Multilevel Electrophysiological Methods in Pathophysiolgy and Management

Studies of diabetes, incontinence, and analgesics

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MULTILEVEL ELECTROPHYSIOLOGICAL METHODS IN PATHOPHYSIOLGY AND MANAGEMENT

STUDIES OF DIABETES, INCONTINENCE, AND ANALGESICS

BY
RASMUS BACH NEDERGAARD

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Multilevel Electrophysiological Methods in Pathophysiolgy and Management

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Ph.D. Dissertation
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Curriculum Vitae

Rasmus Bach Nedergaard

Research interests: In my professional career I have sought to try and understand characteristics of different patient populations and how to help them, it is what pushes me to keep working within science and to try to help where I can.

Education:

- **2014** Master of science (Biomedical Engineering), Aalborg University, Denmark
- **2012** Bachelor of science (Biomedical Engineering), Aalborg University, Denmark

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- **2015-2018** Research Fellow, New Zealand College of Chiropractic
- **2014** Research Assistant, New Zealand College of Chiropractic

Publications:

1. Mark, E.B., **Nedergaard, R.B.**, Hansen, T.M., Nissen, T.D., Frøkjær, J.B., Scott, S.M., Krogh, K. and Drewes, A.M., 2021. Tapentadol results in less deterioration of gastrointestinal function and symptoms than standard opioid therapy in healthy male volunteers. Neurogastroenterology & Motility. DOI: 10.1111/nmo.14131

2. **Nedergaard, R.B.**, Hansen, T.M., Nissen, T.D., Mark, E.B., Brock, C. and Drewes, A.M., 2021. The effects of tapentadol and oxycodone

on central processing of tonic pain. Clinical Neurophysiology. DOI: 10.1016/j.clinph.2021.07.021

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The thesis is based on the following papers:

I. **Nedergaard RB**, Hansen TM, Mørch CD, Niesters M, Dahan A, Drewes AM. Influence of tapentadol and oxycodone on spinal cord and brain: a randomized, placebo-controlled study in healthy volunteers *submitted to Journal of Clinical Neurophysiology*

II. **Nedergaard RB**, Hansen TM, Nissen TD, Mark EB, Brock C, Drewes AM. The effects of tapentadol and oxycodone on central processing of tonic pain *Clinical Neurophysiology*

III. **Nedergaard RB**, Nissen TD, Mørch CD, Meldgaard T, Juhl AH, Jakobsen PE, Karmisholt J, Brock B, Drewes AM, Brock C. Diabetic Neuropathy Influences Control of Spinal Mechanisms *Journal of Clinical Neurophysiology* 2020

IV. **Nedergaard RB**, Haas S, Christensen P, Krogh K, Laurberg S, Brock C, Drewes AM. Cortical processing of tonic ano-rectal distensions in patients with idiopathic faecal incontinence *Not published*

Abbreviations

BESA	Brain Electric Source Analysis
DM	Diabetes Mellitus
DSPN	Diabetic Symmetrical Polyneuropathy
EEG	Electroencephalography
EMG	Electromyography
IFI	Idiopathic Fecal Incontinence
MOR	mu-opioid receptor
NRI	Noradrenaline Reuptake Inhibition
NRS	Numeric Rating Scale
sLORETA	Standardized Low Resolution Brain Electromagnetic Tomography
VAS	Visual Analogue Scale

English Summary

Healthcare has improved, which means an increasing number of people live longer with diseases. Because of this, more people will experience symptoms of chronic diseases. In addition, chronic disease brings with it risks of chronic symptoms, some likely caused by changes in the body's sensory processing. Of these changes, pain is one of the most frequent symptoms in various diseases, affecting approximately 20 % of the population in the Western world. To improve treatments further investigations into the possible changes due to sensory processing are needed. The use of standardised painful stimuli of different modalities such as mechanical, thermal, and electrical applied to the skin has been used to assess various pain pathways and mechanisms. Symptoms caused by disease confounds the characterisation of pain in clinical work, in addition to this, the experience of pain is multidimensional, and the use of subjective self-reporting does not identify the underlying neural mechanisms in the central nervous system.

The objective of this Ph.D. thesis was to investigate changes due to sensory dysfunction measured objectively with electrophysiology. This includes studies of healthy participants and patient populations to assess the effects of drugs and disease on the spinal and cortical nervous system.

Data from two clinical trials and one cross-sectional study were included in this thesis. The first two papers investigated the effects of the two opioids, oxycodone, and tapentadol, on the central nervous system in healthy volunteers. In the first paper, a nociceptive withdrawal reflex was elicited from the plantar side of the foot and recorded at the anterior tibial muscle using electromyography and from the cortex using electroencephalography (EEG). The second paper investigated the effects of a tonic cold pain (hand submerged in 2° C cold water for two minutes) recorded using EEG. The third paper used the same nociceptive withdrawal reflex stimulation of the foot as the first paper to investigate differences between people with diabetic distal symmetric polyneuropathy and a healthy control group. The fourth and last paper investigated the differences between patients with idiopathic

faecal incontinence and healthy age-matched controls using a tonic distension of the anal canal and rectum where the sensory response was recorded using EEG.

Paper one and two found that both oxycodone and tapentadol affect the brain. However, there was evidence that tapentadol had a dual-acting analgesic effect on opioid receptors working mainly in the cortex and norepinephrine reuptake inhibitor that affects the brainstem and spinal nervous system. Paper three found differences between people with diabetic distal symmetric polyneuropathy and a healthy control group in the stimulation strength needed to elicit a reflex, the number of reflexes observed, and the amplitude and latency of the recorded cortical signals at the vertex during electrical stimulation of the foot. Lastly, paper four found that while there is a difference in the compliance of the rectum and anal canal of people with idiopathic faecal incontinence and healthy controls, there were no differences between the two groups in the EEG response.

In conclusion, using experimental models, it was possible to find differences in the effects of different opioids on healthy volunteers and the group of people with diabetes compared to a healthy control group. However, there were no differences in the brain's response to stimuli between the idiopathic faecal incontinence and healthy controls. This is possibly due to altered compliance of the rectum, while cortical possessing of the tonic sensory input itself is intact. This thesis represents an example of advanced signal processing tools in combination with experimental pain models that are able to explain differences in drugs and disease mechanisms based on objective electrophysiological measures.

Dansk Resume

Sundhedsvæsenet forbedres løbende, hvilket betyder et øget antal af mennesker, som lever længere liv med sygdomme. På grund af dette vil flere mennesker opleve symptomer på kroniske sygdomme. Dette medfører risici for kroniske symptomer, som er forårsaget af ændringer i kroppens sensoriske behandling. Af disse ændringer er smerte et af de mest hyppige symptomer i en række sygdomme. Dette rammer ca. 20% af befolkningen i den vestlige verden. For at kunne forbedre behandlinger er der behov for yderligere undersøgelser af de mulige ændringer som sker på grund af sensorisk opfattelse. Anvendelsen af standardiserede smertefulde stimuli af forskellige modaliteter såsom mekanisk, termisk, og elektrisk på huden er blevet brugt til vurdering af forskellige smerteveje og mekanismer. Karakteriseringen af smerte i klinisk arbejde er forurenet af symptomer forårsaget af sygdom, ud over dette er oplevelsen af smerter flerdimensionel, og brugen af subjektiv selvrapportering identificerer ikke de underliggende neurale mekanismer i centralnervesystemet.

Formålet med denne ph.d.-afhandling var at undersøge ændringer på grund af sensorisk dysfunktion målt objektivt ved hjælp af elektrofysiologi. Dette blev gjort ved inklusioner af raske deltagere og patientpopulationer for at vurdere virkningen af lægemidler og sygdomme på det perifere og centrale nervesystem.

Data fra to kliniske forsøg og et tværsnitsstudie blev inkluderet i denne afhandling. De første to artikler undersøgte virkningen af to opioider: oxycodon og tapentadol sammen med et placebo-lægemiddel på det centrale nervesystem hos raske frivillige. I det første studie blev en nociceptiv tilbagetrækningsrefleks fremkaldt fra undersiden af foden og optaget ved tibialis anterior ved hjælp af elektromyografi og fra cortex ved anvendelse af elektroencefalografi (EEG). Den andet artikel undersøgte virkningerne af en tonisk kuldepåvirket smerte (hånd nedsænket i 2°C koldt vand i to minutter) optaget ved hjælp af EEG. Den tredje artikel anvendte den samme nociceptive tilbagetrækningsrefleks af foden til at undersøge forskelle mellem en gruppe

mennesker med diabetisk distal symmetrisk polyneruropati og en sund kontrolgruppe. Den fjerde og sidste artikel undersøgte forskellene mellem en gruppe patienter med idiopatisk fækal inkontinens og en sund kontrolgruppe ved hjælp af en tonisk distension af anal kanalen og endetarmen registreret ved hjælp af EEG.

Artikel et og to fandt, at både oxycodon og tapentadol har en effekt på hjernen. Tapentadol har en dobbeltvirkende analgetisk virkning på opioioid receptorer, som primært virker i cortex og norepinephrine genoptagelses inhibitor som påvirker hjernestammen og det spinale hjernesystem. Artikel tre fandt forskelle mellem mennesker med diabetisk distal symmetrisk polyneuropati og en kontrolgruppe i styrken af den stimulering, der var nødvendig for at fremkalde en refleks, antallet af observerede reflekser, og amplituden og latensen af kortikale signaler registreret på toppen af hovedet. Til sidst fandt artikel fire, at selvom der er en forskel i kompliansen af endetarmen og anal kanalen hos mennesker med idiopatisk fækal inkontinens og sunde kontroller, er der ingen forskelle mellem de to grupper i hjernens fortolkning af tonisk stimulering.

Afslutningsvis ved hjælp af eksperimentelle smertemodeller var det muligt at finde forskelle i virkningen af opioider på raske frivillige og i gruppen af mennesker med diabetes sammenlignet med en sund kontrolgruppe. Der var imidlertid ingen forskelle mellem personer med idiopatisk fækal inkontinens og kontrolpersoner, dette skyldes muligvis ændret komplians af endetarmen, mens kortikalt besiddelse af selve den toniske sensoriske input er intakt. Denne afhandling repræsenterer et eksempel på avancerede signalbehandlingsværktøjer i kombination med eksperimentelle smertemodeller, som er i stand til at skelne mellem forskellige befolkningspopulationer baseret på objektive mål.

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I would like to thank my main supervisor Asbjørn Mohr Drewes. Asbjørn has always been able to see the goal in the horizon and help me steer in the correct direction, even when we encountered problems starting a clinical trial and had to shift focus. Asbjørn has never been more than an email away, and always ready with thorough comments to my papers. Christina Brock has always been there day to day asking where I was in relation to my goals and if I needed any help. Anyone working with Christina can attest to her personality raising the mood of a room, and I have had the pleasure working in the same office as her for two years of my thesis. Carsten Dahl Mørch has been invaluably helpful in my thesis with his extensive knowledge on the methodologies and statistical analysis we have used throughout the thesis.

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from Denmark that I did not see myself. My path to where I am today has not been a straight one, and would never have happened without all of the wonderful people I have met on the way. The PhD salary was funded by The Danish Cancer Society and from Region Nordjyllands Sundhedsvidenskabelige Forskningsfond.

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Finally I would like to thank my family for always supporting me and listening patiently when I explain my work. I would especially like to thank my wife Mette and daughter Olivia for reminding me that research is important, but nothing is more important than family.

Rasmus Bach Nedergaard
Aalborg University, November, 2021

Contents

Chapter 1

Introduction

As healthcare improves an increasing number of people live longer lives with diseases. As a result new problems arise that needs to be addressed. Chronic disease brings with it risks of chronic symptoms some likely caused by changes in the body's sensory processing. Of these changes pain is one of the most frequent symptoms in a variety of diseases, affecting approximately 20% of the population in Western world [1–3]. With the increasing number of people experiencing these changes, it becomes more and more important to develop treatment plans to help mitigate changes in sensory processing. To be able to improve treatments further investigations into the possible changes due to sensory processing are needed.
The use of standardized painful stimuli of different modalities such as mechanical, thermal and electrical applied to the skin, have been used in the assessment of various pain pathways and mechanisms [4].

1 Experimental pain models

The characterisation of pain in clinical work is confounded by symptoms caused by the diseases [5], the experience of pain is multidimensional [6] and the use of subjective self-reporting does not identify the underlying neural mechanisms in the central nervous system and is confounded by psychological factors [7].
Inducing experimental pain in a population of healthy volunteers bypasses symptoms caused by diseases and help to better study pain mechanisms [8]. Experimental pain has been widely used in both healthy and patient populations [9–13]. To better understand the different changes to the nervous system objective analysis based on electrophysiology was used [7, 8, 14]. A visualisation of types of stimulation are available in Figure 1.1. In addition subjective visual analogue scale and numeric rating scales were used to assess

pain [8].

2 The pain system

Pain is the body's response to: *"An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage."* according to The International Association for the Study of Pain. According to Zhu and Lu 2010 [15] pain can be classified as:

- Nociceptive: Activation or sensitization of peripheral nociceptors
- Inflammatory: Inflammation and tumour cells releasing chemicals that affect the nociceptor afferents
- Neuropathic: Injury or abnormalities of peripheral or central neural structures

The nerves responsible for the sensation of pain are called nociceptors they react to: thermal-, chemical- and mechanical-stimulations. The nociceptors are located in the skin, internal organs, tendons, muscles, and joints [16]. Nociceptive pain can be either visceral, originating in the internal organs or somatic, in the skin, tendons, muscles, and joints [8]. The nerve fibres responsible for the transmission of sensation and pain are the A-beta, A-delta and the C-fibers. The A-beta fibres are large-diameter highly myelinated fibres with a low activation threshold able to quickly send information from the periphery to the central nervous system, A-delta fibres are thinly myelinated fibres with a higher activation threshold. Finally, C-fibers are unmyelinated slow conducting nerve fibres with the highest activation threshold [17]. A-delta and C-fibers are nociceptors and transmit painful stimuli to the central nervous system through the dorsal horn of the spinal cord see Figure 1.1.

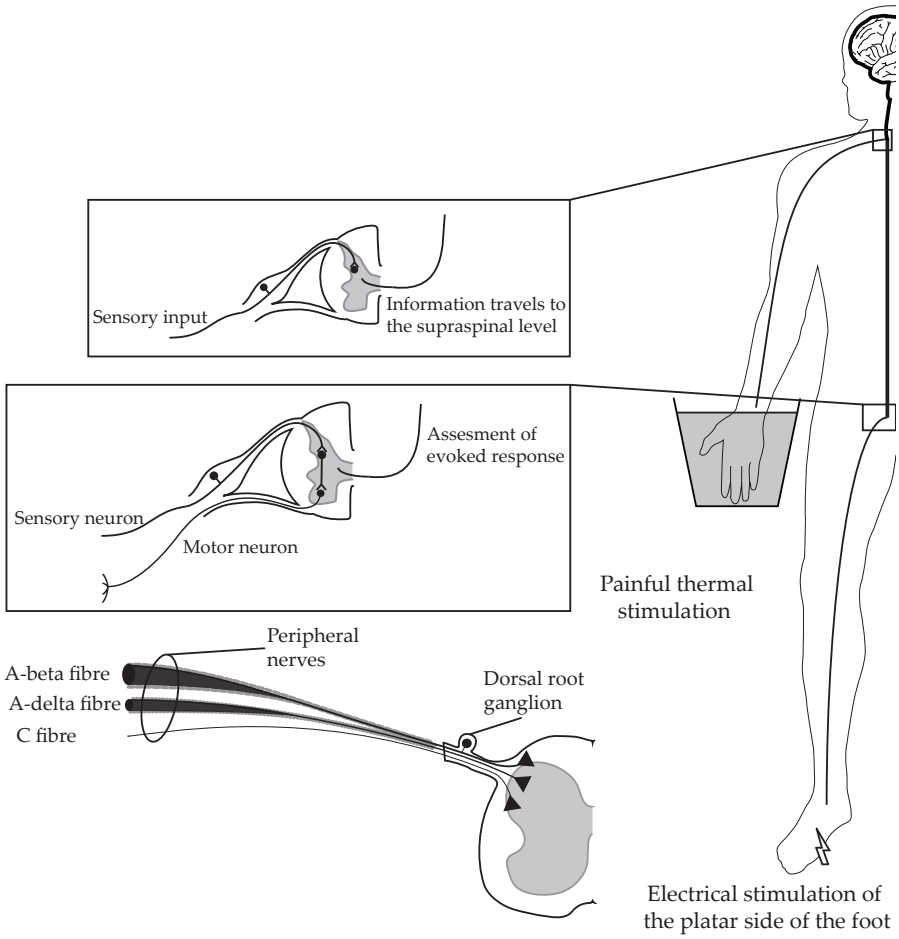

Fig. 1.1: An overview of different types of stimulations to induce experimental pain. One a tonic painful stimulation of the hand using chilled water, this method is described in Section 2.1. The other a polysynaptic withdrawal reflex of the foot, described in Section 3.1. The painful stimuli travel from the periphery through the dorsal horn of the spinal cord into the central nervous system.

Chapter 2

Electroencephalography

Electroencephalography (EEG) was first described by Richard Caton in 1875 in the report *The Electric Currents of the Brain* [18] and was recorded in humans by Hans Berger in 1929 [19]. EEG records the simultaneous firing of neurons from the brain and can be recorded from the scalp. It is a powerful non invasive tool to study the electrophysiological dynamics of the brain [20].
Compared to other modalities that are able to describe brain activity EEG has a high temporal resolution but a relatively poor spatial resolution due to characteristics of the electrical signal traveling through different tissues, see figure 2.1. The high temporal resolution of EEG makes it a good candidate for exploring brain mechanisms, specifically related to chronic pain [21].

1 Generation of EEG

Generation of a measurable EEG at the scalp is a result of synchronised synaptic activity of cortical neurons which are pyramid cells arranged in cortical columns [22], these are visualized in figure 2.1. The recorded EEG at the scalp is a spatially smoothed signal of local field potentials from within the brain [23]. EEG signals decay over distance, meaning most of the measurable EEG is generated by the cortex, it is however possible to record deeper strong electrical fields [22].

2 Types of EEG recording

Once the signals from the local field potentials have travelled through the structures of the brain and arrived at the skull, they are separated from the recording electrodes by layers of poor conductors. These include the skull, dead skin cells, hair, and air. Specialised electrodes that minimises these areas

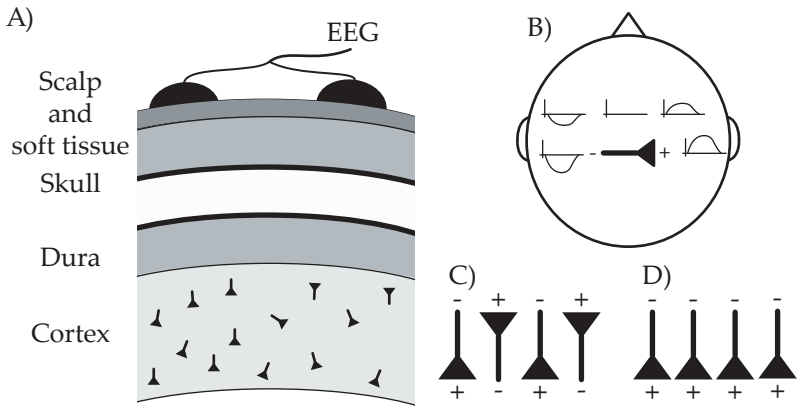

Fig. 2.1: A) Recording electrical activity of the brain from neurons in the cortex from the scalp through electroencephalography (EEG). B) Visualizes the generation of electrical signals from cortical neurons. When cortical neurons are arranged in a reversed pattern C) no signal is generated, aligned neurons sum to a measurable signal D)

of poor conduction such as wet-, dry-electrodes, and electrode gel between the skin and recording electrodes can be used. All of the experiments in this thesis was conducted using electrode gel. The gel fills in the air pockets between hairs creating a conductive path from the scalp to electrodes [22]. Increasing the number of recording electrodes decreases the localisation error of the underlying electrical potentials generating the EEG. The localisation improvements reduce as the number of electrodes increases [24–26]. Increasing the number of EEG electrodes also increase the time to mount electrodes, and increase in cost for the system used. In this thesis, all EEG recordings were conducted using 62 channel EEG mounted in accordance with the 10-20 system.

2.1 Continuous

Continuous EEG is a recording with no time-locked stimulation. The neuronal firing is randomly distributed over time. Continuous recordings can record resting EEG with no external stimulation, or during the induction of a stimulation that is not time-locked. Usually, EEG is divided into frequency bands, the definition of these bands varies slightly but overall they are:

- Delta (1-4 Hz)
- Theta (4-8 Hz)
- Alpha (8-12 Hz)
- Beta (12-32 Hz)
- Gamma (32-70 Hz)

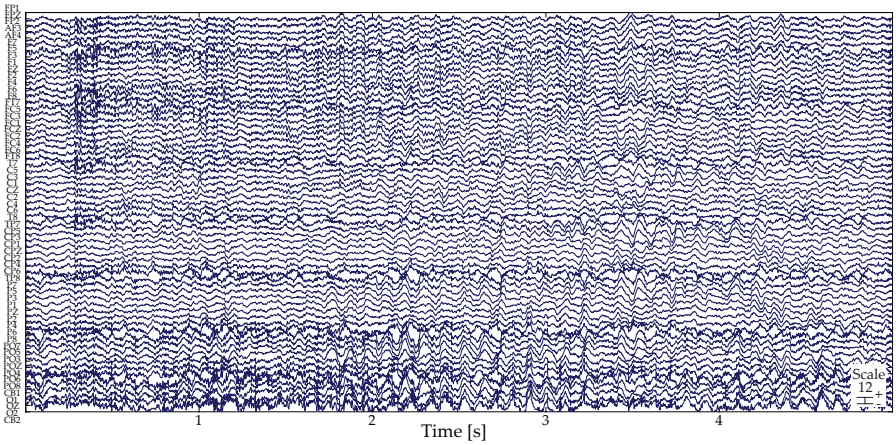

Fig. 2.2: A five second window of continuous electroencephalography (EEG) recorded in a subject at rest with no stimulation. There are 62 EEG channels in a 10-20 arrangement.

A variation of continuous EEG is the use of tonic stimulations. The use of tonic pain stimulation is a physiologically meaningful stimulation that is believed to best mimics chronic pain [27]. However, continuous EEG is susceptible to movement, muscle, eye and heart artefacts which to some extend can be removed using preprocessing techniques. An example of continuous EEG is available in figure 2.2.

2.2 Evoked potentials

Evoked potentials are EEG time-locked stimulations that can either be analysed using single-sweeps or analysis averaged-sweeps. The use of a short stimulation allows for a synchronised neuronal firing resulting in negative and positive peaks. These are often quantified using latencies and peak to peak amplitudes. A visualisation of single sweeps and the corresponding average of multiple sweeps are available in figure 2.3. The number of stimulations can vary between a couple to thousands of stimuli. When using fewer stimulations is often chosen to reduce the discomfort associated with the stimuli.

3 Preprocessing of EEG

When recording EEG signals surrounding electrical noise is also recorded [28]. Among these are biological noise such as: Blinks, eye movement, muscle activity (including the heart) and skin potential along with external sources

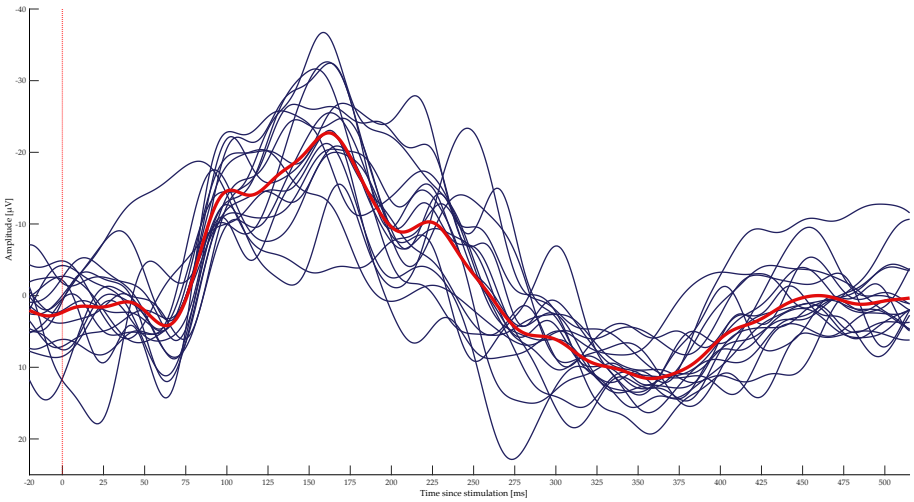

Fig. 2.3: Eighteen single sweep evoked potentials in blue and an average of the sweeps in red displayed.

such as line noise (50-60 Hz). These artifacts decrease the signal to noise ratio and can be either transient or constant. In order to remove artifacts several methods can be deployed. In this thesis filtering and artifact subspace reconstruction [29, 30] along with the standardized preprocessing for large-scale EEG analysis (PREP) pipeline [31] were applied. Additionally filters specific to the stimulations were applied, these are described in detail in papers I-IV. After having filtered the data, channels that have noise or artefacts in more than 10% of the recording were deemed to be bad channels and were interpolated spherically [32, 33]. The use of independent component analysis has become widely used [30, 34, 35], it is however outside the scope of this thesis to be described.

4 Analysis of EEG

Recordings of EEG are normally recorded across multiple electrodes, over a large band of frequencies, these signals are nonstationary with a need to be localized in time, space and scale [36].

4.1 Wavelet

Wavelet analysis differs from traditional frequency analysis such as Fourier analysis since Fourier analysis use sine and cosine waves, these extend infinitely in time. Mother wavelets, which are used to decompose data into

time-frequency coefficients, have a zero mean amplitude (like sine and cosine waves) and finite energy over time (unlike sine and cosine waves). Additionally, mother wavelets have little lower frequency energy relative to higher frequency energy [36]. This results in a high frequency resolution at low frequencies and high time resolution at high frequencies, which is what is of interest in EEG analysis.

4.2 Amplitude and latency

The analysis of areas of interest in the time and amplitude domain of EEG can be performed on the continuous signal, during single sweep analysis of a known stimulation or using averages of multiple single sweeps. The advantage of analysis of averages is that noise is stochastic and therefore non-stationary, which should always average out given enough single sweeps used. The disadvantage is that any jitter in latency of the stimulation and resulting evoked potential will result in a smear of the averaged signal affecting latency and to a potential large extend the amplitude of a signal.

4.3 Inverse modeling

As described in section 1 and visualised in Figure 2.1 EEG at the scalp is a result of synchronised synaptic activity of cortical neurons in the brain, which is a spatially smoothed signal [37]. To investigate the cortical EEG source generators, the "inverse problem" can be solved. There is no single answer to the inverse problem, but it can be approximated given some assumptions are met [22]. To optimise this process several factors needs to be accounted for. Amongst these are: The number of electrodes, Michel et al. [24] has described 63 electrodes to be sufficient. The location of electrodes needs to be monitored closely, and noise, both electrical and biological signals must be minimised.
In this thesis, Brain Electric Source Analysis (BESA) and standardised low resolution brain electromagnetic tomography (sLORETA) software has been used. These inverse models deploy two different strategies to solving the inverse problem. BESA is a dipolar source model and relies on an a priori number of dipoles in a specified time interval. The dipoles are then either varied in time or orientation to optimise the residual variance of the data (data not described by the dipoles). The residual variance is one of four criteria used in BESA to find an optimal inverse solution. The three others are: source activation criterion, energy criterion, and separation criterion. These criteria relate to common activation of sources, activation outside the specified time interval and favour as few sources active at the same time as possible. All of these criteria are sought to be minimised for the optimal solution [38].
sLORETA is a distributed source model that uses standardised current den-

sity which results in a zero localisation error, when using a noiseless source simulation [38, 39]. Compared to a minimum norm approach this also allows sLORETA to have higher accuracy of deep cortical sources [39]. Compared to BESA, sLORETA does not rely on predefined dipoles and can analyse brain activity in different frequency bands.

Chapter 3

Opioids

The use of exogenous opioids has been described throughout history. The first recorded mention was in 3500 BC by the Sumerians (southern Iraq) [40–42]. Opium is derived from the opium poppy, which is believed to originate from modern day Turkey [41]. Today opium derived from the opium poppy is not widely used clinically. Purified alkaloids from opium (morphine and codeine) and the semisynthetic derivatives of them (oxymorphone, oxycodone, hydromorphone, hydrocodone) along with fully synthetic opioids (mepridine, methadone, fentanyl, pentazocine) are used mainly in pain relief [43].
There are four types of opioid receptors: mu-opioid receptors, delta-opioid receptor, kappa opioid receptor, and nociceptin/orphanin receptor [44]. The opioid receptors are distributed in the central nervous system, and enteric nervous system [41, 43].

1 Analgesic effect

Opioids assert their analgesic effect by activating opioid receptors. This results in an indirect inhibition of voltage-dependent calcium channels which blocks the release of pain neurotransmitters from the nociceptive fibres, resulting in analgesia [42].
Clinically morphine has been widely used, but other strong opioids include, e.g. oxycodone, buprenorphine, hydromorphone, methadone, alfentanil, fentanyl and tapentadol. These drugs are predominantly mu-opioid receptor agonists [45]. Pain processing in the brain is altered due to opioids [7]. These changes can be assessed spinally and supraspinally [14]. In order to assess the effects of opioids reproducibly performing the assessments on a homogeneous group is important [4, 5].

1.1 Oxycodone

Oxycodone is a mu-opioid receptor (MOR) agonist which is commonly used in the treatment of moderate to severe pain. Oxycodone has increased in consumption since the beginning of 2000 in the European region [46]. A diagram of the mu-opioid receptor is visualised in Figure 3.1.

1.2 Tapentadol

Tapentadol is a newer opioid that combines the MOR agonist with noradrenaline reuptake inhibition (NRI) [47]. It is used in chronic and neuropathic pain [48]. The effects of tapentadol have been investigated preclinically [48, 49]. The central and peripheral mechanisms of tapentadol are relevant to examine in the human brain compared to classical opioids such as oxycodone. The dual acting effects of tapentadol are visualised in Figure 3.1.

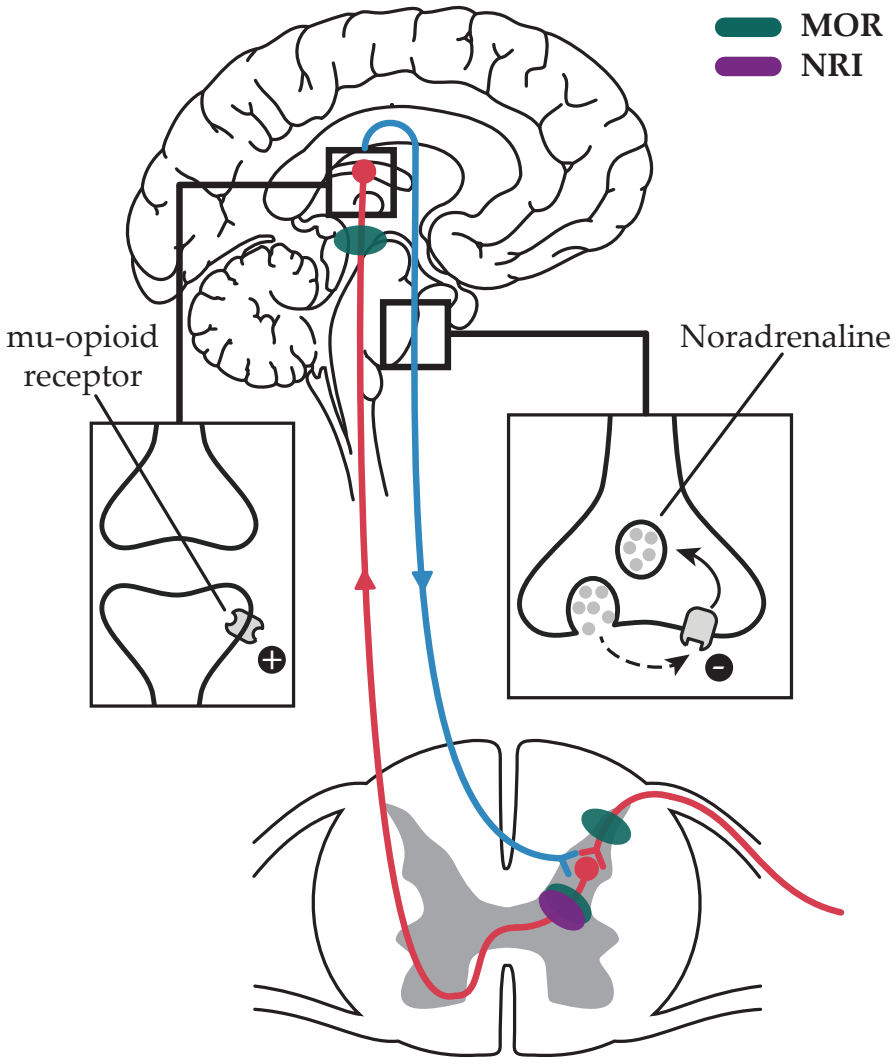

Fig. 3.1: The mu-opioid receptor (MOR) agonist effect of oxycodone and tapentadol interrupts pre- and post-synaptic transmission affecting the ascending pain signal supraspinally [50] this effect is lower in tapentadal compared to traditional opioids [51]. In addition to this tapentadol inhibits noradrenaline reuptake (NRI) which enhances descending inhibion of pain [52].

Chapter 4

Patient populations

1 Diabetes

Diabetes mellitus (DM) is a metabolic disease identified by hyperglycaemia. There are several types of DM. The focus of this thesis is on type 1, which is an autoimmune disease that destroys the insulin-producing cells of the pancreas [53]. The prevalence of type 1 DM is increasing [54, 55]. There are both micro- and macro-vascular complications associated with DM [56]. Of the microvascular complications, polyneuropathy will affect 30-50 % of all people with DM throughout the course of the disease. Diabetic neuropathy most commonly presents as length-dependent diabetic symmetrical polyneuropathy (DSPN) [57]. This type of neuropathy affects the long nerve fibres in the body and commonly presents symptoms in the feet and hands [58, 59].

2 Idiopathic fecal incontinence

Faecal incontinence is a common symptom affecting approximately 50% of nursing home residents [60–62]. The causes of faecal incontinence are manifold, but when unclear the condition is referred to as idiopathic faecal incontinence (IFI). IFI patients have structurally intact but weak sphincters, decreased anal sensation [63, 64] and altered sensitivity [65]. Pudendal neuropathy is considered to be present in many of these patients [66–68]. A part of the possible problem affecting this patient group is a change of the sensory function of the rectum and anal canal [65], which affects the ability to defecate [69].

Chapter 5

Hypothesis and aims

The hypothesis of the thesis is that sensory dysfunction, results in changes which can be measured objectively using electrophysiology. This will be assessed in terms of alterations in the central nervous system, quantified using advanced signal analysis. To investigate sensory dysfunction, models of stimulation were selected and applied in healthy volunteers and patients with expected neurological dysfunctions.
The project aims to investigate differences in processing of standardised stimuli in different patient populations and healthy volunteers. To create a basis for comparison, a clinical trial containing three intervention arms (two opioids: oxycodone (affecting the central nervous system) and tapentadol (affecting both the central and peripheral nervous system)), and placebo was used to test the effects of opioids on phasic and tonic experimental pain stimulations in healthy subjects. Two patient populations were included in the thesis as well, firstly a population of patients with DSPN and a group of women with idiopathic faecal incontinence. The patient population with DSPN represent a group with known sensory dysfunction, whereas the pathogenesis behind idiopathic faecal incontinence to a large degree is unknown.
The aims of the current thesis were:

1. *To investigate the cortical and spinal changes on a healthy control population receiving a treatment of oxycodone and tapentadol using the nociceptive withdrawal reflex.*
2. *To investigate the cortical processing of a tonic pain stimulation using the cold pressor test on a healthy control population receiving a treatment of oxycodone and tapentadol.*
3. *To compare the processing of spinal stimuli between patients with DSPN and healthy controls using the nociceptive withdrawal reflex.*
4. *To compare the cortical processing of tonic ano-rectal distensions in patients with idiopathic faecal incontinence and healthy controls*

Chapter 6

Materials and methods

1 Study design

Three studies from ongoing projects at the centre of Mech-Sense, Aalborg University Hospital contributed to this thesis. The studies are briefly described in Table 6.1. Trial 1 and 2 were conducted according to the rules of Good Clinical Practice and monitored by the Good Clinical Practice unit at Aalborg and Aarhus University Hospitals, Denmark. All subjects provided informed consent prior to the experiments.

Table 6.1: A brief overview of the trial data used in this thesis.

	Subjects	**Study design**	**Period length**
Trial 1 Paper I and II	HV (n=21)	Cross-over	3 x 14 days
Trial 2 Paper III	HV (n=21) DSPN (n=48)	Cross-sectional	Single day
Trial 3 Paper IV	HV (n=20) IFI (n=20)	Cross-sectional	Single day

1.1 Trial 1

Trial 1 was conducted to try to investigate the effect of different analgesics on pain in humans. The trial was designed to activate the pain system at a detailed level comprising the afferent nerves, the spinal cord, the brain and the

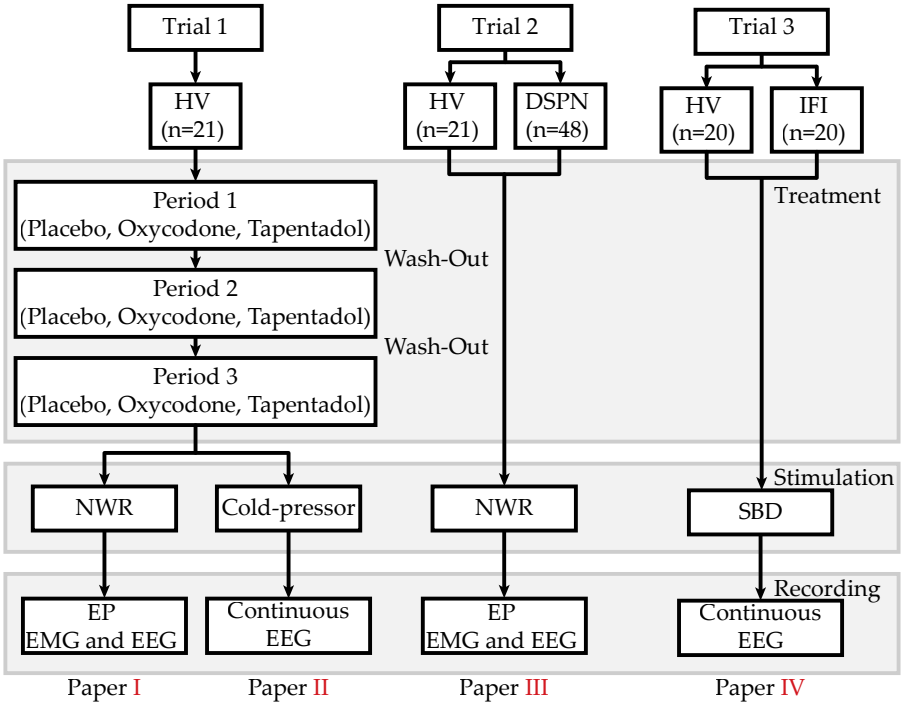

Fig. 6.1: Overview of data used in the current thesis. Trial 1 was a repeated measures cross over trial investigating the effects of opioids on healthy volunteers (HV). Trial 2 and 3 were trials comparing patient populations of diabetic symmetrical polyneuropathy and idiopathic faecal incontinence to healthy volunteers. The stimulations applied were either a nociceptive withdrawal reflex (NWR), a cold-pressor test, or sustained balloon distension (SBD). Recordings were either evoked potentials (EP) or continuous electroencephalography (EEG) and electromyography (EMG)

descending control systems. The trial was registered in the public database EUDRACT (ref 2017-000141-52) and approved by the local ethical committee (N-20170009). The trial included a combination of human experimental pain models, which made it possible to model both spinal and supraspinal activity.
Twenty-one subjects completed the study; the inclusion criteria were: Male, age 20-45 and of Scandinavian descent and opioid naïve. Exclusion criteria were: Known allergy towards pharmaceutical compounds similar to those used in the study, participation in other studies within three months before the first visit, expected need of medical/surgical treatment during the study, history of psychiatric illness, history of persistent or recurring pain conditions, nicotine consumption, daily alcohol consumption, personal or family history of substance abuse, use of any medication including herbal as well as any over-the-counter drugs within 48 hours before the start of the study period, intake of alcohol within 24 hours before the start of the study period, use of prescription medicine, and need to drive a motor vehicle within the treatment periods.
The subjects were treated with tapentadol, oxycodone and placebo for 14 days in a randomized order. Participants were treated with tapentadol extended-release tablets 50 mg (Palexia; Grunenthal GmbH, Aachen, Germany), oxycodone extended-release tablets 10 mg (OxyContin; Mundipharma A/S, Vedbæk, Denmark) and placebo tablets (Hospital Pharmacy Aarhus, Aarhus University Hospital, Aarhus, Denmark) for 14 days. A single tablet was ingested on the morning of days 1 and 14, and two tablets were ingested on days 2-13 (morning and evening). The "wash-out" period between treatments was at least one week. All medication was dispensed by The Hospital Pharmacy Aarhus, Aarhus University Hospital, Aarhus, Denmark.

1.2 Trial 2

The aim of trial 2 was to explore if the drug liraglutide had a neuroprotective effect on people with DSPN. The trial was registered in public databases: EUDRACT (ref 2013-004375-12) and clinicaltrials.gov (ref NCT02138045) and approved by the local ethical committee (N-20130077, N-20090008). In addition, basic pain mechanisms in diabetic neuropathy were investigated. The data used for this thesis was baseline recordings from the trial compared to a healthy age, gender, height, and weight-matched population.
Forty-eight patients with type 1 diabetes were recruited at the Department of Endocrinology, Aalborg University Hospital, Denmark. Potentially eligible patients were prescreened based on a recorded vibration perception threshold above 18 V. DSPN was verified by nerve conduction tests, according to the Toronto criteria [58]. Additional inclusion criteria among others were age above 18 years, a confirmed diagnosis of type 1 DM for a minimum of 2

years, exclusion criteria among others included type 2 DM and other neurologic disorders than DSPN.
Twenty-one age-matched healthy volunteers were included for comparison. Inclusion criteria were age above 18 years and normal peripheral nerve conduction. Exclusion criteria included type 1 and type 2 DM, neurologic disorders, and medication that could alter neuronal function.

1.3 Trial 3

Trial 3 investigated the neural response to rectal and anal stimuli in patients with IFI; the trial was approved by the local ethical committee (N-20090008). Twenty women with IFI were recruited from the Department of Surgery, Aarhus University Hospital. All assessments were performed in the main paper by Haas et al. [65]. All subjects were assessed using the Wexner faecal incontinence score and St Mark's Incontinence Score. Additionally, patients completed a bowel diary three weeks before enrolment, recording urge- and incontinence-episodes, soiling/seepage and use of pads. IFI patients were defined with the Wexner faecal incontinence score of ≥ 9 and/or ≥ 3 faecal incontinence episodes during the 3 weeks. Exclusion criteria were prior colorectal-, pelvic-, spinal-, or brain-surgery; active use of medication known to interfere with gastrointestinal-, hormonal-, or cerebral-function; or an external sphincter defect $> 60°$ when assessed by endoanal ultrasonography. For comparison, 20 age-matched healthy women with no prior history of faecal incontinence were included.

2 Stimulation Methods

2.1 Cold-pressor pain

The cold-pressor pain is a tonic stimulation consisting of submerging the hand in cold water normally ranging between 1 and 7 degrees for an amount of time, e.g. two minutes [70]. The tonic stimulation is transmitted to the brain using the A-beta (sensory) and C-fibers (pain) [71]. Along with, e.g. ischemic muscle pain cold pain is believed to mimic clinical pain well [27]. This is in part due to the length of the stimulation which better mimics chronic pain than a short phasic stimulation [27]. An example of the sensory pathways is shown in Figure 1.1. Cold pain has also been shown to be sensitive to opioid analgesia [8]. In this thesis, objective measures of pain were obtained using EEG. In addition subjects reported subjective measures of pain using a numeric rating scale.

2.2 Sustained balloon distension

Sustained balloon distension was deployed in this thesis using a specially designed inflator device. This device has previously been used as a tool to study cortical processing of visceral sensation and pain using a rapid balloon distension paradigm [65, 72, 73]. As described in 2.1 on the preceding page, tonic stimulations better mimic some chronic diseases due to the length of the stimulation and its unpleasantness compared to a short phasic stimulation [27]. The ability to place an inflatable balloon in the rectum and anal canal enables one to investigate both the visceral sensory system (rectum) and the somatic sensory system (anal canal). The visceral sensory system is sparsely innervated compared to the somatic sensory system. In addition to this, not all of the visceral inputs to the central nervous system are consciously perceived. To stimulate the visceral system, distension of hollow organs activating stretch/tension receptors in the organ wall have been used [74]. An overview of gastrointestinal pain is available in [75]. To our knowledge, the use of a tonic visceral stimulation system in a patient population is novel.

3 Assessment Methods

3.1 Nociceptive withdrawal reflex

The nociceptive withdrawal reflex is a well studied polysynaptic reflex designed to withdraw a limb from potentially damaging stimuli [76]. An example of the sensory pathways is shown in Figure 1.1. The nociceptive signal travels from the site of stimulation to the cortex via A-delta and C-fibers [77]. In recent years it has been possible to objectively assess the presence of single withdrawal reflexes of the peripheral electromyography (EMG) signal [78, 79] These measures have also been proven to be reliable over time [80]. To objectively measure a nociceptive withdrawal reflex different scoring criteria have been investigated. Of these the interval peak z-score: $\frac{NWR_{\text{peak}} - baseline_{\text{mean}}}{baseline_{\text{standard deviation}}}$ and the mean interval z score: $\frac{NWR_{\text{interval mean}} - baseline_{\text{mean}}}{baseline_{\text{standard deviation}}}$ were found to be the most accurate [78]. The nociceptive withdrawal reflex has been used to test pain in many patient populations, among them painful diabetic neuropathy [81].

3.2 Questionnaires

The Visual Analogue Scale (VAS) or Numerical Rating Scales (NRS) were used in papers I-IV. In all cases they were used to gauge the immediately perceived sensation or pain of the experimental pain model. The use of VAS

and NRS in chronic pain is not reliable [82], it does however have high reliability in the assessment of acute pain measurement [83]. The VAS and NRS was used to obtain a subjective measure of pain along with the objective electrophysiology measures used in in each paper. The numerical rating scale is visualised in Figure 6.2.

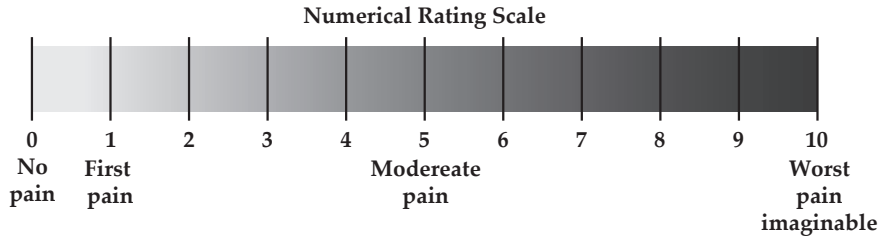

Fig. 6.2: The numeric rating scale. Displayed are the guides the subjects were given to assess the sensation.

Chapter 7

Key results

1 Aim 1

Aim: To investigate the cortical and spinal changes on a healthy control population receiving a treatment of oxycodone and tapentadol using the nociceptive withdrawal reflex.

Paper I: Using the nociceptive withdrawal reflex, we were able to identify a decrease in the number of reflexes observed of (p = 0.001; [95% CI: -1.46, -0.32]) in the tapentadol (MOR agonist and NRI) treatment. No other differences were observed in the peripheral EMG measures (latency and area under the curve of the reflex).
Cortically, there was a decrease of the N1 component of the sensory evoked potential (p = 0.003; [95% CI: 3.37, 21.69]) during oxycodone (MOR) treatment.
Applying inverse modeling to the sensory evoked potentials revealed a caudal movement of the anterior cingulate cortex of all treatment arms (placebo: p = 0.012; [95% CI: -23.10, -2.10], oxycodone: p < 0.001; [95% CI: -36.32, -15.24], tapentadol: p = 0.001; [95% CI: -26.58, -5.48]). The dipole placed in the insula region also moved caudally, but only during tapentadol (p = 0.001; [95% CI: -10.88, -2.17]) and oxycodone (p = 0.022; [95% CI: -9.20, -0.51]) treatments.
The subjective measures of sensation and pain perception did not change between baseline and treatment.

Interpretation: Only tapentadol induced changes in the spinal component of the central nervous system. Both oxycodone and tapentadol affected cortical measures. The anterior cingulate cortex and insula are part of a brain system involved in the processing of sensory stimuli, this system is not only

pain-specific [84]. The insula component only changed in the active treatment groups with opioids suggests that this is a drug-related effect driven by the mu-opioid receptor agonist.

2 Aim 2

Aim: To investigate the cortical processing of a tonic pain stimulation using the cold pressor test on a healthy control population receiving a treatment of oxycodone and tapentadol.

Paper II: Both active treatments changed the pain perception of submerging the subjects hand in chilled circulated water at day 4 after treatment; oxycodone ($p = 0.006$; [95% CI: -1.13, -0.16]) and tapentadol ($p = 0.039$; [95% CI: -1.12, -0.02]). There were no differences between days in the placebo arm. This change persisted for the oxycodone treatment ($p = 0.039$; [95% CI: -1.12, -0.02]) at day 14, but not for the tapentadol treatment.
Both of the active treatments changed the spectral power of the cortex when submerging the hand in chilled water. Oxycodone differed from placebo in the delta ($p < 0.01$; [95% CI: -3.83, -1.46]), theta ($p = 0.03$; [95% CI: -1.35, -0.72]), alpha1 1.47 ($p < 0.01$; [95% CI: 1.1, 1.8]), alpha2 ($p < 0.01$; [95% CI: 0.62, 1.29]) and beta1 ($p = 0.025$; [95% CI: 0.07, 0.94]) bands. Tapentadol increased compared to placebo in the alpha1 band 0.62 ($p < 0.001$; [95% CI: 0.26, 0.98]).
Cortical sources were investigated using sLORETA inverse modelling. Oxycodone was different from placebo in the temporal and limbic area in the delta band and the frontal region in the beta1 frequency band. Tapentadol differed from placebo in the temporal lobe close to the insula in the alpha2 band.

Interpretation: Oxycodone appears to have a stronger cortical effect than tapentadol. This is likely due to the dual-acting effects of tapentadol affecting the limbic system, which is visible in the inverse modelling.

3 Aim 3

Aim: To compare the processing of spinal stimuli between patients with DSPN and healthy controls using the nociceptive withdrawal reflex.

Paper III: People with length-dependent DSPN when compared to healthy controls had a higher perception threshold: 5 mA; [range: 2 - 40] vs. 3 mA; [range: 2 - 6], ($p = 0.001$) and reflex threshold: 22 mA; [range: 5 – 50]

vs. 15 mA; [range: 6 – 37], (p = 0.012). The ability to elicit the nociceptive withdrawal reflex was reduced for people with length-dependent DSPN by 0.045, (p=0.014; [95% CI: 0.004–0.54]). When it was possible to elicit the withdrawal reflex there were no differences in latency and area under the curve of the reflex. Cortically, no differences were observed at the Oz electrode, located at the base of the skull. At the vertex, the Cz electrode revealed length-dependent DSPN increased the latency of the N1 peak 115.1 ms, (p = 0.013) and decreased the P1-N1 amplitude 23.72 mV, (p = 0.021) compared to healthy controls. The disease duration did not correlate with the latency of the P1 peak or P1-N1 amplitude.

Interpretation: These findings indicate that diabetes length-dependent DSPN affects both the spinal and cortical central nervous system. In addition, the fact that there is a significant difference in the odds ratio of eliciting a reflex between healthy and patients could potentially be used as a screening tool for small fibre neuropathy, which is not measured using conventional nerve conduction techniques. No correlation between the selected cortical measures and disease duration suggest that the disease duration is not the main reason for length-dependent DSPN.

4 Aim 4

Aim: To compare the cortical processing of tonic ano-rectal distensions in patients with idiopathic faecal incontinence and healthy controls

Paper IV: It was possible to record changes in the cortical processing while distending the anal canal and rectum, but there were no differences in the EEG response between the patients and controls.

Interpretation: The above finding could suggest that a sustained ano-rectal distension results in different activation of afferent nerves compared to a rapid balloon distension which has previously proven to have a different cortical response when patients with IFI were compared with controls [65].

Chapter 8

Discussion

The overall objective of this thesis was to investigate changes in the cortical and spinal components of the central nervous system due to changes in sensory function either due to medication or disease. The nociceptive withdrawal reflex and tonic stimulations were used in healthy volunteers and two patient populations. The discussion contains methodological considerations, experimental settings, clinical implications, and future perspectives of the current thesis.

1 Methodological considerations

1.1 The nociceptive withdrawal reflex

The use of the nociceptive withdrawal reflex allows for analysis of both the spinal and cortical parts of the central nervous system. The nociceptive withdrawal reflex is a polysynaptic reflex involving mainly A-delta and C fibres [85]. Combining an evaluation of the peripheral motor response, mediated through the spinal reflex using EMG and the interval peak z-score and the central response using EEG allows for granular analysis of the effects of sensory dysfunction. The peak interval z-score has been suggested as an objective measure of the nociceptive withdrawal reflex [78, 79]. The use of the interval z-score has made it possible to quantify the number of reflexes as a result of stimulations for each subject. Sensory evoked potentials have been used previously to investigate the effects of DM [86, 87] and different drugs [88, 89].

Spinal effects

In paper I, the nociceptive withdrawal reflex analysis revealed a reduction in the number of observed reflexes in the tapentadol arm of treatment. This

finding supports the dual-acting effects of tapentadol in contrast to oxycodone. In addition to affecting the ascending pathway by manipulating the mu-opioid receptor, tapentadol also affects the descending pathway by inhibiting noradrenaline reuptake which increases available noradrenaline [48]. A schematic the tapentadol and oxycodone is available in Figure 3.1.
In Paper III the same approach was applied in the patient population with DSPN and revealed a reduction in the odds ratio of eliciting a nociceptive withdrawal reflex. In the patient population, the effects of neuropathy increased the perception and reflex threshold resulting in some patients having a reflex threshold of at least 50 mA. Due to safety limitations of the electrical stimulator that used it would not be able to deliver the currents needed for the stimulations above the reflex threshold. In the case of participants exceeding the 50 mA threshold or experienced the stimulation pain/unpleasantness to be unbearable the experiment was not completed. No healthy participants exceeded the 50 mA threshold or experienced intolerable pain or unpleasantness. Of the patient participants, 29% did not finish the study. In the participants who were able to complete the stimulations, there were no differences in latency and area under the curve of the reflex. This highlights the differences between the patient population and healthy controls. Comparing the number of reflexes between patients and healthy controls patients had fewer reflexes. Comparing the characteristics of reflexes recorded (latency and area under the curve) between patients and healthy controls revealed no differences between groups. This indicates that while it was more difficult to elicit the nociceptive withdrawal reflex in the patient population, there are no differences in the response once the reflex is elicited.

Cortical effects

In paper I the only observed change was a decrease in the latency of the N1 component during oxycodone treatment. This is either due to a jitter effect of opioids [90] or an actual effect of the stronger centrally acting oxycodone. In addition to changes to the evoked potentials inverse modelling was investigated. The anterior cingulate and insula components changed significantly after intervention. These brain regions are involved in the sensory processing of the intensity of stimuli [84], where the anterior cingulate is also involved in the affective pain response [91]. The rostral anterior cingulate cortex along with the brainstem has been shown to be linked to a placebo response as well [92]. The insula component only changed during the active treatments, which indicates an opioid effect.
The diabetes patients in paper III displayed a prolonged latency and amplitude of the N1 component of the Cz electrode. There were no differences in early evoked potentials recorded at the Oz electrode close to the brainstem. This suggests that the changes observed between the signal entered the brainstem and reaching the Cz electrode result from differences in cortical

processing of later cortical signals.

1.2 Tonic stimulations

Two different tonic stimulations were used in the current studies. Generally, continuous recordings of electrical brain activity using EEG in combination with a tonic pain stimulation have been used to demonstrate changes in the central nervous system [93]. Tonic stimulations are believed to better mimic clinical pain than short phasic stimuli and are sensitive to treatment with, e.g. opioids [94, 95].
In paper II the cold pressor test was used to investigate differences in the central processing of healthy volunteers when administering oxycodone and tapentadol. There was a decrease in pain perception on day 4 for both active treatments, which persisted to day 14 for oxycodone, but not tapentadol. The cold pressor test has previously been shown to be a good tool to assess opioid analgesia [9]. The tapentadol treatment did not significantly change the analgesic effect on day 14, possibly due to too low a number of subjects and thus a type 2 error. In the spectral analysis of cortical measures, there was a difference in the baseline recordings before correcting for multiple comparisons. The cold pressor test has previously been proven to be reproducible [93]. Thus this change between baselines is believed to be a type 1 error. Differences were observed in the delta, theta, alpha1, alpha2, and beta1 bands during oxycodone treatment and a change in the alpha1 band in the tapentadol treatment. The change observed in the delta band in the oxycodone treatment has previously been found to correlate with unpleasantness and increase between a resting state recording, and the cold pressor test [93, 96]. This suggests the decrease in the delta band could reflect the decreased perception of pain. This is supported by the reduction in theta band activity that previously was shown to correlate to the subjective pain score [93]. The changes in the alpha frequency of both oxycodone and tapentadol are believed to be an adaptive response to pain [85] and as such differentiated the effect of the two drugs on the resting EEG during tonic pain compared to the placebo arm. The inverse model analysis using sLORETA revealed increases in the delta, alpha2 and beta1 bands in the temporal lobe, limbic structures and frontal lobes between oxycodone and placebo. Tapentadol treatment only resulted in an increase in the alpha1 band in the temporal lobe compared to placebo. Overall this may reflect a weaker effect of the MOR agonist in tapentadol compared to oxycodone.

The use of rapidly inflated balloon distension has previously been used to investigate cortically evoked potentials to balloon distension in a healthy population and people with IFI [65, 72, 73]. In paper IV no differences were observed in the cortical processing of people with IFI compared to healthy

volunteers. This indicates that while IFI changes are observable using rapid balloon distensions, there were no differences using a tonic mechanical stimulation. The tonic stimulation could potentially last long enough for the body to accommodate the change in volume. Thus the findings indicate that this part of the regulatory system in people with IFI is likely intact.

2 Experimental settings

2.1 Pharmacological treatment

The opioid doses in trial 1 were chosen to be a drug dose high enough to induce neurological changes but still be ethically justifiable. The doses of 10 mg oxycodone and 50 mg tapentadol were deemed to be equipotent [48]. Previous studies have investigated oxycodone and venlafaxine (serotonin and norepinephrine reuptake inhibitor) for five days in similar experimental designs [89, 96]. The choice to measure the effects over 14 days was to allow for the noradrenaline reuptake inhibition of tapentadol to take full effect.

2.2 Experimental models in patient populations

The choice to include people with confirmed DSPN in trial 2 underlined the differences between this population and healthy controls. However, some of the people in the study were so affected by neuropathy that it was not possible to record a withdrawal reflex. Using a method of defining the stimulations applied by the individual subjects reflex threshold displayed the fact that if a reflex is elicited the resulting response does not differ between groups. The current needed and the reflexes observed however differed between groups.
In the experimental paradigm in trial 3, the balloon used to create the sensory stimuli was inflated to a level equivalent to the sensation when needing to defecate for the individual subject. This level was then kept throughout the stimulation. The rectum and anal canal are able to accommodate a volume resulting in a loss of sensation over time for some of the participants in the trial. In future studies monitoring the sensation throughout the stimulation could help track when the accommodation occurs. Another approach could be to increase the balloon volume in accordance with the accommodation, this could potentially result in a dangerous distension of the tissue and was consequently not used in trial 3.

3 Clinical implications

Preclinical and clinical studies have shown a difference between tapentadol and traditional opioids like oxycodone [48]. These results have been disputed due to confounding factors relating to species differences and confounders in clinical settings [45]. This thesis has helped confirm that the two opioids exert different effects on the brain's pain processing. These findings support that tapentadol's dual properties, which impact the opioidergic and noradrenergic systems, differ from classical opioids on the brain and the spinal systems processing. Although the results in this thesis cannot be translated directly to patients with chronic pain, they support that opioid rotation may be beneficial in some cases.
The use of the nociceptive withdrawal reflex can enable the diagnosis of small fibre neuropathy since the traditional evaluations rely on the thickly myelinated A-beta fibres. In contrast, the nociceptive withdrawal reflex is transmitted by the A-delta and C fibres that are affected by small fibre neuropathy. Additionally, a decrease in the latency and amplitude at the Cz electrode was observed in patients, while no differences were observed at the Oz level adjacent to the brainstem. This suggests a change in cortical processing for people with DSPN. There was no correlation between disease duration and latency of the first peak of the evoked potential; this tells us that the duration of diabetes alone does not predict the development of neuropathy. Other factors such as: age, body mass index, smoking, hypertension, and hyperglycaemia also increase the risk [97–99].

4 Future perspectives

Future studies investigating the effects of opioids should have females included since chronic pain is more prevalent in females than males, and studies suggest women have a lower sensitivity to analgesia than men [100, 101]. In addition to the inverse modeling, that has been used in paper I and II it is possible to investigate communication between areas of the brain, one method of doing this is the phase lag index and weighted phase lag index. In addition to the EEG source generators from inverse modeling phase lag index investigates the functional connectivity of areas of the brain. Phase lag index has been used in the past to detect pain presence [102]. It would be a new insight on how opioids change the processing of painful stimuli.
We were able to detect differences between healthy volunteers and people with DSPN underlining the effects of neuropathy on the central nervous system. A number of projects that focus on the implementation of EEG measures in clinical settings are underway. An effort to use resting EEG and the cold-pressor pain stimulation to further understand the effects of neurolog-

ical disease on the brain and see if it is possible to use measured changes and use them as a screening tool is being developed. In order for this to be implemented in a meaningful way clinically the complexity and preparation time needs to be minimised. One way of doing this is to reduce the number of electrodes. Methods measuring the coherence between areas of the brain such as phase lag index along with statistical methods such as machine learning and artificial intelligence are being tested to improve the electrode locations of fewer, but well placed electrode. In addition to EEG a number of other experimental pain models are being included among these is quantitative sensory testing used to test somatosensory function. A collaboration with Haukeland University Hospital in Bergen is investigating differences between people with diabetes and healthy controls using a rapid balloon distension of the rectum as a stimulation method.

Chapter 9

Conclusion

This PhD thesis had four aims investigated in three trials. It was possible to record cortical changes in a healthy volunteer group using the nociceptive withdrawal reflex and the cold-pressor test. Overall oxycodone had a greater effect on the cortical system, but tapentadol also affected the peripheral nervous system (Aims 1 and 2). Using the nociceptive withdrawal reflex differences were found between people with DSPN and healthy controls. The neuropathy changed the number of reflexes observed and the latency and amplitude recorded at the vertex (Aim 3). Lastly there were no cortically recorded differences between people with idiopathic fecal incontinence and healthy controls using a sustained balloon distension (Aim 4).

All in all EEG and EMG were sensitive enough to detect changes in various experimental pain models. The mechanisms behind chronic disease are complex, and knowledge obtained from the combination of multiple experimental pain models will likely contribute to an expanded understanding of these mechanisms and hopefully help in the detection and diagnosis of complications related to chronic disease.

Chapter 10

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