in the Hodgkin and Huxley equations depend on the opening and closing of activation and inactivation gates of the voltage gated ion channels. It is important to note that inactivation lags activation (66). The activation and inactivation gates serve as key

structures in the initiation and development of the action potential and therefore affect nerve fiber excitability. The opening and closing of activation and inactivation gates depends on the ion channel structure, which differs between different subtypes of the ion channels (69). Therefore, the expression of ion channel subtypes affects the excitability of nerve fibers to electrical stimulation.

The "standard" recruitment order of nerve fibers, which depends on the fiber diameter, is due to factors that affect nerve fiber activation that indeed depend on nerve fiber diameter, i.e. changes in extracellular potential and membrane resistance (65,70). Larger diameter fibers therefore become more depolarized for a given electrical current and action potential generation will occur at lower currents (e.g. 65,70). The results from the above mentioned models of nerve fiber activation are based on electrical stimulation with rectangular electrical currents.

The accommodation phenomenon relates to nerve fiber depolarization with low intensity or slowly rising electrical currents, which hinder action potential generation and increase activation threshold (51,52). Accommodation has only been shown to affect large diameter fibers (16,42,50). Therefore, in this PhD project slowly rising depolarizing pulses were used in an attempt to decrease large fiber excitability by utilizing large fiber accommodation and thereby reverse the activation order of small and large fibers (46,50).

Accommodation is modulated by the resting membrane potential and is most likely generated by the voltage gated ion channels (71) involving both inactivation of sodium (72,73) and potassium channels (74,75). Accommodation is therefore considered as an important excitability property that depends on the cellular and molecular biology of the ion channel subtypes and the fact that they are also regionally distinct as they are expressed differently on small and large diameter fibers (reviewed in Catterall et al. 2005).

Nine Na_V subtypes have been identified (76), i.e: $Na_V1.1$-$Na_V1.9$ according to the Goldin nomenclature (77). The Na_V subtypes have different molecular and physiological characteristics (78) and therefore have different voltage-dependence for activation and inactivation, pharmacological properties, and time constants for activation and inactivation (76,78). Subtypes $Na_V1.6$- $Na_V1.9$ are mainly expressed in the sensory nerve fibers (76) and their kinetics is therefore in focus regarding excitability of sensory nerve fibers. Subtypes, which are tetrodotoxin (TTX)-resistant, Na_V 1.8 (79,80) and $Na_V1.9$ (81) are preferentially expressed in small and medium sized dorsal root ganglion (DRG) neurons and their axons (80,81). $Na_V1.7$ has also been shown to be expressed in majority of small neurons and act as the predominant TTX-sensitive current in small fibers (82) whereas $Na_V1.6$ is the predominant TTX-sensitive current in large fibers (83). Different from large fibers, where action potential is mainly driven by TTX-sensitive currents, action potential in small nociceptive fibers is mostly generated by TTX-resistant Na_V 1.8 (Blair and Bean 2002).

A recent study showed that accommodation could be generated in computational model of large Aβ fibers when only TTX-sensitive ion channels were implemented, illustrating the importance of these Na_V subtypes in accommodation (42). TTX-resistant ion channels have slower activation and inactivation than TTX-sensitive ion

channels (41), which could partly explain why accommodation is more pronounced in large versus small fibers (42).
Less is known about the expression of potassium channel subunits on the different sensory nerve fibers (84) and the nomenclature for the K_V subtypes is not as clear as for the Na_V channels. The slow inactivation time constant measured in DRG neurons likely relates to several K_V subtypes and has been shown to vary greatly in sensory neurons (85). It has furthermore been found that some subtypes are selectively expressed in small nociceptive neurons whereas others are selectively expressed in larger non-nociceptive fibers (84,85).
Different excitability properties of small and large fibers may therefore play a role in determining activation order of the fiber types with electrical stimulation. In this PhD project the evidence regarding different activation and inactivation kinetics of ion channel subtypes, which are expressed differently on the small versus the large fibers, were utilized to target small fibers with slowly rising electrical pulses that could activate small fibers whilst forcing large fibers to inactivate (accommodate).

2.2. ELECTRICAL STIMULATION EQUIPMENT

Electrical stimulation was applied in all experiments. The equipment for delivering and controlling electrical stimulation (shown in Fig. 1) was composed of an electrical stimulator (DS5, Digitimer), which has been developed to stimulate peripheral nerve fibers, a digital acquisition card (NI-DAQ, Usb-6351, National Instruments), personal computer, and electrodes. The software used to control the stimulation was a customized LabView program made at Aalborg University.
In all studies, two-electrode system was applied with the cathode (driven negative) acting as a working electrode for delivering the electrical current and the anode (driven positive) acting as a counter electrode. The electrodes are in this thesis referred to as cathodes and anodes. When current flows from the cathode to the anode through the electrolytic solution an electrical field is generated, which distribution in the surrounding tissue depends on the dimensions of the electrode and the electrode-tissue interface (43,44,86). The electrode selection therefore plays a major role in determining the distribution of the electrical field, which affects which nerve fibers have a potential to activate to the delivered current (43).
To achieve widespread electrical field in the dermal skin layer for targeting the large-diameter Aβ fibers, well known self-adhesive patch electrodes were applied in papers 1 and 2. The cathodes were 2 cm^2 Neuroline 700 electrodes (Ambu A/S Ballerup, Denmark) and the anodes were 10 cm^2 Pals Neurostimulation electrodes (Axelgaard, co., Ltd., Fallbrook, CA, USA) (64). For targeting the small-diameter nociceptive Aδ and C fibers, which are mainly located in the skin epidermis, the intracutaneous electrical stimulation was originally proposed by Bromm and Meier in 1984. The electrode configuration was based on an intra-epidermal high current density electrical stimulation via 1.2 mm electrodes inserted to a small hole, which was drilled into the epidermis (87). Their results regarding detection and pain threshold as well as pain sensation support preferential activation of nociceptive fibers. The current

intensity needed to obtain pain- and detection threshold was 0.38 mA and 0.08 mA, respectively, and decreased 10 fold compared to the standard superficial stimulation where the pain and detection thresholds were 4.6 mA and 1.2 mA, respectively (87). Therefore, a decreased absolute current pre-pain range was observed (i.e. the difference between pain and detection threshold). Furthermore and most importantly, the intracutaenous stimulus caused a definite and well localized pain characterized with a stabbing, hot and sharp sensation (87). This was in sharp contrast with the conventionally applied currents, which elicited an unpleasant paresthesia and consequently the stimuli were not tolerated by the subjects if intensity exceeded the 2.5 fold individual pain threshold (87). The idea of the intracutaneous stimulations has been further developed and in 2000, a modified surface electrode with small diameter pins was presented, which allowed a noninvasive activation of small diameter fibers involved in the blink reflex (88). In 2002, Inui and colleagues proposed yet another intra-epidermal electrode consisting of needle protruding the epidermis. They were able to show that evoked potential latencies for this type of stimulation was longer than for conventional transcutaneous electrical stimulation (302 ± 17 ms and 245 ± 22 ms, respectively), but still shorter than for laser stimuli (341 ± 21 ms). Based on these recordings, conduction velocity of the intra-epidermal stimulation electrode was estimated to be 15.1 m/s, which corresponds to the conduction velocity of Aδ fibers (15). In 2009, the same research group reported that the electrode was capable of activating C fibers, since a slower conduction velocity (1.5 ± 0.7 m/s) was reported (89). The study used a triangular pulse, which may have played a role in the enhanced selectivity towards the C fibers (89).

To activate a larger field than usually possible with needle or pin like electrodes, the cutaneous field stimulation was composed of 16 needle-like electrodes, fixed 2 cm a part in a matrix (90). The sensation was described as pricking and burning and the burning sensation remained after applying A-fiber block, which indicated activation of C-fibers (90). Similar to this design and also utilizing the concept of spatial summation, but with pins that do not penetrate the skin, Klein and colleagues have repeatedly applied a ring electrode composed of 10 pins placed in a small circle (25). Inspired by this design and utilizing both spatial summation and providing a narrow distribution, high density electrical field in the epidermis of the skin, 15 small-diameter stainless steel pins were used as cathodes in papers 1 and 2. Surrounding the pins, a concentric stainless steel plate was used as anode in papers 1 and 2. This electrode configuration will be referred to as the pin electrode, which was designed and built at Aalborg University (see Fig 2-1). The pin electrode has been used in several research projects including EEG measurements of latencies, which match the conduction velocities of the small-diameter Aδ fibers (45). In papers 3 and 4, only the cathodal part of the pin electrode was used and the anode was a 10 cm^2 Pals Neurostimulation patch electrode, therefore matching the electrode used in HFS studies by Klein and colleagues (25).

2.3. PERCEPTION THREHSOLD AS A MEASURE OF NERVE FIBER EXCITABILITY

When stimulating a peripheral neuron the response of the cell to the change in extracellular field is one thing and the steps from stimulation to perception is another thing, involving several mechanical, chemical, physiological and psychological processes (91). To compensate for the infeasibility of measuring direct cell responses in humans, psychophysical measures such as the quantitative sensory testing (QST) have been routinely used for studying pain and sensation in humans. QST consists of various subjective threshold- and intensity measures such as detection- and pain threshold to heat or mechanical stimuli and have been widely used to assess indirectly certain functions of both the peripheral and the central nervous system (92). Threshold tracking measurements have been used to assess function of large sensory and motor fibers by measuring the compound action potential (62,93). Threshold tracking tracks for instance the threshold electronous by indirectly measuring the voltage outside the nerve as a proportion of the membrane potential. Therefore, the measurements do not rely on individual thresholds but on the shape of the "electronous" (62). Inspired by the threshold tracking technique, Hennings and colleagues recently presented the perception threshold technique to assess membrane properties in sensory nerve fibers indirectly by the utilizing of subjective perception thresholds (64). A critical assumption of this technique relates to the electrodes used for activation of the different sensory fibers and also the central aspect of perception which involves complicated and also unknown mechanisms. Even though the threshold tracking method comes closer to measuring the actual membrane voltage than the perception threshold tracking, the idea of measuring the shape/or change in threshold is similar.

As described in section 2.1, the distance from the electrical current to the fiber affects selectivity of fiber activation and the excitability of the fiber depends on the distance to the electrode and electrode geometry. This is difficult to control for when determining the perception threshold, but to allow for comparison between stimulation parameters, the electrode location has to remain constant. In papers 1 and 2, the perception threshold was obtained for two electrode types described in sec. 2.2, which remained in the same position throughout the experiment. The electrodes cannot be compared per se, but instead relative changes in perception thresholds to the different parameters were compared between the electrodes (papers 1 and 2). Therefore, factors related to the distance from current to fiber can mostly be neglected. However, this factor would limit the possibilities for comparison between activation of different fiber types with the same electrode and comparisons between measurements are not possible if the electrode is moved between positions.

In papers 1 and 2, activation of small nociceptive and large nonnociceptive fibers was indicated with subjective perception thresholds of stimuli delivered with pin- and patch electrodes, respectively, see setup in figure 2-1. The perception threshold to the electrical stimulation is defined as the current intensity at which there is an approximately 50% change of perceiving the current (62). In all of the studies, perception threshold to the electrical stimulation was identified using the method of

limits, which is explained in details in papers 1 and 2. All experimental subjects were trained in perceiving the electrical current and indicating their perception during a training sequence, which was performed prior to the actual experiments. The method of limits is based on averaging over current intensities of above threshold, i.e. when the current is perceived and below threshold which is the current intensity where perception dissipates (94). The method of limits has been used in QST protocols (92) and is considered appropriate for determining sensory thresholds as it relies on both supra- and sub threshold intensities. The method therefore captures somewhat the stochastic behavior of the perception threshold as it is also possible to perceive stimuli below the identified threshold, but it is less likely. Additional support for this method can be obtained from computational models of small and large sensory fibers where the perception threshold changes, especially for the patch electrodes, have been correlated to computed changes in extracellular field potentials (42). In a recent study from our research group, altered perception thresholds were observed during a cooling condition compared to normal skin temperature, which indicates that the perception threshold tracking method is capable of detecting different fiber excitability when electrode location is kept constant (95).

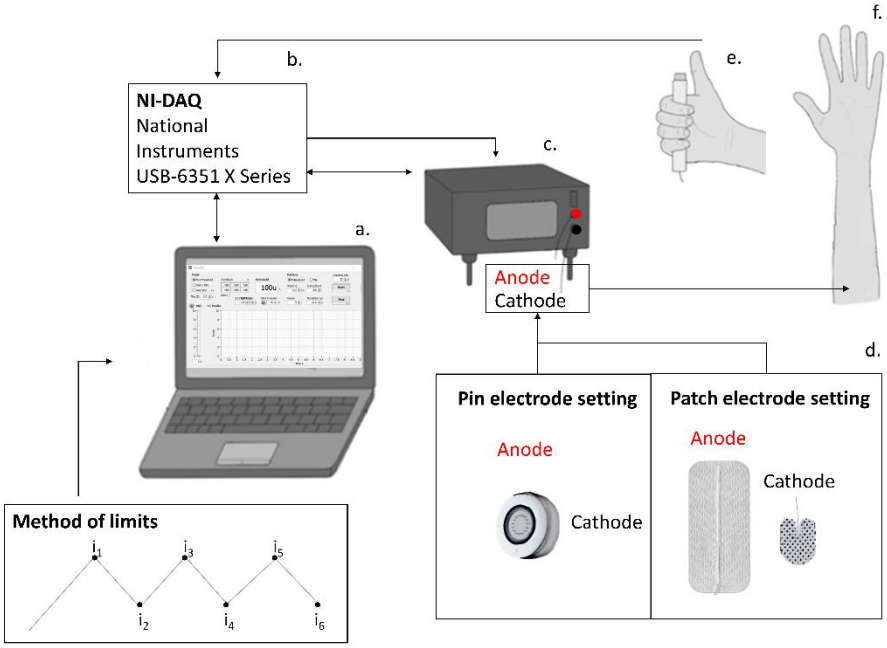

Figure 2-1. Experimental setup in papers 1 and 2. a) Control and acquisition, Personal computer with a custom-made program to control method of limits (LabVIEW; National Instruments). b) Data acquisition system: NI-DAQ USB-6351, National instruments. c) Digitimer DS5 Stimulator (Isolated Bipolar Current Stimulator). d) Electrode settings. e) Handheld push button to indicate perception. f) Electrode-participant connection.

2.4. STIMULATION PARAMETERS FOR PREFERENTIAL SMALL FIBER ACTIVATION

In papers 1 and 2 the pulse shape and stimulation duration for preferential activation of small fibers were examined using perception threshold to indicate nerve fiber activation with the pin and patch electrodes. Paper 1 investigated different pulse shapes; exponential, linear, bounded exponential (B.EXP) and rectangular pulse. Paper 2 examined the effect of the B.EXP duration on preferential small fiber activation in more details. In both studies, high intensity stimulation was also applied to investigate pain responses and qualitative description.

2.4.1. PULSE SHAPE AND DURATION

When applying slowly rising currents for preferential small fiber activation, it is currently unknown how slowly the current should increase and how the shape of this slow increase should be. If a fixed duration is applied, the rate of current increase is always changed by the current intensity both with linear pulses and exponential pulses, but with exponential pulses the time constant also decides the shape of the pulses. Studies have used pulses with an undetermined duration and investigated different rates of current increase for B.EXP pulses (52) and linear pulses (71) as well as fixed duration of linear- (50) and exponential pulses (53). These methods likely utilize large fiber accommodation, but the shape and the rate of current increase to maximize accommodation in large sensory fibers is currently unknown.

The purpose of study I was therefore to compare different pulse shapes by investigating the perception thresholds to linear-, exponential-, B.EXP, and rectangular pulses of 5 and 50 ms. Table 1 in paper 1 shows in details the shapes of the pulses and the formulas used to create them. The main outcome was the perception threshold ratio between the patch- and the pin electrodes, which is described in more details in paper 1.

In paper 2, the perception threshold of the B.EXP pulse shape and rectangular pulses were examined for durations from 1 ms to 100 ms to create strength-duration curves (paper 2). In paper 2, the accommodation slope was calculated for the patch electrode perception thresholds, which manifested a linear relationship in the ‘accommodation curve’, which is a special form of the strength-duration curve observed for B.EXP pulses with the patch electrode (paper 2).

To further examine the difference in accommodation between the pulse shapes in paper 1, the accommodation slopes based on the perception thresholds for 5- and 50 ms B.EXP, linear, and exponential pulses were calculated in this thesis (the method is provided in paper 2). In this thesis, the accommodation slopes were compared between the pulse shapes using a non-parametric Friedman test according to the data distribution for both the patch- and pin electrode. Post hoc comparisons were performed between each pulse shape using a Wilcoxon sign rank test and a Bonferroni corrected p value ($p < 0.017$ was considered significant). Furthermore, to allow for comparison between results from paper 1 and paper 2, the accommodation slopes for

paper 2 were also calculated using only perception thresholds for 5- and 50 ms B.EXP pulses. The slopes were compared between the studies using a non-parametric test for independent samples (Mann Whitney test).

2.4.2. INTENSITY AND FREQUENCY

In paper 1 and 2, high intensity stimulations were tested to evaluate the qualitative sensation to the stimulation pulses (paper 1) and pain ratings for different intensities (paper 2). In paper 1, all stimulations were performed at 10 times perception threshold and the sensation to the stimulation evaluated using the short-form pain McGill Questionnaire (96). In paper 2, pain responses were obtained for stimulations of various intensities from very low intensity (0.1 times perception threshold) to high intensity (20 times perception threshold) (paper 2). The intensities were within the range that has been tested with a similar electrodes (87). The stimulations consisted of four paradigms including single 1 ms rectangular and 40 ms B.EXP pulses and to mimic stimulations used in experimental models, a 10 Hz paradigm for 1 s was also tested for each shape (97) (paper 2).

2.5. STIMULATION PARAMETERS: EFFECT ON PREFERENTIAL ACTIVATION

2.5.1. PULSE SHAPE AND DURATION

The results on perception threshold ratio between the patch and the pin electrode indicated largest accommodation with the B.EXP pulse shape applied through the patch electrode (paper 1). In paper 2, significant large fiber accommodation was shown for pulse durations longer than 20 ms, but largest threshold increase was observed for 100 ms pulse. Instead of the standard strength-duration curve observed for rectangular pulses, an accommodation curve was observed for B.EXP pulses where an increase in threshold was observed for increased duration (paper 2). Since, only two pulse durations were applied in paper 1 it was not possible to create as detailed accommodation curve as in paper 2.

Since accommodation is considered to be the key mechanism when current threshold increases for increased pulse duration, a positive accommodation slope will be used in this thesis to indicate accommodation and a negative accommodation slope indicates no accommodation. The accommodation slopes for the patch electrode, calculated in this thesis based on data from paper 1 (new analysis, figure 2-2), were shown to depend on the pulse shape ($p < 0.001$). Accommodation slope of the B.EXP pulse was the only positive slope observed and post hoc comparisons revealed that the accommodation slope for the B.EXP pulse shape was larger than for both linear pulse ($p = 0.004$) and exponential pulse ($p < 0.001$). Significant difference was not observed between the accommodation slope for the exponential and linear pulse shapes ($p = 0.056$). As expected, accommodation slopes with the pin electrode were negative

indicating no accommodation. No differences were found between the accommodation slopes of the different pulse shapes for the pin electrode, ($p = 0.130$).

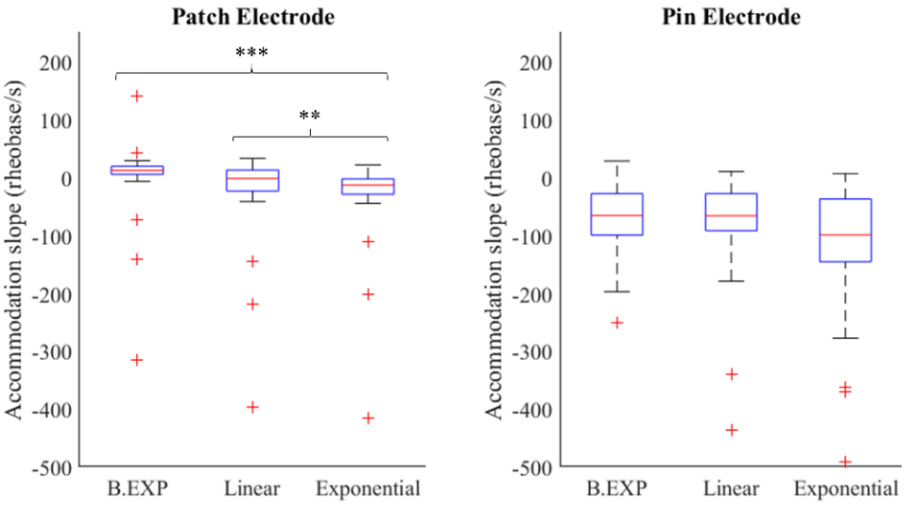

Figure 2-2. Accommodation slopes. Median and interquartile range of the accommodation slope for the three slowly rising pulse shapes in paper 1 for the patch electrode on the left and the pin electrode on the right.

The accommodation slope in paper 2 was 23.52 ± 2.84 rheobase/s, which is consistent with the early findings on accommodation slope of 21.2 ± 0.46 rheobase/s (52). No differences were found between the accommodation slopes of the B.EXP pulse shape between paper 1 and paper 2 (data not shown for study 2, patch electrode: $p = 0.984$, pin electrode: $p = 0.401$). Therefore, accommodation slope data for the B.EXP pulse shape in papers 1 and 2 were pooled for all 49 participants (figure 2-3). The accommodation slope was positive for the patch electrode and negative for the pin electrode indicating that accommodation was only observed for the patch but not the pin electrode.

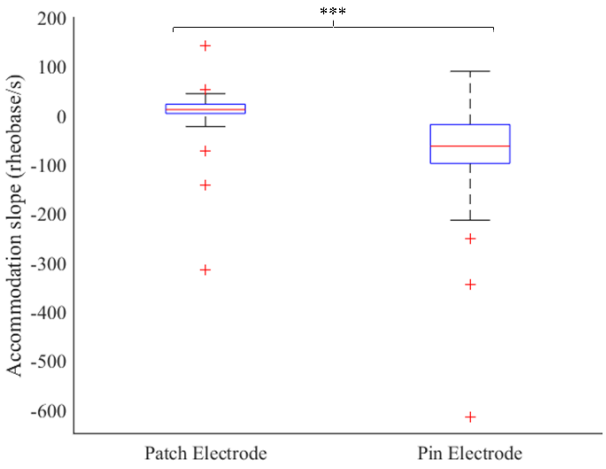

Figure 2-3. Pooled accommodation slope for papers 1 and 2. The accommodation slopes are shown for the patch and the pin electrodes (n = 49).

Both results on perception threshold as seen in figure 2 in paper 1 and the results on accommodation slopes shown in figure 2-2 in this thesis indicate that when the longest pulse duration is 50 ms, accommodation is observed for the majority of subjects for the B.EXP pulse shapes but not for linear and exponential pulses. However, it is likely that accommodation would have been generated for longer durations of linear pulses. This is supported in a study from Hennings and colleagues, where accommodation to linear pulses was shown to increase with longer durations (50). Furthermore, a recent study showed that longer durations (100 and 200 ms) were needed to cause accommodation to linear pulses delivered through identical patch electrodes as used in paper 1 and 2 (42). Results from paper 2 however showed that accommodation was observed for 20 ms B.EXP pulses, which allows for greater usability and flexibility compared to the application of very long pulse durations. No experimental studies have to the author's knowledge been performed on accommodation to exponential pulses but increased selectivity towards small fibers had previously been indicated in a computational study (53). In paper 1, the longest duration was 50 ms and it therefore remains unknown whether longer durations will result in accommodation for the exponential pulse shape. It is fair to speculate whether the initial very slow rise of the exponential pulse followed by a fast increase towards the end of the pulse is not ideal to cause accommodation. During maintained depolarization, the fibers are forced to inactivate and remain in the refractory state, which increases the activation threshold. The sudden increase at the end of the exponential pulse may therefore reach the increased activation threshold and activate the fibers anyway. With the B.EXP shape the current however does not increase to a great extent towards the end of the pulse and therefore the current likely maintains below the increased activation threshold due to accommodation.

Unfortunately, no subjective ratings were observed to evaluate the quality of these stimuli as the stimulus intensity was low (around perception threshold) and therefore it is difficult to detect any differences with subjective ratings. This is one of the limitations of using the subjective perception threshold as a measure of the nerve fiber activation, because neither electrodes are completely selective despite their preference towards each of the fiber types. However, a way to overcome this limitation is to create- and use a perception threshold curve as the outcome measure instead of each individual perception threshold.

2.5.2. INTENSITY AND FREQUENCY

Results on high intensity stimulation in paper 1 showed, that qualitative sensation for stimulations with the pin electrode were mainly described as stabbing, shooting, and sharp (paper 1). This was expected due to the selective nature of the pin electrode towards the nociceptive fibers. The descriptions were however not different between the pulse shapes when delivered at high intensity (paper 1). An explanation for this could be that the large fiber accommodation obtained with the slowly rising pulses at intensities close to perception threshold (paper 1 and 2) is not achieved for high intensity, since the current rises much faster than low intensity currents of the "same" shape. Detailed stimulus-response curves are shown for the paradigms tested in paper 2 in figure 6 and table 1 in paper 2. Importantly, pain was observed for low intensity, but higher intensity was needed to obtain maximum pain rating. Pain ratings were larger for 10 Hz pulse trains (around 5/10 on NRS) compared to single pulses (around 3/10), likely due to temporal summation of pain as discussed in paper 2. Even though the results on high intensity stimulations in both studies give some information about the pain to the stimulations with different shapes, the results do not add substantial information regarding the selectivity of the stimulation. It is however likely, and consistent with previous studies, that the selectivity decreases with increased intensity due to the spread of current to deeper skin layers (37,44,88) and also due to loss of accommodation with a steeper current increase compared to perception threshold intensities (paper 1).

In papers 1 and 2, only single pulses were used to determine the perception threshold, which was the main outcome to determine the activation selectivity. Previous studies have however showed that the nerve fiber selectivity can be stimulation frequency dependent, i.e. the perception threshold is different when multiple pulses are applied (54,98). It is also important to consider the effect of frequency when developing experimental pain models using electrical stimulation as discussed in more details in the following chapter on the HFS experimental model.

The knowledge on mechanisms related to the frequency-dependence in selective electrical stimulation is rather sparse. It has been speculated weather high frequency bursts can mimic afferent firing immediately after an injury (28,99). In a series of papers, the Neurometer stimulation device has claimed to be able to activate specific nerve fibers depending on the applied stimulation frequency (e.g. 98,100). The stimulation frequencies proposed were 5 Hz for activating C-fibers, 250 Hz for

activating Aδ fibers and 2000 Hz for activating Aβ fibers (98). Recent evidence furthermore indicate that 4 Hz sine-wave stimulation can activate C fibers selectively based on results from microneurography and cell imaging in pigs and mice (54). It can however be speculated whether it is the stimulation frequency or simply the shape of how the current rises, that generates the selectivity of small fibers with the paradigm (54). In figure 2-4, it is illustrated how the ascending phase of a 4 Hz sine wave matches to a great extent the B. EXP pulse shape (of 60 ms), which was found in papers 1 and 2 to activate small fibers rather preferentially compared to other pulse shapes (paper 1) and shorter durations (paper 2).

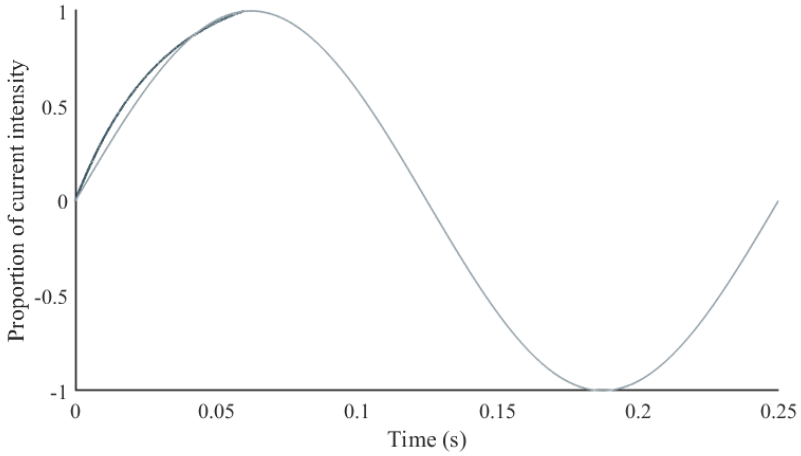

Figure 2-4. Comparison to sine wave stimulation. The figure shows a one second plot of a sine wave (gray) and the ascending phase of the B.EXP pulse shape applied in this PhD project (black) illustrating the similarities of how the current increases.

2.6. SUMMARY ON PREFERENTIAL ELECTRICAL STIMULATION OF SMALL FIBERS

According to former evidence on fiber excitability, the small and large nerve fibers are prone to different mechanisms during slowly rising electrical stimulation. In large fibers, depolarization to slowly rising pulses causes accommodation, which causes elevated activation threshold, whereas in small fibers depolarization can cause increased excitability and spontanous activity. The recruitment order of the fibers therefore not only depends on fiber diameter, but on the combination of fiber diameter and excitability properties, the parameters used for electrical stimulation and the applied electrode. The main results from papers 1 and 2, which investigated the parameters for electrical stimulation, indicate that accommodation in large fibers depends on the rate of current increase. The bounded exponential pulse has an increasing form of exponential current decay, with an initial steep increase followed by a slow increse towards the end of the pulse (B.EXP pulse shape). The B.EXP pulse was shown to cause greater increase in perception threshold (accommodation) in large

fibers than linear and exponentially increasing currents. It is furthermore concluded that greatest large fiber accommodation, with concurrent small fiber activation, was achieved when 100 ms pulses were applied.

CHAPTER 3. ELECTRICAL HIGH FREQUENCY STIMULATION (HFS) PAIN MODEL

HFS has been used to intensely activate nociceptive fibers at high frequency, mimicking intense nociceptive input during injury. HFS has routinely been used as a model of secondary hyperalgesia (e.g. 25–27,101), a phenomenon related to chronic pain. The intense nociceptive signaling to the dorsal horn of the spinal cord potentiates responses in spinal neurons heterosynaptically which can be assessed directly from spinal cord neurons in animals (24). In humans, facilitated mechanical sensitivity in the area around HFS (secondary hyperalgesia) has been considered as a perceptual correlate of these potentiated heterosynaptic responses (25).

Most evidences indicate that the induction of secondary hyperalgesia in humans is due to activation of mainly C-fibers (17,18,35) and to some extent Aδ-fibers (18). Contribution from "silent" mechano-insensitive C-fibers is also considered crucial (102,103). The standard HFS model relies solely on rectangular pulses at high intensity, which likely cause intense co-activation of all cutaneous sensory fiber types (18,37). When all cutaneous sensory afferents are activated, multiple mechanisms may be induced at spinal- and higher levels, which complicates interpretation of results (58). Preferential activation towards the nociceptive fibers with HFS would enable more "class-specific" input to the spinal cord and thereby most likely mimic the clinical pathophysiological conditions and efficiency of the model. Moreover it could allow for more detailed, mechanism-specific interpretation of results.

The results from papers 1 and 2 indicated that the B. EXP shape increased preferential activation of small nociceptive fibers. Supporting those results, a recent study relying on measures with microneurography suggested that sine wave with almost identical stimulation phase as the B.EXP pulse could activate C-fibers selectively (54).

In paper 3 it was examined whether a preconditioning B.EXP prepulse, which based on results from papers 1 and 2 is assumed to increase the selectivity towards the small nociceptive fibers, could be utilized to increase effectiveness of the HFS electrical stimulation pain model (HFS+B.EXP). Moreover, to find out if the amount of hyperalgesia could be even further facilitated, the effect of priming with heat/capsaicin before HFS paradigm was investigated (HFS+HEAT/CAP) in paper 4. In addition to the HFS+B.EXP and HFS+HEAT/CAP sessions, the golden standard HFS paradigm (25) was applied. One study (study III) was designed as a three session crossover study with one week between sessions 'HFS', 'HFS+B.EXP' and 'HFS+HEAT/CAP'. All stimulations and tests were performed on the subject's forearm and the site for stimulation was randomized in the first session between the dominant and non-dominant arm. The same site was used in the HFS and HFS+B.EXP sessions but the opposite site was used in the HFS+HEAT/CAP session. This was to

avoid any potential carryover desensitizing effect of capsaicin (104), even though effects from short lasting capsaicin application likely have already washed out (105). In this chapter, the methods used in this PhD project to modify the electrical HFS model of secondary hyperalgesia (sections 3.1-3.2) and related findings (sections 3.3-3.4) are presented (papers 3 and 4). Potential mechanisms will furthermore be presented and summary will be provided.

3.1. CONDITIONING STIMULATION

Different methods have been used to induce mechanical secondary hyperalgesia in humans. Electrical stimulation is a commonly used method (18,25,28,106) and use of algogens, such as capsaicin (29,107) and mustard oil (108) and heat burn stimulation (31) are also models to induce secondary hyperalgesia. In study III (papers 3 and 4), three different conditioning paradigms were used to induce secondary hyperalgesia (a protocol timeline is provided in figure 3-4). In all sessions, the golden standard HFS stimulation pattern was utilized were five 100 Hz, 1 s bursts of 2 ms rectangular pulses were applied every 10 s through the pin electrode, which are described in section 2.2 (25). To ensure intense nociceptive activation, the intensity of the HFS conditioning stimulation was set to 10 times perception threshold or a minimum of 1.5 mA (109) in case of perception threshold < 150 uA, (paper 3 and paper 4). In the 'HFS' session, the standard HFS was applied without modifications.

In the 'HFS+B.EXP' session, HFS was preconditioned with a 100 ms B.EXP pulse to accommodate large fibers and thereby forcing them to inactivate (paper 1 and 2) prior to the conditioning stimulation of the small fibers (paper 3). A limitation of this method relates to the fact that findings in paper 1 and 2 are mainly based on large fiber accommodation with a patch electrode. When determining the intensity of the B.EXP prepulse for generating large fiber accommodation prior to HFS, the properties of the pin electrode needed to be considered. Since pin electrodes in general are rather selective for small fibers, especially for intensities close to perception threshold, it was assumed that current at the level of perception threshold would not affect/accommodate the large fibers. A recent study showed that the perception threshold to linear pulses applied through the pin electrode was at least three times larger when lidocaine patch was placed on the skin to block the small fibers (42). Coactivation of large fibers has furthermore been shown for intensities larger than two times perception threshold (37). The intensity of the B.EXP prepulse was therefore set to 3 times perception threshold allowing the electrical field to spread into the dermis where the large fibers terminate (Provitera et al. 2007, paper 3). The stimulation paradigm is illustrated in figure 3-2. Results from the 'HFS' and 'HFS+B.EXP' sessions were presented in paper 3.

In the 'HFS+HEAT/CAP' session, heat/capsaicin priming was performed adjacent to the pin electrode for inducing secondary hyperalgesia prior to HFS in the area of HFS conditioning. Results from the 'HFS' and 'HFS+HEAT/CAP' sessions were presented in paper 4. Heat/capsaicin sensitizing method was selected to avoid the discomfort associated with direct capsaicin injection (29,30) or heat-burn (31,32), but

still induce sufficient peripheral and central priming (33). Heat stimulation of 45 degrees was applied for 5 minutes using a 3x3 cm thermode (Pathway, Medoc Ltd., Ramat Yishai, IL) and subsequently, a 4x4 cm cutaneous 8% capsaicin patch (transdermal patch, 'Qutenza', Astellas) was placed in the same location for 30 minutes, see figure 3-3) (paper 4). Capsaicin (methyl-n-vanillyl nonamide) is a chemical irritant and the active component of chili peppers in genus capsicum (111), which acts on the TRPV1-positive C and Aδ-fibers (17,103,112) and can cause burning pain sensation (107).

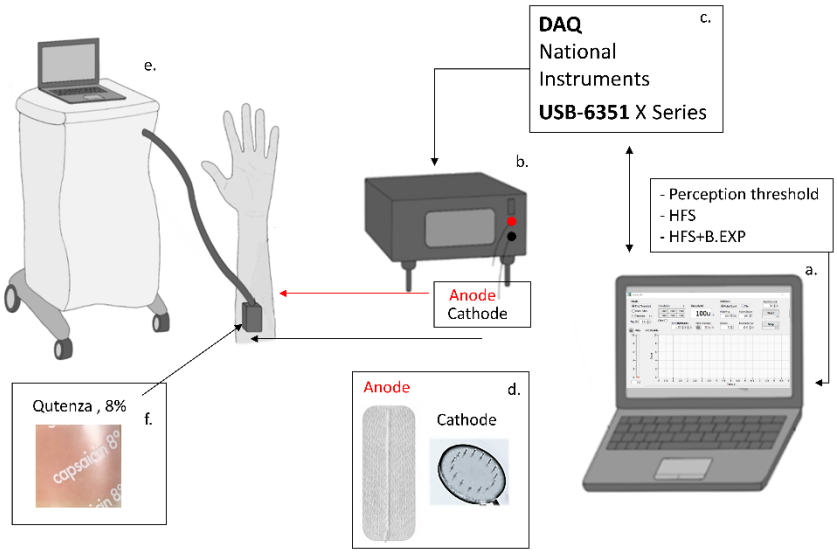

Figure 3-1. Illustration of the setup of the conditioning paradigms, used in study III. a) A customized program implemented in a personal computer to control the electrical stimulation, b) DS5 electrical stimulator, c) Data acquisition, NI-DAQ, USB-6351, d) Pin electrode cathode and large patch anode, e) Thermode of the Medoc system for delivering heat stimulation, f) Qutenza 8%, cutaneous capsaicin patch.

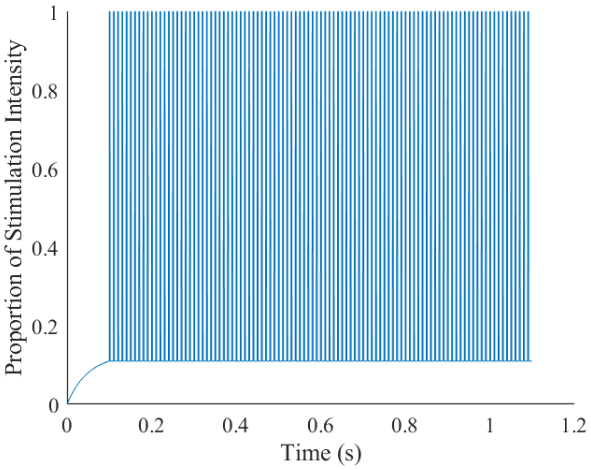

Figure 3-2. Illustration of the HFS stimulation with a B.EXP prepulse. The figure shows the active one second HFS paradigm with a B.EXP prepulse. The current intensity is shown as a proportion of the intensity applied with the DS5 stimulator. The exemplified intensities for 2 ms rectangular HFS pulses and the B.EXP prepulse are: 1.5 mA and 180 µA, respectively.

3.2. ASSESSMENTS

The main outcome for evaluating mechanical secondary hyperalgesia was mechanical pain sensitivity (MPS) to pinprick stimulation. The heat pain threshold (HPT) was applied to examine primarily the peripheral effect due to heat/capsaicin in the area of heat/capsaicin application. Furthermore, Full-Field laser perfusion imaging (FLPI) technique was used to examine blood perfusion in the area of HFS stimulation. Ratings for MPS and HFS conditioning stimulation were assessed on a verbal numerical rating scale (NRS) where the subjects were asked to rate the sensation to the stimulation on a scale from 0 to 10 with 5 being the threshold where sensation changed from being non-painful to painful (paper 3 and 4). MPS, HPT, and FLPI were assessed in all sessions at baseline and at four timepoints after HFS. In addition, the same measurements were carried out immediately after removal of capsaicin in the 'HFS+HEAT/CAP' session.

3.2.1. MECHANICAL PAIN SENSITIVITY

Mechanical pain sensitivity (MPS) to pinprick stimulation was assessed using custom-made pinprick stimulators with a round tip, 0.2 mm in diameter (SMI, Aalborg University). Two weights (128 mN and 256 mN) were used in all sessions. More robust results have been observed when using low pinprick weights (113) but 256 mN was also applied to obtain ratings closer to a painful sensation on the NRS. For each stimulation, one weight was applied three times and with an approximal contact time of one s and one s in between stimulations and subjects were asked to rate the

sensation on a NRS. MPS was tested in four areas in all sessions; one proximal (A2) and one distal (A1) to the HFS pin electrode, an area distal to the area of heat/capsaicin application (A3) and a remote unconditioned control area (A4) in random sequence. The areas for MPS are shown in figure 3-3. MPS to both weights was obtained two times in the four areas, all in a randomized order and the average of the two rating was used for analysis.

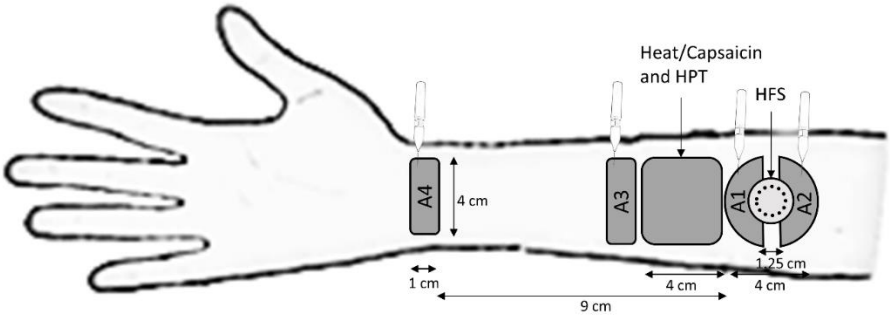

Figure 3-3. Stimulation areas. Mechanical pinprick stimulation was applied in areas A1-A4 on the subject's volar forearm. The placement of the pin electrode to deliver high frequency stimulation (HFS) and the area for heat/capsaicin conditioning and heat pain threshold (HPT) testing are also shown.

3.2.2. HEAT PAIN THRESHOLD

Heat pain threshold (HPT) was mainly obtained to assess peripheral sensitization following the heat/capsaicin priming in paper 4. HPT was measured to investigate changes in thermal perception using a contact thermode (30x30 mm ATS; Pathway; Medoc Ltd; Ramat Yishai, Israel). The thermode was placed on the same location as the heat/capsaicin application (and therefore adjacent to HFS stimulation), see figure 3-3. From a starting temperature of 32 degrees, the heat increased at 1 degree/s until the participants indicated that their sensation changed from being only warm to being painful by pressing a response button. The heat returned to baseline at 8 degrees/s and the procedure was repeated to obtain an average of three temperature thresholds as the HPT.

Contradicting results have been obtained regarding the effect of HFS on changes in thermal sensation. Most studies have observed no differences in HPT after HFS (27,28), but a recent study found increased sensitivity to small-spot laser stimulation following HFS in the area of mechanical secondary hyperalgesia (114). The increased heat sensitivity was however not as pronounced as the increase in mechanical sensitivity (114). As secondary mechanical hyperalgesia is most likely mainly mediated by the capsaicin-insensitive fibers (17), which are not sensitive to heat, the HPT was not hypothesized to decrease in the secondary hyperalgesia of HFS. The

HPT was nevertheless obtained in this area to standardize the experimental procedure throughout sessions.

3.2.3. SUPERFICIAL BLOOD PERFUSION

Neurogenic inflammation was assessed in all sessions by measuring the superficial blood perfusion at the area of the pin electrode (1.5 cm^2). The superficial blood perfusion was measured using a Full-Field Laser Perfusion Imager (FLPI), (MoorFLPI; Moor Instruments Ltd, Axminister, Uk). The blood perfusion is indicative of peripheral processes due to activation of peptidergic C-fibres (115,116). When activated, the peptidergic C-fibers release of neuropeptides such as substance P and calcitonin-gene related peptides, which can result in neurogenic inflammation (117,118). The purpose of the blood perfusion measurements was to examine the extent of activation of peptidergic C-fibers to the HFS stimulation, but also to obtain information about peripheral effects in relation to the development of secondary hyperalgesia after HFS.

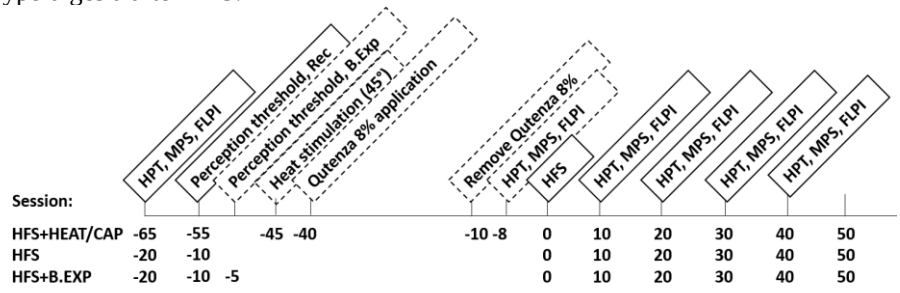

Figure 3-4. Timelines for the three experimental sessions in study III. Dashed boxes indicate stimulations/tasks that were only performed in one of the sessions; Perception threshold of B.EXP was only identified in the 'HFS+B.EXP' session, and heat- and Qutenza application were only applied in the 'HFS+HEAT/CAP' session. Heat pain threshold = HPT, Mechanical pain sensitivity = MPS, Full-field laser perfusion imaging = FLPI, Rectangular = Rec, Bounded exponential = B.EXP, high frequency stimulation = HFS.

3.3. STIMULATION PARAMETERS: EFFECT ON HFS-INDUCED SECONDARY HYPERALGESIA

Electrical stimulation experimental pain models of secondary hyperalgesia have been widely used in experimental studies with the purpose of investigating underlying mechanisms (18,27,109,119), pharmacological effects (119,120) and even the effect of genotype (121). Even though a "rather standardized" paradigm has been used in most studies, different electrical stimulation parameters to induce secondary hyperalgesia have been examined (25,28,120,122). As aim two of this PhD study was to investigate the effect of using a slowly rising B.EXP prepulse to increase small fiber selectivity of the HFS pain model, this section will focus on the effect of especially pulse shape and other parameters on the developed secondary hyperalgesia

with the model. As there are different mechanisms involved in the induction- and facilitated pathway of secondary hyperalgesia (17,18,123), the immediate action on the peripheral fibers and the maintained central sensitizing effects after HFS have to be considered.
In line with results from previous studies, increased sensitivity to mechanical pinprick stimulation, indicating mechanical secondary hyperalgesia, was observed for up to 50 minutes after HFS in all sessions of study III (paper 3, paper 4).

3.3.1. PULSE SHAPE

In paper 3, the pulse shape for HFS stimulation via pin electrodes was modified with an attempt to increase the selectivity towards the nociceptive fibers. The intensity level usually applied for HFS with pin electrodes to induce secondary hyperalgesia (25,124) is above the intensity level required for selective small fiber activation (37). To compensate for this loss of selectivity, HFS was pre-conditioned with a long duration B.EXP pulse to inactivate the large fibers (paper 3). The most frequently applied paradigm is based on 2 ms rectangular pulses delivered in bursts of 100 Hz (25) and similar results have been obtained with 1 ms rectangular pulses (28).
Results on the MPS with 128 mN pinprick stimuli showed that larger difference from baseline and control area was observed for the HFS+B.EXP session compared to the HFS session (paper 3) (see also figure. 3-6 in this thesis). For 256 mN pinprick stimuli however, no difference was observed between HFS and HFS+B.EXP (paper 3) (figure. 3-7 in this thesis).
The immediate effect of applying HFS following a slowly rising (100 ms) B.EXP prepulse compared to the traditional HFS was examined with the pain ratings to HFS and the blood perfusion after HFS. New analysis was performed to compare the pain ratings to HFS (figure 3 in paper 4) and HFS+B.EXP (figure 3-5 in this thesis) and no significant difference was observed ($F(1,19) = 1.52$, $p = 0.23$). The results from the superficial blood perfusion showed higher blood perfusion in the HFS+B.EXP session compared to the HFS session (paper 3), which may indicate that the HFS+B.EXP paradigm activated more peptidergic C fibers (paper 3), but this could not be supported with the pain ratings to HFS. It is possible that the intensity is predominant in determining the amount of pain to HFS (25).
Due to the different shape of the stimulations, greater charge was delivered with the HFS+B.EXP paradigm then with the HFS paradigm, which may have increased the pH level at the electrode-tissue interface. This may also contribute to the larger blood perfusion observed following HFS+B.EXP compared to HFS, but interestingly, the difference in blood perfusion was only observed immediately (10 min) after HFS, and not for the following time points (paper 3). In contrast, increased MPS was observed 20 and 30 minutes after HFS+B.EXP compared to HFS but not immediately (10 min) after HFS. It is therefore speculated whether increased blood flow and increased secondary hyperalgesia may have a causal relationship as sensitization due to inflammation processes develops over time (31,125). Interestingly, a recent study showed that charge balancing does not affect the amount of induced secondary

hyperalgesia with HFS (124). This indicates that the induced secondary hyperalgesia is not linked to the potential blood flow increase due to accumulation of charge and thereby changes in pH, but rather due to the preferential activation of C-fibers related to the induction pathway (paper 3). Taken together, the results from paper 3 indicate that preferential activation of small nociceptive fibers during HFS can increase the amount of mechanical secondary hyperalgesia induced with the model provided that it is assessed with 128 mN mechanical pinprick stimulation.

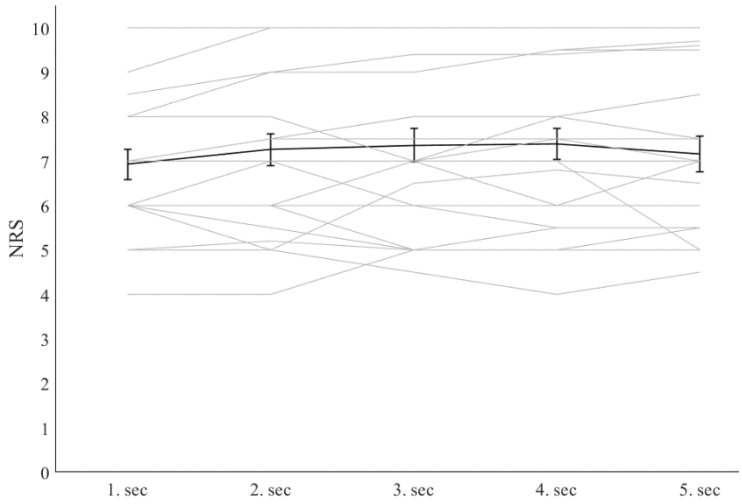

Figure 3-5. The pain ratings to HFS+B.EXP conditioning stimulation (not shown in paper 3). Mean and standard error is shown in black and subjective ratings are shown with the gray lines, illustrating the between subject variation.

3.3.2. FREQUENCY

The stimulation frequency for HFS (100 Hz) used in most studies with electrical stimulation to induce secondary hyperalgesia, is based on protocols for inducing LTP in spinal synapses of rats (22,23,126). The discharging response of nociceptors to mechanical stimulation have been examined and initial responses, mimicking the beginning of an injury, showed discharges at 100 Hz or at even higher rates (99). Within a few seconds, a more stochastic frequency pattern from low (< 1) to high (> 100 Hz) was observed (99). It is therefore questionable whether nociceptive fibers are capable of following such high frequency of stimulation for a full second not to say in five repetitive bursts.

Different frequencies have also been shown to induce secondary hyperalgesia and the optimal frequency pattern to induce secondary hyperalgesia in the most efficient way has to the author knowledge not yet been determined. In the study by Klein and colleagues, 1 Hz stimulation induced decreased sensitivity indicating long term depression of pain at the area of HFS stimulation, but outside the conditioning

stimulation a small amount of secondary mechanical hyperalgesia was observed (25). The 100 Hz HFS pattern was recently compared to a constant 10 Hz stimulation for 50 seconds paradigm (28). Interestingly the effect was similar and the 10 Hz stimulation, which may be closer to the natural firing frequency of the nociceptive fibers (99), therefore increases physiological relevance of the model (28). 10 Hz stimulation using only the B.EXP stimulation pulses was attempted in a pilot study related to this PhD, but significant secondary hyperalgesia could not be generated (127). This could be due to inefficient conditioning current intensity (127).

Van den Broeke and colleagues have also investigated the frequency of HFS stimulation in two very recent studies (122,124). Results suggested that (50 Hz) burst stimulation was more efficient than (5 Hz) continuous stimulation (122). Furthermore, they showed that intermediate frequencies (20 and 42 Hz) were more efficient to induce secondary hyperalgesia than 5 Hz and interestingly also more efficient than the 100 Hz paradigm (124). The study by (124) was a between-subjects design and the same participants did therefore not participate in the different paradigms. As there is high variability in the hyperalgesic response between participants (128), this could have affected the results on frequency dependence. In a study from the same research group, similar NRS ratings to 128 mN pinprick were observed after 100 Hz HFS (114) compared to the recent 42 Hz HFS study (124).

Using a different model, secondary hyperalgesia has also been induced using 0.5 Hz for 35 minutes and 1 Hz for 17 minutes (129) and also by using very low frequencies of 1/20 Hz for targeting especially the "silent" C-fibers (102). A difference in the low frequency models compared to HFS is that ongoing electrical stimulation is needed to observe secondary hyperalgesia, therefore it does not induce secondary hyperalgesia that outlasts the conditioning stimulation (102,129).

3.3.3. INTENSITY

The intensity of HFS was set to 10 times individual perception threshold for the 2 ms rectangular pulse or a minimum of 1.5 mA in all sessions (paper 3 and 4). In most previous studies, stimulation intensity varies from either 10 or 20 times the perception threshold (18,25,106) and studies have also used a fixed intensity of 2 mA (119) and 1.5 mA (109).

The stimulation intensity selected for the HFS is probably the stimulation parameter with greatest effect on the selectivity of nociceptive fibers (37). As mentioned in chapter 2, the recruitment order of fiber types depends on fiber diameter (large before small) assuming that the fibers are in the same location. Traditionally when C-fibers are targeted, high intensity, often termed "C-fiber intensity", is used (58,130). The spread of the electrical field is however dependent on both intensity and electrode type (43,44). With the pin electrode, small diameter fibers in the epidermis (131,132) are preferentially activated at low intensity but with high intensity the currents also spreads to deeper layers, which may cause co-activation of large fibers (37). Optimal stimulation intensity would be restricted to superficial layers, but still of sufficient intensity to recruit enough small fibers to induce secondary hyperalgesia via spatial

activation. This is one of the reasons why a relatively low intensity was used for the conditioning stimulation in this PhD, but unfortunately there is a risk of recruiting less C-fibers. Furthermore, as an accommodating pre-pulse was applied to increase the threshold of the large fibers, a lower intensity was considered less likely to reach that threshold and activate the large fibers anyway. This is however speculative as the degree of accommodation with the pin electrode is yet unknown.
The applied conditioning stimulation intensities in papers 3 and 4 were 1.61 ± 0.08 mA, 1.64 ± 0.10 mA, and 1.63 ± 0.07 for HFS, HFS+B.EXP, and HFS+HEAT/CAP, respectively. A minimum of 1.5mA was set to ensure sufficient effect of the model since low perception thresholds are observed for some participants, which have been experienced to cause ineffective stimulation intensity for HFS (unpublished data). The pooled perception threshold for combined sessions from papers 3 and 4 was 115.08 ± 8.03 µA, which is consistent with the perception threshold of 110 ± 60 µA obtained in the original paper by Klein et al. 2004. On the contrary, van den Broeke and colleagues in general observe perception threshold for stimulation in the same location on the forearm, which is close to twice as high (122–124). In addition, the perception threshold is multiplied by 20 and therefore the HFS conditioning intensity is approximately four times larger compared to the current study (122–124). Greater effect of HFS was also indicated for higher intensity in the original study (25). It is therefore possible that a greater hyperalgesia would have been observed had such a high intensity been applied. Based on the above speculations, the induced mechanism may therefore also to some extent be intensity dependent.

3.4. EXPERIMENTAL PRIMING WITH HEAT/CAPSAICIN

The heat/capsaicin sensitization caused decreased HPT and a larger blood perfusion after HFS, both indicating sensitization of peripheral nerves (paper 4). HFS+HEAT/CAP also caused larger increase in MPS assessed with 128 mN pinprick stimuli after HFS compared to the standard HFS paradigm (paper 4) (figure 3-6). Additional analysis was performed to compare the MPS in stimulation site A1 (see figure 3-3) between HFS+HEAT/CAP and HFS+B.EXP and no differences were observed between the paradigms (figure 3-6, RM-ANOVA, main effect of paradigm, $F(1,18) = 0.404$, $p = 0.533$). The differences observed, i.e. larger MPS for both HFS+B.EXP and HFS+HEAT/CAP paradigms compared to the standard HFS, were only found for 128 mN pinprick stimulation and not for 256 mN (figure 3-6 and 3-7) (paper 3,4).
The sensation to the Qutenza 8% capsaicin patch was rated rather mild compared to ratings to capsaicin injection (figure 3-8) (107). The ratings to the Qutenza patch also had a different temporal response compared to capsaicin injection, which causes an instant, high pain followed by an exponential decline over 5-15 minutes (depending on dose) (107), whereas the ratings to the Qutenza patch in the current study increased throughout the 30 minute application (figure 3-8).

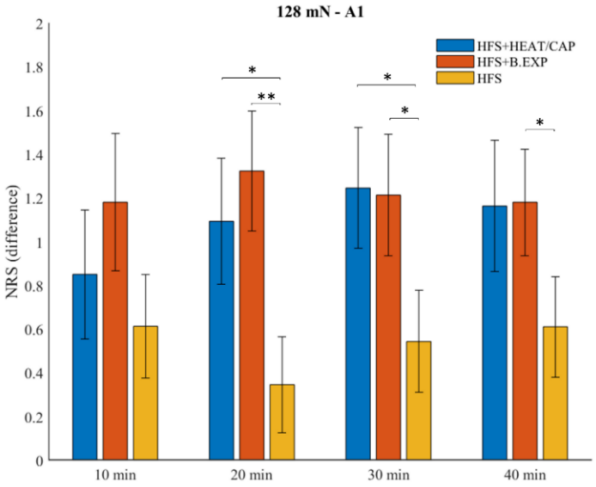

*Figure 3-6. The mechanical pain sensitivity for 128 mN pinprick stimulation in A1, the area proximal to high frequency stimulation (HFS), in all three experimental sessions of study III. Ratings are on a numerical rating scale (NRS) and are subtracted from baseline and unconditioned control area. HFS+HEAT/CAP = HFS with heat/capsaicin priming, HFS+B.EXP = HFS with a B.EXP prepulse. Asterisks indicate differences from post hoc comparisons with Sidak correction, * p < 0.05, ** p < 0.01. (Paper 3, 4).*

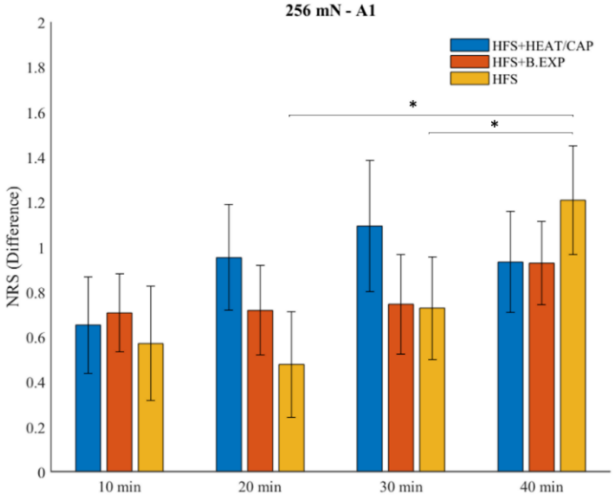

*Figure 3-7. The mechanical pain sensitivity for 256 mN pinprick stimulation in A1, the area proximal to high frequency stimulation (HFS), in all three experimental sessions of study III. Ratings are on a numerical rating scale (NRS) and are subtracted from baseline and unconditioned control area. HFS+HEAT/CAP = HFS with heat/capsaicin priming, HFS+B.EXP = HFS with a B.EXP prepulse. Asterisks indicate differences from post hoc comparisons with Sidak correction, * p < 0.05 (Paper 3, 4).*

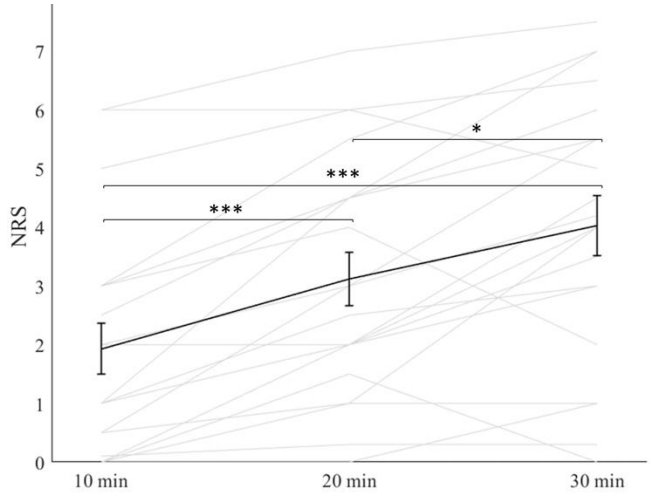

*Figure 3-8. Sensation to heat/capsaicin. Ratings to the Qutenza 8% capsaicin patch are shown after 10-, 20-, and 30- minutes of patch application (data is not shown in paper 4). Mean and standard error is shown in black and as individual subject ratings in gray. Asterisks indicate significant differences from a RM-ANOVA, post hoc comparison with Sidak correction, * $p < 0.05$, *** $p < 0.001$.*

3.5. MECHANISMS INVOLVED

Both paradigms tested in paper 3 and 4, i.e. HFS+B.EXP and HFS+HEAT/CAP, were shown to facilitate the secondary hyperalgesia induced with HFS. Different mechanisms likely contribute to this facilitation as the paradigms were developed utilizing different approaches. The B.EXP prepulse was assumed to increase prefrential activation of primary afferent nociceptive fibers, which likely reduced counteracting pain relieving mechanisms related to activation of Aβ fibers and gate control (56–58). Heat/capsaicin caused a pre-sensitization at central and peripheral level. Since secondary hyperalgesia is generally considered a central mechanism (29,107) the central priming is more likely to have influenced the developed secondary hyperalgesia, but effect of peripheral priming cannot be excluded. The mechanisms involved in secondary hyperlgesia induced with HFS and capsaicin are discussed in greater details in papers 3 and 4.

3.6. SUMMARY ON ELECTRICAL HIGH FREQUENCY STIMULATION PAIN MODEL

Results indicate that the mechanical pain sensitivity to pinprick after HFS can be facilitated compared to the standard 100 Hz model. Results showed that sensitivity was increased from baseline and control area when preconditioning with a B.EXP 100 ms prepulse (paper 3) and central and peripheral priming with heat/capsaicin (paper

4) were applied prior to 100 Hz HFS. The two paradigms HFS+B.EXP and HFS+HEAT/CAP, however likely enhance the mechanical sensitivity through different mechanisms. The long duration B.EXP pulse increases preferential activation of small primary afferent fibers, which likely minimizes coactivation of mechanisms related to Aβ fiber activation. Activation of large Aβ fibres might lead to pain reduction through gating control, and therefore, blocking the large fibers may lead to greater more secondary hyperalgesia. The heat/capsaicin may act to prime the system, centrally or peripherally, and therefore facilitate secondary hyperalgesia to HFS. Highly interesting for development of future study is to consider the combination of the heat/capsaicin priming and HFS with a B.EXP prepulse to induce secondary hyperalgesia.

CHAPTER 4. LIMITATIONS, CONCLUSION AND PERSPECTIVES

4.1. LIMITATIONS

Due to the indirect nature of the HFS model used in the current studies and former studies, questions still remain unanswered regarding the exact mechanism/s induced with the model. By increasing selectivity towards the nociceptive fibers, as attempted in this PhD project, the physiological relevance of the model is increased by limiting unknowns related to co-activation of large fibers. The results from papers 1 and 2 on pulse shape and duration for increasing selectivity towards the nociceptive fibers are however also indirect as they rely on subjective measurements of perception threshold. Highly important step towards increasing the relevance of the current work would be to evaluate the nerve fiber selectivity towards the small nociceptive fibers with the B.EXP pulse utilizing more direct nerve measurements in animals or the microneurography technique (54).

4.2. CONCLUSION

In papers 1 and 2, the perception threshold was used as an indirect measure of the activation threshold of the small (Aδ- and C) fibers and large (Aβ) fibers stimulated rather selectively at low intensities with the pin and patch electrodes, respectively. In paper 1, different pulse shapes: linear, exponential, bounded exponential (B.EXP), and rectangular were compared and results showed that for 50 ms B.EXP pulse shape, perception threshold with the patch electrode increased compared to 5 ms, which is most likely attributed to the accommodation phenomenon. For the other pulse shapes with the patch electrode and all pulse shapes with the pin electrode, perception threshold either decreased or did not differ from 5 ms to 50 ms (paper 1). This suggests that the large Aβ fibers can be somewhat inactivated during B.EXP stimulation, which may increase preferential activation of small nociceptive fibers.
In paper 2, the duration of the B.EXP pulse was further examined also using the perception thresholds of the pin- and the patch electrodes. Results showed that perception threshold with the patch electrode formed a curve of accommodation contrary to the standard strength-duration curve that was observed for rectangular pulses. The largest perception threshold was observed for 100 ms pulses with the patch electrode and as expected, no threshold increase was observed for long durations with the pin electrode indicating absence of accommodation (paper 2). It is likely that the time constant of current increase for the B.EXP pulse, which increases with increased pulse duration, determines the degree of accommodation as it determines how the current increases for this specific pulse shape and duration.

In paper 3 and 4 it was attempted to increase the amount of secondary hyperalgesia induced with the HFS human experimental pain model by utilizing two different approaches. In paper 3 preferential nociceptor activation was applied be preceding HFS with 100 ms B.EXP pulse (HFS+B.EXP). In paper 4, a novel combination of the heat/capsaicin sensitization followed by HFS (HFS+HEAT/CAP) was applied. Results indicated that secondary hyperalgesia could be facilitated after both HFS+B.EXP (paper 3) and HFS+HEAT/CAP (paper 4), provided it was assessed with 128 mN pinprick stimulation. The HFS+B.EXP paradigm is believed to increase the physiological relevance of the model due to decreased co-activation of large fibers, which otherwise likely counteracts the desired pain facilitation of the HFS pain model.

4.3. FUTURE PERSPECTIVES

4.3.1. PREFREENTIAL ELECTRICAL STIMULATION OF SMALL NOCICEPTIVE FIBERS

The initial finding of this PhD study relates to the special B.EXP pulse shape and its ability to accommodate large fibers with the patch electrode in paper 1 and 2. In paper 3 and 4, the pulse shape was utilized to increase preferential activation of small fibers in the HFS pain model and thereby increasing its mechanism-based value for inducing secondary hyperalgesia, which effect was furthermore shown to increase. This is just an example of possible usability related to the B.EXP for preferential small fiber activation. Selective small fiber activation with electrical stimulation has remained a challenge and despite comprehensive research related to both stimulation parameters (46,49,50,53) and electrodes (15,37,43,44), optimal stimulation paradigm is still to be established. The B.EXP stimulation shape shown in this PhD to accommodate large nerve fibers could provide a valuable contribution for future paradigms for selective small fiber activation. To move forward in this field it is essential to gather knowledge related to electrode design and stimulation parameters and importantly, current research also focuses on the electrode development (43,133).
The B.EXP pulse shape could contribute profoundly to the perception threshold tracking technique that utilizes perception thresholds in combination with computational models to investigate nerve fiber excitability, for instance with accommodation (42,64). The future scope of the perception threshold tracking method is to reveal peripheral mechanisms related to neuropathic pain (42,64).

4.3.2. HFS HUMAN EXPERIMENTAL PAIN MODEL

LTP in pain pathways has been hypothesized to be a mechanism underlying chronic pain originating from peripheral inflammation, neuropathy, acute postoperative pain or another acute pain event (21). Human experimental pain models, such as the HFS model currently applied in this PhD study, can be used to investigate mechanisms of the human pain system. To date, they have mostly been applied for gaining deeper understanding of underlying mechanisms.

Taking secondary hyperalgesia for an example, by utilizing different experimental protocols a great understanding has been revealed regarding primary afferent fiber types and how different types are involved in the induction and facilitation (17,18,35). Similar pathways have been shown to be involved in secondary hyperalgesia when experimentally induced and in neuropathic pain patients and secondary hyperalgesia has therefore been considered as a model of neuropathic pain (134). The facilitating pathway, i.e the induction pathway, has been shown to involve intense activation of mainly capsaicin-sensitive C fibers with little or no contribution of the A-fibers (18,35,36). The facilitated pathway however, most likely involves exclusively high-threshold mechanoreceptive A-fibers (HTMs) (17,35,123). A treatment of conditions related to secondary hyperalgesia would therefore gain from treatments focused on targeting the HTMs specifically, which as Magerl et al. 2001 nicely speculated, for instance do not include the opioid receptor (17,135). Since the capsaicin-sensitive fibers are not involved in the facilitated pathway, the capsaicin desensitization treatment paradigm may therefore only work to block the initiation of the mechanism (17,18,136).

If underlying mechanisms are properly established and induction and assessment techniques are well controlled, the HFS experimental pain model could also serve as an important tool for pharmacological testing and for diagnosis of specific underlying mechanisms.

Considering that the facilitated secondary hyperalgesia observed following HFS+B.EXP (paper 3) and HFS+HEAT/CAP (paper 4) was mediated through different mechanisms, it is possible that even greater effect would have been observed in a model combining the two methods, i.e. priming the central- and peripheral nervous system with heat/capsaicin followed by HFS with a B.Exp prepulse. However for the purpose of increasing the specificity of the model and for using this model to investigate more specifically the role of heterosynaptic LTP in secondary hyperalgesia in a human experimental model, the HFS+B.EXP indeed limits confounding mechanisms reltaed to activation of large Aβ fibers. The heat/capsaicin priming on the other hand may add mechanisms as facilitated secondary hyperalgesia is likely observed due to additive mechanisms. This comes with an obvious limitation, that unknown dimensions may be involved in the model, complicating interpretation of results even more than in the orignial HFS paradigm. Despite plausible implication, such as using the HFS+HEAT/CAP model to investigate secondary hyperlagesia with HFS in healthy subjects who are already centrally sensitized with heat/capasaicin, the method requires further study.

The current study investigated secondary hyperalgesia for 50 minutes after HFS similar to many other similar studies (28,106). Results from a study on the time-course of secondary hyperalgesia, induced with HFS, showed a time course of around 24 hours in most subjects (137). In few subjects the estimated time for recovery was much longer (> 10 days) and it was speculated whether the long time course may reflect susceptibility of the subjects to develop chronic pain (137). Accordingly, it would be interesting to investigate the time-course of hyperalgesia developed after

HFS+HEAT/CAP paradigm to examine the potential of modeling susceptible subjects that could develop longer lasting secondary hyperalgesia to HFS.

The HFS model has yet, not been used in any clinical applications, but potentials should be discussed. Initially, when designing the current PhD project, ideas came to mind regarding the use of the HFS model as a contribution to methods already established for predicting postoperative pain (138). More research was however needed to improve the selectivity of the stimulation and further research on healthy subjects was needed to test the feasibility of the model before investigating the model on patients undergoing surgery. By utilizing the idea of using the heat/capsaicin sensitization to 'mimic' susceptible subjects, an interesting study would be to test the feasibility of the HFS model for predicting whether healthy participants are primed with the heat/capsaicin sensitization.

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