The Brain's Response to Pain and Morphine

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THE BRAIN'S RESPONSE TO PAIN AND MORPHINE

– A STUDY BASED ON EEG AND MRI

BY
TINE MARIA HANSEN

DISSERTATION SUBMITTED 2015

AALBORG UNIVERSITY
DENMARK

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Tine Maria Hansen

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II. Hansen TM, Graversen C, Frøkjaer JB, Olesen AE, Valeriani M, Drewes AM. Single-sweep spectral analysis of contact heat evoked potentials: A novel approach to identify altered cortical processing after morphine treatment. Br J Clin Pharmacol. 2014. Dec 31. doi: 10.1111/bcp.12579. [Epub ahead of print]

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ABBREVIATIONS

ACC	Anterior cingulate cortex
ASL	Arterial spin labeling
BOLD	Blood oxygen level-dependent
CHEPS	Contact Heat Evoked Potential Stimulator
CNS	Central nervous system
CP	Chronic pancreatitis
DTI	Diffusion tensor imaging
EEG	Electroencephalography
EP	Evoked potential
fMRI	Functional magnetic resonance imaging
HV	Healthy volunteers
IC	Insula cortex
ISI	Inter-stimulus interval
PET	Positon emission tomography
PFC	Prefrontal cortex
SPECT	Single photon emission computed tomography
MEG	Magnetoencephalography
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
VAS	Visual analogue scale

ENGLISH SUMMARY

Approximately 20% of the population in Europe suffers from chronic pain and this is often treated with opioids or other analgesic drugs. The effect of opioids is individual and some people do not respond adequately to treatment and therefore pain management is often unsuccessful. More knowledge about the pain processing and response to opioids would therefore be beneficial to understand pain and its management.

The objectives of this PhD thesis were to investigate the pain response to skin heat stimulation using a Contact Heat Evoked Potential Stimulator (CHEPS) in patients suffering from painful chronic pancreatitis and healthy volunteers, and to investigate morphine modulation of the pain response in healthy volunteers.

Data were collected from two experimental studies. In study I, central pain processing and habituation to CHEPS stimulations in chronic pancreatitis patients and in healthy volunteers were assessed using electroencephalography (EEG). In study II, the pain response was further investigated in healthy volunteers using EEG and magnetic resonance imaging (MRI) obtained by the blood oxygen level-dependent (BOLD) signal and brain metabolites (spectroscopy). Morphine-induced analgesia was further assessed by these three neurophysiological measurement methods.

The main findings were increased amplitudes of the EEG N2/P2 complex during repeated stimuli for chronic pancreatitis patients. This indicates impaired habituation as a part of neuroplastic/neuropathic brain changes in chronic pain. On the other hand, expected habituation (decreased amplitudes) was observed for healthy volunteers. In healthy volunteers the BOLD signal revealed pain-induced activation in the anterior cingulate cortex, secondary somatosensory cortex/insula cortex, thalamus and cerebellum. Pain stimulation induced an increase in the brain metabolite N-acetylaspartate/creatine ratio in anterior cingulate cortex. Following morphine treatment, low frequency oscillations in the EEG decreased, whereas high frequency oscillations increased. Morphine reduced pain-induced BOLD activation in the insula cortex, anterior cingulate cortex and inferior parietal cortex and the brain metabolite concentrations of glutamate/creatine, myoinositol/creatine and N-acetylaspartate/creatine ratios decreased in anterior cingulate cortex.

In conclusion CHEPS-induced changes in pain responses before and after treatment with morphine were detectable by EEG, the BOLD signal and spectroscopy. Despite limitations of the designs, the presented modalities were useful to investigate mechanisms of pain and analgesics. Knowledge from more modalities may enhance our understanding of the complex mechanisms in chronic pain and plays an important role in development of new drugs and optimisation of treatment strategies of chronic pain.

DANSK RESUME

Omkring 20% af befolkningen oplever kroniske smerter, og disse bliver ofte behandlet med stærkt smertestillende morfinlignende stoffer (opioider). Den smertelindrende effekt af opioider er meget individuel, og en del personer har ingen eller kun begrænset effekt af opioider. Derfor er smertebehandling ofte utilstrækkelig. Mere viden om smertesystemets funktion vil være gavnlig for at kunne optimere og individualisere smertebehandlingen hos den enkelte patient.

Formålene med dette ph.d.-projekt var at undersøge smteresponset på stimulering med varme på huden ved brug af en kontakttermode (kaldet CHEPS) hos patienter med kronisk bugspytkirtelbetændelse og hos raske forsøgspersoner, samt at undersøge morfineffekten på smteresponset hos raske forsøgspersoner.

Data fra to eksperimentelle studier blev inkluderet i dette projekt. Den centrale smerteprocessering og habituering udløst af CHEPS stimuli blev i studie I undersøgt med elektroencephalografi (EEG) hos patienter med kronisk bugspytkirtelbetændelse og raske forsøgspersoner. I studie II blev smteresponset yderligere undersøgt både med EEG og magnetisk resonans billeddannelse (MRI), hvor blood oxygen level-dependent (BOLD) responset, og metabolitkoncentrationer i hjernen (spektroskopi) blev målt. Morfins smertestillende effekt blev ligeledes undersøgt med disse tre neurofysiologiske målemetoder.

Hovedresultaterne viste en øgning i amplituderne af EEG N2/P2 komplekset ved gentagne stimuli hos patienter med kronisk bugspytkirtelbetændelse, hvilket indikerer nedsat habituering som et udtryk for neuroplastiske/neuropatiske ændringer i hjernen. Som forventet faldt amplituderne (intakt habituering) af EEG N2/P2 komplekset hos raske forsøgspersoner ved gentagne stimuli. Hos raske forsøgspersoner viste BOLD responset ved smertestimulering aktivering i anterior cingulate cortex, sekundær somatosensory cortex/insula, thalamus og cerebellum. Smertestimulering viste desuden øgning i N-acetylaspartat/kreatin ratio i anterior cingulate cortex. Morfinbehandling nedsatte lavfrekvent aktivitet og øgede højfrekvent aktivitet i hjernen målt med EEG, reducerede det smerteinduceret BOLD respons i insula cortex, anterior cingulate cortex and inferior parietal cortex, og et fald i metabolit ratioer blev fundet for glutamat/kreatin, myoinositol/kreatin og N-acetylaspartat/kreatin i anterior cingulate cortex.

Det var således muligt at måle CHEPS-inducerede ændringer af smteresponset ved brug af EEG og MRI ved at måle BOLD responset og metabolitkoncentrationer i hjernen før og efter behandling med morfin. Trods metodemæssige begrænsninger er de præsenterede metoder brugbare til at undersøge mekanismerne bag smerte og smertestillende behandlinger. Kombination af information fra flere målemetoder er vigtig for at øge vores viden om smertenetværkets komplekse funktion, hvilket i fremtiden kan spille en vigtig rolle i udviklingen og valideringen af nye typer smertebehandling.

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CHAPTER 1. INTRODUCTION

Pain is defined by The International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey, 1994). Acute pain arises suddenly and is usually treatable, whereas chronic pain persists over time and treatment can be challenging. Pain lasting for periods longer than three month is often defined as chronic pain. Chronic pain is a major problem (Breivik, Collett, Ventafridda, Cohen, & Gallacher, 2006; Trescot, Helm, et al., 2008) and 19% of the population in Europe suffers from chronic pain (Breivik et al., 2006). Chronic pain has not only a health, social and economic impact on individuals, but is also an enormous cost for the society (Breivik et al., 2006; Eriksen, Jensen, Sjøgren, Ekholm, & Rasmussen, 2003). Chronic pain is often controlled by using opioids or other strong analgesic drugs. In Denmark, 12% of persons suffering from chronic pain use opioids (Eriksen et al., 2003). Genetic factors influence on opioid efficacy, metabolism and adverse effects (Tremblay & Hamet, 2010) and Maier et al. reported that 29% of persons with chronic non-malignant pain were non-responders to morphine (Maier et al., 2002). Due to the complexity of pain and the individual analgesic effect of opioids, the pain management is often inadequate. To identify abnormal pain processing and obtain better pain management, identification of objective biomarkers of the individual analgesic effects are highly warranted (Woolf & Max, 2001; Woolf, 2011). Studies have shown that the pain processing in the brain is altered due to opioids, but the opioidergic pathways are inadequately explored (Balasubramanian, Morley-Forster, & Bureau, 2006; Sprenger, Berthele, Platzer, Boecker, & Tölle, 2005) and further investigations of the pathways from the periphery to the brain response to pain and its treatment are highly warranted.

1.1. PAIN PROCESSING

A complex network of neurons is involved in pain processing. Pain information is transmitted from the periphery via primary afferent fibres to the central nervous system (CNS). The following sections describe the pain system in a simplified way to give an overview of the very complex system from the stimulus is transmitted from the periphery to the spinal cord and the supraspinal levels (see Figure 1).

1.1.1. THE PAIN SYSTEM

Sensory receptors (primary afferent fibres) in the periphery, which detect potentially dangerous signals, are called nociceptors. Nociceptors are peripheral free nerve endings and provide information about the stimulus intensity and location (Zhu & Lu, 2010). Nociceptive pain is divided into somatic and visceral pain, where somatic pain originates from skin, muscle or bone damage and visceral pain originates from internal organs (A. E. Olesen, Andresen, Staahl, & Drewes, 2012). The focus in this thesis will be on somatic pain. Primary afferent fibres are divided into different types of fibres: Aβ-fibres, Aδ-fibres and C-fibres. Aβ-fibres have a large diameter and conduct signals (action potentials) quickly as Aβ-fibres are highly myelinated. Aδ-fibres are smaller in diameter, thinly myelinated and conduct signals slower than Aβ-fibres. C-fibres are smallest in diameter and unmyelinated, thus slowly conducting fibres. (D'Mello & Dickenson, 2008) Most nociceptors are C-fibres or Aδ-fibres (Zhu & Lu, 2010). Nociceptors can be specific responding to mechanical, thermal or chemical stimuli. Nociceptors which respond to more modalities are called polymodal and nociceptors which do not respond to any of the modalities are called silent. The silent nociceptors cannot be activated in general but only in connection with pathological conditions. (Zhu & Lu, 2010) A thermal painful stimulus in hairy skin of the arm leads to a double pain sensation; a pricking pain ("first pain") followed by a burning sensation ("second pain"). The "first pain" is mediated by the myelinated Aδ-fibres, thus conducted faster than the "second pain", which is mediated by the unmyelinated C-fibres.

The primary afferent fibres terminate at the dorsal horn in the spinal cord and project to secondary neurons. The dorsal horn is organised into different physiologically distinct layers (laminae I-VI). Nociceptive Aδ-fibres and C-fibres mostly terminate in the superficial layer of the dorsal horn (laminae I-II) and the deeper laminae are mainly supplied by a smaller number of Aδ-fibres and C-fibres. Aβ-fibres mostly terminate laminae III-VI. (Craig, 2003; D'Mello & Dickenson, 2008) Secondary neurons can be nociceptive specific or Wide Dynamic Range neurons. The nociceptive specific neurons respond only to nociceptive stimulation mediated by Aδ-fibres and C-fibres. The Wide Dynamic Range neurons respond both to innocuous and nociceptive stimulation mediated by Aβ-fibres, Aδ-fibres and C-fibres. These neurons are dynamic and respond to the stimulus gradually. (Marchand, 2008) Neurotransmitters transmit signals between neurons. Glutamate is an important neurotransmitter (among others) at all levels of the nervous system (D'Mello & Dickenson, 2008). Signals from secondary neurons are mainly transmitted via two different pathways to the thalamus; the spinothalamic (lateral) tract transmitting signals to the lateral nuclei of the thalamus and the spinorecticular (medial) tract transmitting signals to the medial nuclei of thalamus and the brainstem (to e.g., nucleus raphes magnus and periaqueductal grey). Both tracts cross immediately in the spinal cord. (Marchand, 2008)

When the signals reach the supraspinal level many cortical regions are involved in the perception of pain. The activated regions depend on the particular stimulus (D'Mello & Dickenson, 2008). However, the most commonly activated cortical regions include the primary somatosensory cortex (SI), secondary somatosensory cortex (SII), insula cortex (IC), anterior cingulate cortex (ACC) and prefrontal cortex (PFC) (Apkarian, Bushnell, Treede, & Zubieta, 2005). These regions are commonly referred to as the "pain matrix" (Fomberstein, Qadri, & Ramani, 2013). The different cortical regions play different roles in the pain processing. SI is associated with the intensity of the stimulus and SII plays a role in coding the intensity of the stimulus (Bornhövd et al., 2002). IC also plays a role in pain intensity (Bornhövd et al., 2002; Coghill, Sang, Maisog, & Iadarola, 1999) and it can be difficult to dissociate IC from SII (Peyron et al., 2002). ACC has a connection with the emotional content of the stimulus and involved in coding of stimulus perception (Bornhövd et al., 2002). PFC plays a role in directing attention towards the stimulus and has a connection to the working memory processing (Bornhövd et al., 2002). However, due to plasticity, modulation of nociceptive signals is possible at all levels of the CNS (Marchand, 2008).

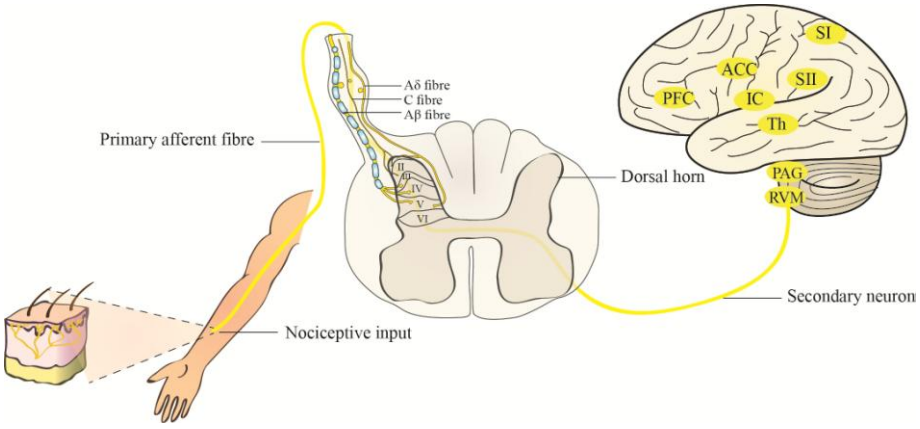

Figure 1: The somatic sensory system. The nociceptive stimulus is transmitted from the periphery (in this example, the skin) via primary afferent fibres, which terminates at the dorsal horn (organised into different physiologically distinct layers I-IV) in the spinal cord. The signal is then transmitted to secondary neurons and reaches the supraspinal level, where many cortical regions are involved in pain processing. PFC: prefrontal cortex, ACC: anterior cingulate cortex; IC: insula cortex; SI: primary somatosensory cortex; SII: secondary somatosensory cortex; Th: thalamus; PAG: periaqueductal grey; RVM: rostral ventromedial medulla.

1.1.2. CHRONIC PAIN

Pain processing is altered in chronic pain disorders and often involves changes in the nervous system at different levels. Detailed description of the complex pain mechanisms behind chronification and chronic pain are beyond the scope for this thesis. Sensitisation of the nervous system plays a role in chronic pain disorders. Chronic pain disorders can lead to structural, functional and metabolic changes of the CNS (Borsook, 2012; Henry, Chiodo, & Yang, 2011). When the CNS is sensitised (central sensitisation), the pain response will be amplified, which leads to more pain (hyperalgesia). Studies have shown alterations of the CNS and central sensitisation in chronic pain disorders (Woolf, 2011). In this thesis painful chronic pancreatitis will serve as a model (prototype disease) for chronic pain (even though the initiating pain is visceral of origin).

Chronic pancreatitis

Chronic pancreatitis (CP) is a major source of morbidity in the Western world. The incidence of CP is approximately 10 per 100,000 inhabitants (Andersen, Pedersen, Scheel, & Worning, 1982). Chronic abdominal pain is the most common symptom and CP is characterised by progressive destruction of the pancreas tissue with significant impairment of exocrine and endocrine functions (Lieb & Forsmark, 2009). Thus, the origin of pain is considered to be visceral. However, studies have shown CNS alterations to play a key role in CP (Frøkjær, Olesen, et al., 2011; Frøkjær et al., 2012; Lelic, Olesen, Hansen, Valeriani, & Drewes, 2014). Pain management in CP is difficult and it is associated with in impaired psychosocial functioning, physical disability, decreased quality of life, hospitalisation and is costly for the society (Pasricha, 2012). Opioids are often used in treatment of CP but limited effectiveness and undesirable side-effects are common (Paisley & Kinsella, 2014). Hence, optimised pain management is highly desirable and more knowledge would be beneficial to enhance the understanding of the underlying pain mechanisms.

1.2. PAIN MANAGEMENT

Modulation of the nociceptive signal with the aim to reduce the perception of pain can be reached using analgesics. The strategy for analgesic treatment normally follows the "pain relief ladder" provided by the World Health Organization (WHO) initially formulated for pain relief in cancer patients, where the potency of analgesic drugs (non-opioids, weak-opioids and strong opioids) are titrated increasingly until pain relief is obtained (WHO, 1996). Opioids are used to control moderate to severe

acute pain and chronic pain. Opioids mainly act on the CNS, however, opioid receptors are also located in the periphery (Stein et al., 2009; Trescot, Datta, Lee, & Hansen, 2008). The term "opioid" is used to describe compounds that activate opioid receptors (Trescot, Datta, et al., 2008). Opioid receptors can be activated by endogenous opioids or exogenous administrated opioids. Endogenous opioids are naturally occurring substances (dynorphins, enkephalins, endorphins) (Gutstein & Akil, 2006) and this will not be further described. Morphine is the "gold standard" exogenous opioid (Lugo & Kern, 2002) and is described in section 1.2.2. Opioid receptors are subdivided into μ-receptors, δ-receptors, κ-receptors and the opioid receptor like-1 (ORL_1) (Corbett, Henderson, McKnight, & Paterson, 2006; Gutstein & Akil, 2006). Most clinical relevant opioids (e.g. morphine) exert the main effect on the μ–receptors, thus, the distribution of this receptor type will be in focus in the next sections describing this type of opioid receptors at the peripheral, spinal and supraspinal levels.

1.2.1. OPIOID RECEPTORS

Peripherally, opioid receptors are located at peripheral sensory nerve terminals. Opioid peptides or exogenous opioids bind to opioid receptors and this leads to analgesia. (Stein, Schäfer, & Machelska, 2003) Spinally, opioid receptors are localised at the presynaptic and post synaptic sites in the spinal cord dorsal horn (Inturrisi, 2002). The superficial laminae I (around the termination of C-fibres) and substantia gelatinosa of the dorsal horn mostly contain the highest concentrations of opioid receptors and receptors are predominantly located presynaptic on the central terminals of the primary afferents but also postsynaptic at the secondary neurons and on interneurons. The neurotransmitter release of glutamate (and other neurotransmitters) is blocked when opioid receptors are activated, thus leading to analgesia (Trescot, Datta, et al., 2008), but other mechanisms are also involved in the hyperpolarisation of the neurons. Supraspinally, opioid receptors are found in the brain stem, thalamus and cortex (Inturrisi, 2002). The periaqueductal grey and rostral ventromedial medulla (both opioid-rich regions) are involved in descending control of the nociceptive signal as the periaqueductal grey transmits the nociceptive signal to the rostral ventromedial medulla and opioids inhibit the nociceptive signal transmitted to the dorsal horn laminae. (Heinricher, Tavares, Leith, & Lumb, 2009) Imaging studies have shown especially ACC, IC, PFC and thalamus to be opioid-rich regions (Apkarian et al., 2005; Firestone et al., 1996; Jones et al., 1991; Petrovic, Kalso, Petersson, & Ingvar, 2002; Zubieta et al., 2005). These areas are as mentioned in section 1.1.1 also a part of the "pain matrix".

1.2.2. MORPHINE

Morphine is a widely used opioid to treat acute and chronic pain. Morphine is a naturally occurring compound found in the opium poppy plant (Papaver somniferum) and first isolated in 1804. Morphine acts on different levels of the nervous system, such as the periphery, spinal cord and brain regions and exerts its main effect on the μ-receptors (Inturrisi, 2002). Administration of morphine includes oral, rectal, subcutaneous, intravenous, epidural and intrathecal routes. However, due to simplicity, convenience and economy oral administration is preferred (Donnelly, Davis, Walsh, & Naughton, 2014). Morphine passes the blood-brain-barrier (which is the interface between the blood and the brain) slowly and only approximately 40 to 50 percent of an oral administered dose of morphine reaches the CNS, within 30 minutes to 90 minutes (Trescot, Datta, et al., 2008). The elimination half-life is approximately 2 hours (Trescot, Datta, et al., 2008). Morphine is mainly metabolised into morphine-6-glucoronide (M6G) (10-15%) and morphine-3-glucuronide (M3G) (45-55%). M6G has a certain analgesic effect, whereas it is now believed that M3G is inactive such as the other metabolites (Christrup, 1997; Trescot, Datta, et al., 2008). Although morphine is widely used in the clinic, around 1/3 patients with chronic non-malignant pain are defined as non-responders to morphine and side-effects are common (Maier et al., 2002). Opioids affect mood, rewarding behavior, the respiration system, cardiovascular system, neuroendocrine system and gastrointestinal function (Gutstein & Akil, 2006). The most common side-effects are sedation, constipation and nausea (Brock et al., 2012). Knowledge about morphine modulation of central pain processing of the nociceptive input may provide further insight into the multiple complex mechanisms (Woolf & Max, 2001).

1.3. EXPERIMENTAL PAIN MODELS

It is difficult to evaluate the analgesic effects in clinical trials because pain has an impact on a number of personal factors (e.g. psychological, cognitive and social aspects) and is confounded by systemic reactions such as fever and general malaise. Furthermore, patients suffering from pain are often treated with different drugs, which can also influence pain perception. (Drewes, Gregersen, & Arendt-Nielsen, 2003) Experimental models are advantageous for evaluating analgesic effects because both the experimentally induced pain can be controlled and the evoked response can be assessed in detail (Arendt-Nielsen, Curatolo, & Drewes, 2007). Pain can be induced electrically, thermally, mechanically and chemically and stimulation can be applied in muscles, bones, skin and viscera. Thus, pain intensity, duration, frequency, and localisation are parameters, which can be designed and controlled by the investigator and psychophysical, behavioural and

neurophysiologic responses can be assessed (Arendt-Nielsen, 1997). The pain system can also be modulated by e.g. pharmacological intervention. The assessment of the pain response and the analgesic effects can be quantified by subjective and objective methods. Subjective methods such as standardised scales and questionnaires describe the experience of pain. Objective methods, such as electroencephalography (EEG), magnetic resonance imaging (MRI), magnetoencephalography (MEG), positron emission tomography (PET) and single photon emission computed tomography (SPECT) describe the neurophysiologic response to pain. Figure 2 shows the concept of an experimental model.

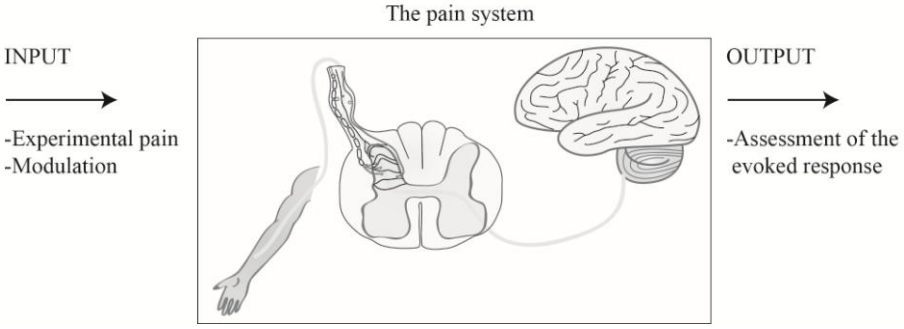

Figure 2: Illustration of the concept of experimental pain models. The pain system is modeled by an experimental painful stimulus (e.g. skin heat stimulation) with a specific pain intensity, duration, frequency, and localisation. The pain system can be modulated (e.g. pharmalogically) and the evoked response can be assessed using subjective (e.g. standardised scales) and objective methods (e.g. electroencephalography and magnetic resonance imaging).

CHAPTER 2. HYPOTHESES & AIMS

To investigate the pain system, experimental models were applied using skin heat as pain stimulation and morphine for modulation of the pain system. It was hypothesised that the electrophysiological (EEG) response of painful skin heat stimulation is altered in a patient group suffering from painful chronic pancreatitis (CP) compared to healthy volunteers (HV). In HV it was also hypothesised that pain stimulation would increase brain activation (the blood oxygen level-dependent (BOLD) response) in pain-specific areas and alter concentrations of brain metabolites measured by MR spectroscopy (MRS) in ACC. Finally, it was hypothesised that morphine-induced analgesia would modulate these neurophysiologic variables measured during pain stimulations. Such an objective assessment approach would likely provide complementary information in understanding pain and cortical analgesic mechanisms. An overview of aims, papers and studies (described in the next chapter) are illustrated in Figure 3.

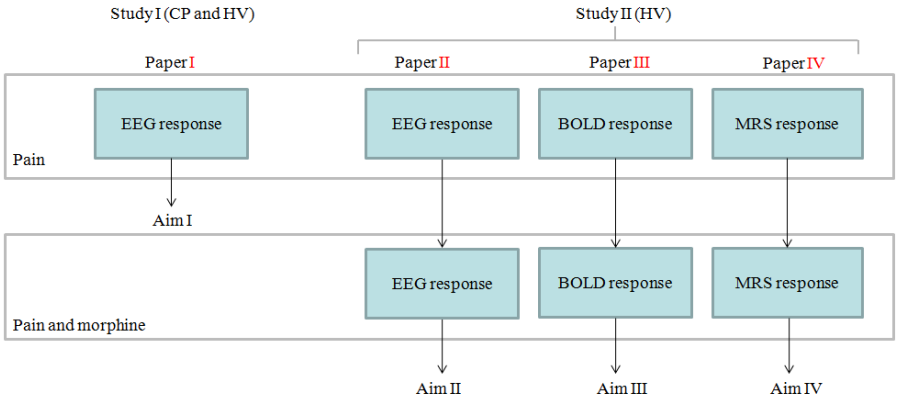

Figure 3: Overview of aims which are investigated in study I (including patients suffering from chronic pancreatitis (CP) and healthy volunteers (HV) and study II (including only healthy volunteers) presented in papers I-IV. EEG, electroencephalography; BOLD, blood oxygen level-dependent; MRS, magnetic resonance spectroscopy.

Hence, the aims were:

I. *To investigate habituation and the brain's response to phasic painful skin heat stimulation in patients suffering from chronic pancreatitis and in healthy volunteers using electrophysiological measurements (paper I).*

II. *To investigate the effect of morphine on heat pain induced sensory processing with electrophysiological measurements in healthy volunteers (paper II).*

III. *To investigate the blood oxygen level-dependent response induced by painful skin heat stimulation and the effect of morphine on this response in healthy volunteers (paper III).*

IV. *To investigate the magnetic resonance spectroscopy response induced by painful skin heat stimulation and the effect of morphine on this response in healthy volunteers (paper IV).*

CHAPTER 3. MATERIALS & METHODS

3.1. MATERIALS

Two studies contributed to this thesis. The studies were approved by the local Ethics Committee (reference no. N-20090008MCH (study I) and N-20100046 (study II)) and conducted according to the Declaration of Helsinki. Study II was further approved by the Danish Medicines Agency (reference no. 2612–4319) and registered at ClinicalTrials.gov (NCT01245244, EUDRACT no. 2010-020894-17). Study II was conducted according to the rules of Good Clinical Practice and monitored by the Good Clinical Practice unit, Aarhus University Hospital, Denmark. All subjects provided informed consent prior to the experiments. Study I was conducted from July 2010 through March 2011 and Study II was conducted in the period from November 2010 to April 2012 at the Research Laboratory at Mech-Sense, Department of Gastroenterology and Hepatology and Department of Radiology at Aalborg University Hospital, Denmark.

3.1.1. STUDY I

Fifteen healthy volunteers and 15 patients diagnosed with painful CP were included for study I. None of the healthy volunteers were suffering from pain-related diseases or receiving medication. Patients suffering from CP were diagnosed according to the Lüneburg diagnostic score (Lankisch et al., 2009). Patients had upper abdominal pain corresponding to the Th10 dermatome reflecting the referred pain area for pancreas (the "viscerotome") lasting for more than 3 days per week for at least 3 months. Patients on stable opioid medication and patients on non-opioid analgesics were included. Exclusion criterias were other acute or chronic pain diseases and previous surgery in the stimulation area at Th10.

3.1.2. STUDY II

Study II was a randomised, double-blinded, placebo-controlled cross-over study with morphine and consisted of two study arms (now termed study IIa and study IIb). Forty healthy volunteers participated in study IIa and 20 of the 40 healthy volunteers participated in study IIb. Inclusion criterias were normal blood pressure, no history of abuse of alcohol, opioids and other drugs, no history of allergy to opioids, no planned treatment or surgery during the study period, no history of pain disorders or mental illness, and no intake of analgesic 24 hours prior to the experiment. Female subjects used safe contraceptive medication and they were investigated in the same phase of the individual menstrual cycle. Subjects were

asked to avoid eating and drinking for at least four hours before the experiment. All subjects participated in screening session where they received the experimental stimuli and they were scanned in the MRI scanner to familiarise them with the experimental environment and to reduce anxiety.

The overall rationale behind study II was to investigate the modulation of the pain response (peripherally, spinally and centrally) by use of morphine. Hence, the overall study design consisted of multiple pain tests (skin heat, reflexes, bone and muscle pressure, multimodal test of rectum (electrical, heat, pressure), cold pressor) and measurements (visual analogue scale (VAS), electromyography, EEG, MRI). The total length of each session of study IIa and study IIb was approximately 4 and 3 hours, respectively. For this thesis only a part of these tests and measurements were used and other findings in study II are reported elsewhere (Kristiansen et al., 2014; Lelic, Olesen, Gregersen, et al., 2014; A. E. Olesen, Brock, Sverrisdóttir, Larsen, & Drewes, 2014; Sverrisdóttir et al., 2014). Therefore, only tests measurements relevant for this thesis are described further.

3.2. METHODS

A Contact Heat Evoked Potential Stimulator (CHEPS) was used for thermal skin heat stimulation and morphine was used to modulate the pain response. Both subjective and objective measurements were applied in both studies to assess the evoked response to thermal skin heat stimulation. The subjective experience of pain perception was assessed by a standardised scale, the VAS, for both studies. EEG was used for objective assessment in study I and study IIa and in study IIb objective assessments were obtained using functional MRI (BOLD) and proton magnetic resonance spectroscopy (1H-MRS, termed MRS). These methods are described in further details in the following sections and Figure 4 shows an overview of the two studies.

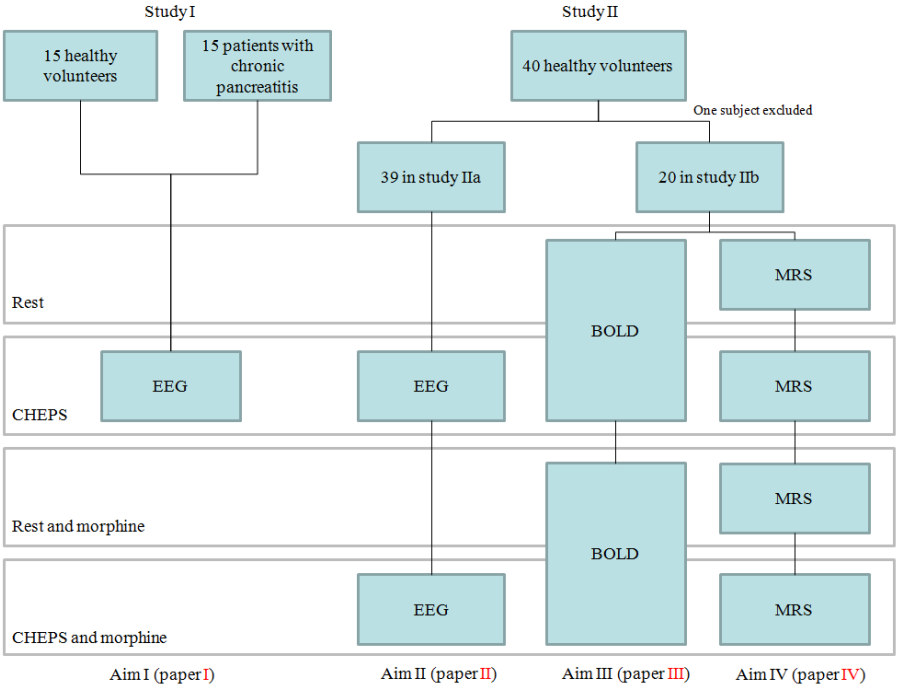

Figure 4: Overview of study I and study II. In study I pain was induced using CHEPS (Contact Heat Evoked Potential Stimulator) and EEG (electroencephalography) was recorded. In study IIa CHEPS-induced pain before and after administration of morphine were also assessed by EEG. In study IIb CHEPS-induced pain before and after administration of morphine were assessed by BOLD (blood oxygen level-dependent) signal. These recordings included both measurements during rest and pain in a so-called "on-off" paradigm. Furthermore, both a resting state condition (no pain stimulation) and a painful condition were assessed by MRS (magnetic resonance spectroscopy) before and after morphine administration.

3.2.1. CONTACT HEAT EVOKED POTENTIAL STIMULATOR

Thermal skin stimulations were applied using a Pathway Stimulator (CHEPS, Medoc Ltd, Ramat Yishai, Israel) with a thermode activation area of 573 mm^2. A MRI compatible thermode was used in the MRI scanner. The heating rate was 70 °C/s and the cooling rate was 40 °C/s. Both myelinated Aδ-fibres and unmyelinated C-fibres are activated during painful contact heat stimulations (Chen, Niddam, & Arendt-Nielsen, 2001; Le Pera, Valeriani, Niddam, Chen, & Arendt-Nielsen, 2002). Different stimulation paradigms have been applied using contact heat with variable pain intensity, duration, frequency, and localisation depending on the method used for assessment of the response (either EEG, BOLD or MRS). Several studies have

used phasic stimuli (Chen et al., 2001; Roberts et al., 2008; Valeriani, Le Pera, Niddam, Chen, & Arendt-Nielsen, 2002) or tonic stimulation (Brooks, Nurmikko, Bimson, Singh, & Roberts, 2002; Kupers, Danielsen, Kehlet, Christensen, & Thomsen, 2009; Tran, Wang, Tandon, Hernandez-Garcia, & Casey, 2010), stimulation of the hand (Brooks et al., 2002), arm (Chen et al., 2001; Greffrath, Baumgärtner, & Treede, 2007; Roberts et al., 2008; Warbrick, Derbyshire, & Bagshaw, 2009) or leg (Kupers et al., 2009; Tran et al., 2010; Warbrick et al., 2009), moving thermode position between stimuli (Greffrath et al., 2007; Roberts et al., 2008; Tran et al., 2010; Warbrick et al., 2009) or fixed position (Greffrath et al., 2007; Warbrick et al., 2009) and different inter-stimulus interval (ISI) (Chen et al., 2001; Valeriani et al., 2002).

We used different stimulation paradigm designs for the different research questions in study I and study II. In study I we applied 31 phasic stimuli on the forearm and upper abdominal area (as this area, Th10, share spinal segmental innervations with the pancreatic gland) with variable ISI (8-12 s) and the thermode was moved between stimuli (see paper I for details). This sequence was repeated three times at each area. Using phasic stimuli the evoked brain potentials can be recorded by EEG (Chen et al., 2001). The advantage of moving the thermode slightly between stimuli is that potential local skin habituation or sensitisation can be avoided or reduced (Chen et al., 2001; Roberts et al., 2008). The initial idea of study II was to design a stimulation paradigm allowing comparison of EEG and BOLD results. Thus, we had to compromise the quality of the measured response as the two measurement modalities require different set-ups. It was not possible to move the thermode inside the scanner during an experiment as the thermode was too sensitive to movements. Furthermore, compared to EEG, more blocks of stimuli were needed for the stimulation paradigm to obtain an acceptable signal-to-noise ratio for the BOLD analysis. Longer and repeated stimulations can likely introduce local skin habituation (Kleinböhl, Trojan, Konrad, & Hölzl, 2006). Thus, for study IIa (EEG) the stimulation paradigm was designed with only 15 phasic stimuli on the forearm with short ISI (1 second) and the thermode was at a fixed location (see paper II for further information). For the BOLD analysis in study IIb stimuli was delivered on the forearm in 9 blocks with same ISI between stimuli (1 second) within a stimulation block (18 seconds in total for one block) with 18 s between stimulation blocks (details described in paper III). A tonic stimulation paradigm (5 minutes) was used for MRS analysis in study IIb as the MRS recording takes several minutes. The stimulus was applied to the upper leg (see paper IV for more information). A similar design has been used by Kupers et al. (Kupers et al., 2009). An overview of the different stimulation paradigms, location and response assessment is shown in Table 1.

Table 1: Overview of CHEPS paradigms in study I-II, paper I-IV.

	Study I	Study II		
	EEG (paper I)	EEG (paper II)	BOLD (paper III)	MRS (paper IV)
Location	Forearm/upper abdominal area	Forearm	Forearm	Upper leg
Type	Phasic	Phasic	Phasic	Tonic
Stimuli per sequence	31	15	9	1
Number of sequences	3	1	9	1
ISI (s)	8-12	1	1	-
Thermode position	Moved	Fixed	Fixed	Fixed
Temperature (°C)	51	52	52	Max 45

ISI: inter-stimulus interval; EEG: electroencephalography; BOLD: blood oxygen level-dependent; MRS: magnetic resonance imaging.

3.2.2. MORPHINE

In study II, morphine and placebo were orally administered (double-blinded) in a randomised order. 30 mg of morphine (15 mL morphine oral liquid mixture 2 mg/mL, The Hospital Pharmacy, Aalborg University Hospital, Denmark) or placebo (15 mL placebo solution, The Hospital Pharmacy, Aalborg University Hospital, Denmark) was administered. To mask any taste and colour 5 mL orange juice concentrate was mixed together with both solutions. Side-effects were monitored during both sessions and one day after each session by questionnaire and phone interview. The time interval between the two sessions was at least one week to allow washout.

3.2.3. PAIN PERCEPTION

Two different VAS methods were used in study I and study II. Study I was a pure pain study whereas analgesia was expected in study II with the possibility of the heat stimulus to be perceived as a non-painful sensation after morphine administration. Thus, another scale was more suitable for study II. In study I: 0 = no pain to 10 = worst pain imaginable. This scale has been used in a similar study (Greffrath et al., 2007). In study II, the pain threshold was set at 5, with the following anchor words on the scale: 0 = no sensation; 1 = vague perception of mild sensation; 2 = definite perception of mild sensation; 3 = vague perception of moderate sensation; 4 = definite perception of moderate sensation; 5 = pain detection threshold; 6 = slight pain; 7 = moderate pain; 8 = medium pain; 9 =

intense pain; and 10 = unbearable pain. This scale has been validated for reliability and robustness in both somatic and visceral pain studies and is described elsewhere (Drewes et al., 2003). To support the memory of the VAS inside the scanner, a modified electronic VAS was used and operated by the subject inside the scanner using control buttons. The modified VAS was displayed in goggles mounted onto the head coil as a vertically oriented scale with numbers 0-10 and anchor word at values 0, 5 and 10.

3.2.4. ELECTROENCEPHALOGRAPHY

EEG provides an objective method to study altered central pain processing and has proven to be a useful method to study analgesic effects (Knott, 2000; Malver et al., 2014). EEG has high temporal resolution but poor spatial resolution. Electrical activity is generated in the brain by neuronal firing within the brain and this electrical activity can be measured by EEG. In a resting state condition (spontaneous EEG), the neuronal firing is randomly distributed in time, but this neuronal firing can be synchronised and activated sequentially when an external stimulus is applied (such as short heat pulses induced by the CHEPS). The latter is called brain evoked potentials (EPs). The resting state EEG have been used to describe abnormal CNS processing in chronic pain patients (S. S. Olesen, Graversen, et al., 2011; S. S. Olesen, Hansen, et al., 2011) and altered pain processing during pharmacological intervention (Knott, 2000) but this will not be described further in this thesis. EPs are typically quantified by their peak latencies and amplitudes, power spectrum, scalp topographies and brain source localisation. EPs have also been used to study altered brain response to pain stimulation (Blauenfeldt, Olesen, Hansen, Graversen, & Drewes, 2010; Frøkjær, Egsgaard, et al., 2011; Valeriani, Pazzaglia, Cruccu, & Truini, 2012) and during pharmacological intervention (Staahl et al., 2011). Traditional EP analysis is typical performed as an average procedure of the EEG response to several repeated stimuli in the time domain. Thus, the signal-to-noise ratio is improved (Dawson, 1951). However, this procedure cancels out non-phase-locked signals and is therefore mostly valid when the main evoked components are phase-locked. In study I the traditional average procedure was applied and peak latencies and amplitudes of the main components of the EPs were assessed (paper I). As described in section 3.2.1 the stimulation paradigm was slightly different in study II and more latency variation (jitter) among sweeps was present (see Figure 5). Thus, a more advanced analysis was used. Previously, studies have extracted information from EPs using single-sweep analysis of EPs in the frequency domain rather than the time domain in diabetes mellitus patients (Graversen, Frøkjaer, Brock, Drewes, & Farina, 2012) and in assessment of the analgesic effect of buprenorphine and fentanyl (Gram et al., 2013). Thus, inter-trial phase alignment and phase-resetting properties of the EPs are preserved (Digiacomo, Marco-Pallarés, Flores, & Gómez, 2008). In study

IIa single-sweep analysis of EPs was used to identify alterations induced by morphine (paper II).

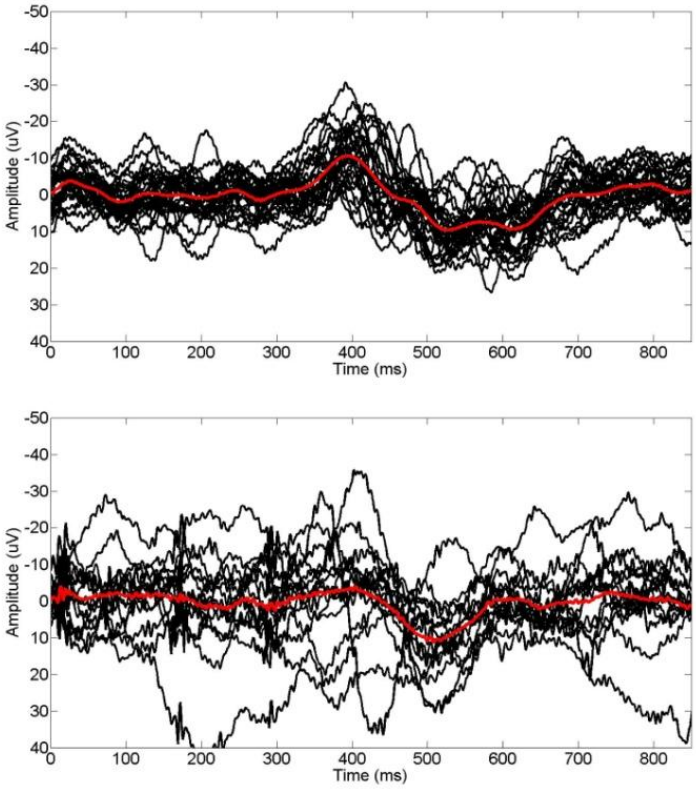

Figure 5: Single-sweeps (black) and the corresponding average evoked potential (red). An example of CHEPS evoked potentials from study I (top) and study IIa (bottom). The two main peaks (N2 and P2) of the average evoked potentials are present in both signals around 400 ms and 500-520 ms after stimulus onset, respectively. More latency variation (jitter) is present in the recording from study IIa illustrated in the bottom figure.

3.2.5. MAGNETIC RESONANCE IMAGING

In experimental pain models, MRI can be used to image brain structures (e.g. diffusion tensor imaging (DTI) tractography, volumetry, grey matter density), functional brain activity (e.g. BOLD, arterial spin labeling (ASL)) and MRS. MRI reveals a high spatial resolution and allows non-invasive and non-radioactive

assessment. Only BOLD and MRS, the methods used in Study IIb, are described further.

Functional MRI is widely used to estimate brain activity and is typically obtained from the BOLD signal where changes in the hemodynamic response are measured. Increased neuronal firing requires increased oxygen level and the blood flow and volume are increased to deliver more oxygen. Oxygenated blood displaces deoxygenated blood a few seconds after neural activity is increased. Oxygenated blood and deoxygenated blood have different magnetic properties as oxygen is carried by hemoglobin in the blood and oxygenated hemoglobin is less magnetic (diamagnetic) than deoxygenated hemoglobin (paramagnetic). Thus, changes in this relationship can be detected using an MRI scanner. The BOLD signal (see Figure 6) can for instance be modulated by experimental thermal noxious stimuli (Brooks et al., 2002; Helmchen et al., 2008; Roberts et al., 2008) and pharmacological intervention (Becerra, Harter, Gonzalez, & Borsook, 2006; Gear et al., 2013; Wanigasekera et al., 2012; Wise et al., 2002). CHEPS-induced pain was used to investigate the brain response before and after morphine administration (paper III).

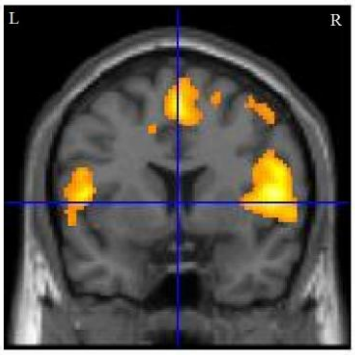

Figure 6: An example of brain activation during CHEPS stimulation. Activity is seen in the anterior cingulate cortex and insula cortex/secondary somatosensory cortex. L: left; R: right.

Proton magnetic resonance spectroscopy (1H-MRS, termed MRS in this thesis) is a method for measurement of brain metabolite concentrations in vivo such as N-acetylaspartate, glutamate, glutamine, choline, creatine, myoinositol, γ-aminobutyric acid (GABA). MRS can also be used to measure metabolite concentrations in other tissue than the brain and MRS can be obtained from different nuclei than protons, but this will not be explained in this thesis. MRS is widely used in the brain because of high sensitivity and abundance. Protons in different molecules resonate at different frequencies, which results in a small chemical shift when a magnetic field is applied. Thus, a MR spectrum is obtained (see Figure 7) with metabolites appearing at specific ppm (parts per million). The area under the curve refers to the metabolite concentration. (Fayed, Olmos, Morales, & Modrego, 2006) Assessment of brain metabolites using MRS have been used in experimental studies in healthy volunteers during acute pain stimulation

(Gussew et al., 2010; Gutzeit et al., 2011, 2013; Kupers et al., 2009; Mullins, Rowland, Jung, & Sibbitt, 2005) and brain metabolite concentration changes has been investigated in long-term opioid dependent subjects (Haselhorst et al., 2002; Yücel et al., 2007). We investigated MRS in the ACC in response to pain and morphine administration.

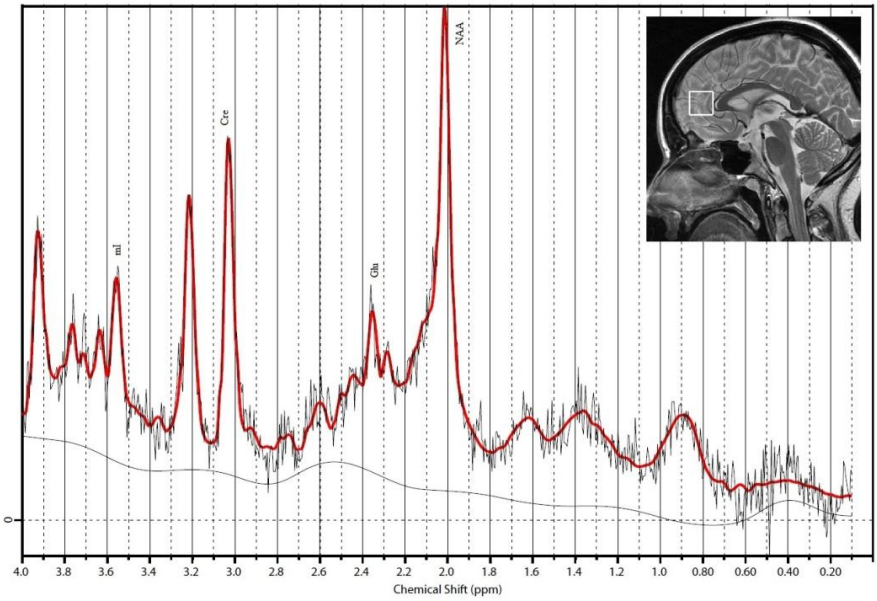

Figure 7: Example of MRS spectrum with a 2×2×2 cm single voxel placed in anterior cingulate cortex. Only relevant metabolites for this thesis are presented in the figure. mI, myoinositol; cre, creatine; glu, glutamine; NAA, N-acetylaspartate; ppm, parts per million.

3.2.6. STASTITICAL ANALYSES

Different analyses approaches were used for the specific EEG, BOLD and MRS related outcomes and the statistical analyses of the studies are reported in details in paper I-IV. As the studies could be considered as explorative studies including multiple end-points, exact sample size calculations were difficult. As a common approach, the subjective pain perception (VAS) was included in all the studies. Considering the change in VAS rating as an outcome, and including knowledge from our previous CP patient and pharmacology intervention studies, it was realistic to set the minimal detectable difference between groups to 25% of the mean VAS and the standard deviation of the mean to 25%. Based on this effect size, this resulted in 16 subjects per group (alpha=0.05, power=0.80) using a two-sided t-test. Additionally, previous studies, which assessed the analgesic effects have typically

included 10-20 subjects for EEG analysis (Malver et al., 2014) and 8-13 subjects for BOLD analysis (Becerra et al., 2006; De Simoni et al., 2013; Gear et al., 2013; Wise et al., 2002). The literature is more limited for MRS assessment of the opioid effects in healthy volunteers. Thus, many factors can influence the size of the required sample and the above mentioned considerations and feasibility considerations were taken into account to decide the sample sizes.

CHAPTER 4. RESULTS

The key results from the studies are presented in this chapter. More detailed results are found in paper I-IV. An overview of the results is illustrated in Figure 8.

4.1. AIM I

Aim: To investigate habituation and the brain's response to phasic painful skin heat stimulation in patients suffering from chronic pancreatitis and in healthy volunteers using electrophysiological measurements (paper I).

Key results:

- N2/P2 amplitudes increased 25% in CP patients and decreased 20% in HV during repeated pain stimulation in the referred pancreatic area (Th10 dermatome; $P = 0.006$).
- N2/P2 amplitudes increased 3% in CP patients and decreased 20% in HV during repeated pain stimulation of the forearm ($P = 0.06$).
- N2/P2 amplitudes were unchanged in CP patients ($F = 2.0$; $P = 0.2$) and decreased in HV ($F = 4.6$; $P = 0.02$) during the second and third sequences of stimulation of the referred pancreatic area (Th10 dermatome).
- N2/P2 amplitudes were unchanged in CP patients ($F = 2.0$; $P = 0.8$) and decreased in HV ($F = 4.1$; $P = 0.04$) during the second and third sequences of stimulation of the forearm.

Interpretation: Patients suffering from CP revealed impaired habituation, whereas HV showed habituation to repeated pain stimulation as expected. Thus, altered central pain processing was demonstrated in CP patients.

4.2. AIM II

Aim: To investigate the effect of morphine on heat pain-induced sensory processing with electrophysiological measurements in healthy volunteers (paper II).

Key results:

- Compared with placebo, morphine decreased the spectral indices in the delta and theta bands during pain stimulation by 13% (P = 0.04) and 9% (P = 0.007), respectively.
- Compared with placebo, morphine increased the spectral indices in the beta and gamma bands during pain stimulation by 10% (P = 0.006) and 24% (P = 0.04), respectively.

Interpretation: Decreased low frequency and increased high frequency oscillations in indicate diminished pain response in response to morphine treatment.

4.3. AIM III

Aim: To investigate the blood oxygen level-dependent response induced by painful skin heat stimulation and the effect of morphine on this response in healthy volunteers (paper III).

Key results:

- Pain stimulation induced activation in the anterior cingulate cortex, secondary somatosensory cortex/insula, thalamus and cerebellum ($P < 0.05$).
- In response to morphine treatment the spatial extent of the activated pain specific areas decreased.
- Reduced pain-induced activation was seen in the right insula, anterior cingulate cortex and inferior parietal cortex after morphine treatment compared to before treatment ($P < 0.05$).
- No effect on pain-induced brain activation was seen after placebo treatment compared to before treatment ($P > 0.05$).

Interpretation: Brain areas of the "pain matrix" were activated by pain stimulation and morphine reduced activation in pain specific and opioidergic dominant areas.

4.4. AIM IV

Aim: To investigate the magnetic resonance spectroscopy response induced by painful skin heat stimulation and the effect of morphine on this response in healthy volunteers (paper IV).

Key results:

- Pain stimulation induced an increase in N-acetylaspartate/creatine ratio (F = 5.5, P = 0.04) in ACC.
- During morphine treatment painful stimulation induced decreased glutamate/creatine (F = 7.3, P = 0.02), myoinositol/creatine (F = 8.38, P = 0.02) and N-acetylaspartate/creatine ratios (F = 13.8, P = 0.004).
- During placebo treatment pain stimulation induced an increase N-acetylaspartate/creatine ratio (F = 6.1, P = 0.04).

Interpretation: N-acetylaspartate/creatine ratio increased during pain and decreased during morphine treatment together with decreased levels of myoinositol/creatine and glutamate/creatine. Thus, these metabolites may play a role in pain processing and opioid-induced analgesia.

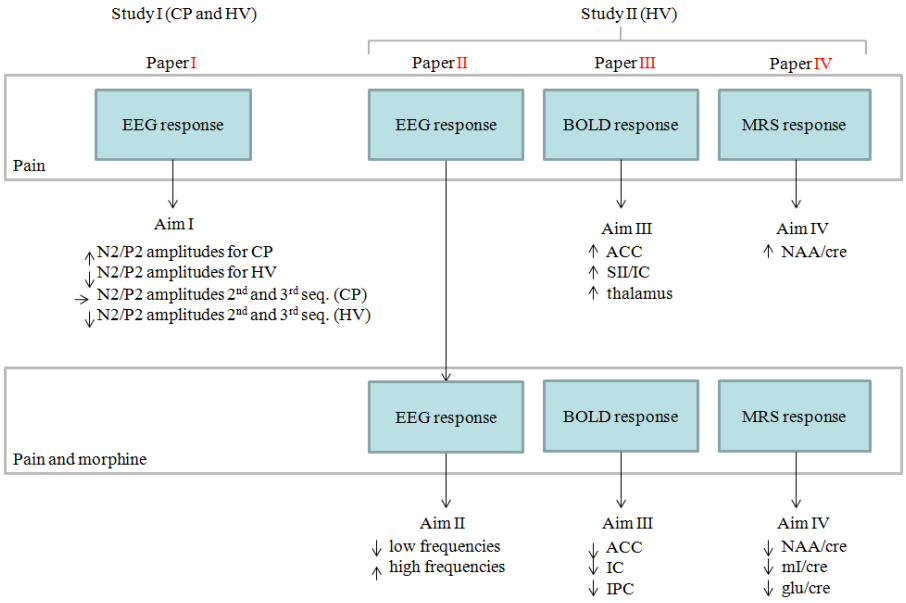

Figure 8: Overview of results of the two studies divided by papers and aims. EEG, electroencephalography; BOLD, blood oxygen level-dependent; MRS, magnetic resonance spectroscopy; ↑, increase; ↓, decrease; →, unchanged; seq., sequence; CP, chronic pancreatitis; HV, healthy volunteers; ACC, anterior cingulate cortex; SII, secondary somatosensory cortex; IC, insula cortex; IPC, inferior parietal cortex; NAA, N-acetylaspartate; mI, myoinositol; glu, glutamate; cre, creatine.

CHAPTER 5. DISCUSSION

CHEPS stimulations were used to investigate the pain system in patients with CP and HV, and morphine was administered to modulate the pain system in HV. The pain response was assessed by EEG, BOLD and MRS. The first part of the discussion contains methodological considerations regarding these elements for modeling, modulation and assessment of the pain system, followed by a discussion of the actual pain and morphine response found by these different modalities.

5.1. METHODOLOGICAL CONSIDERATIONS

CHEPS stimulations

Different settings of the CHEPS were used in the two studies to evoke painful stimulations. CHEPS stimulation introduced habituation. In study I (paper I) the stimulation paradigm was designed to evoke habituation during repeated sequences of stimuli, whereas this was not the case for study II. Due to habituation it was difficult to provide stimuli which were painful during the entire stimulation period (as seen in paper III). Higher stimulation temperatures were not possible, as this would increase the risk of skin injury. Local skin habituation is introduced much faster when stimulating the same skin area compared to the use of variable locations (Greffrath et al., 2007). Local skin habituation could have been minimised by moving the thermode between each stimulation (as mentioned in section 3.2.1, this was not practically possible inside the scanner as the thermode was highly sensitive to movements) and central habituation would still be present as repeated blocks of stimulations were required (paper III). An event-related design with long and variable ISI could also have been used to minimise central habituation and this would be preferable for future studies as fewer stimuli are required. A similar design with long and variable ISI would be possible for EEG recordings of evoked potentials (paper II), however, with long ISI, this is more time consuming and was not possible for the present study. Even though habituation was present, we showed activation of the "pain matrix" and hence the use of slightly different CHEPS paradigms (dictated by the individual setup of the studies) was able to evaluate different aspects of pain processing.

Other parameters such as anticipation, attention and anxiety also influence the individual pain perception. Anticipation can be minimised with variable ISI. Attention confounders were minimised as subjects were asked to count the number of stimuli to keep attention at a constant level. Furthermore, subjects participated in a screening session in study IIa and study IIb before the actual experiment to reduce anxiety.

Morphines effects

The dose (30 mg) and administration (oral) of morphine in this study was chosen based on experience from previous studies (A. E. Olesen, Staahl, Arendt-Nielsen, & Drewes, 2010; Staahl, Christrup, Andersen, Arendt-Nielsen, & Drewes, 2006). A dose of 30 mg morphine is clinically relevant, and avoids too many gastrointestinal side-effects such as nausea, vomiting, feeling of euphoria. In paper II-IV only limited morphine effect was demonstrated on pain perception during CHEPS stimulations. This can be due to several reasons. 1) The inter-individual variability in analgesic effect (Rakvåg et al., 2005; Staahl, Olesen, Andresen, Arendt-Nielsen, & Drewes, 2009). Thus, the response from non-responders (which could me more than 30% in healthy volunteers) might blur the true analgesic effect as group-level analyses were performed. 2) A single oral dose of morphine was administered, and the individual time to reach the maximal analgesic effect is variable. Thus, the optimal time point for measuring varies, but measurements were assessed at the same time point (60 minutes after administration for EEG and BOLD and 80 minutes after administration for MRS) for all subjects. The onset of analgesia is typically 30-40 min after oral morphine administration and the duration is 4 hours (Bennett et al., 2005). Blood samples were collected at several time points in study IIa (this was not feasible in study IIb) and maximum plasma concentration of morphine was found 45 minutes after administration (Sverrisdóttir et al., 2014). Thus, the maximum morphine concentration in the brain was reached after this time point. 3) Habituation also affects the pain perception but this has already been discussed. 4) Opioids may in some cases cause hyperalgesia (however not typically after a single dose) and this may confound the results together with other effects such as sedation (Khodayari-Rostamabad et al., 2015). 5) It can also be speculated that contact heat might not be the optimal type of stimulation as it has been demonstrated that deeper and tonic stimulations are more sensitive to morphine analgesia (Staahl et al., 2009). However, CHEPS stimulation was chosen to detect changes in objective measurements. 6) Higher doses, repeated doses or other administration routes of morphine might be more effective to reveal changes in behavioral and perhaps the objective measurements. However, higher doses may lead to side-effects and high dropout rates. 7) Finally, sample size could be the limiting factor. Previously, studies demonstrated analgesic effect of this dose of oral morphine in 24 subjects (A. E. Olesen et al., 2010; Staahl et al., 2006) and the limited effect on pain perception could be due to the low number of subjects included in paper III-IV and future analysis should include more subjects.

Electroencephalography analysis

In paper I we used the traditional averaging procedure of EPs, which has been used in several other studies (Chen et al., 2001; Valeriani et al., 2002). This procedure was not suitable for EP analysis in paper II due to lower EP amplitudes and latency variability (jitter) as shown in Figure 5 (jitter) as shown in Figure 5. Low EP amplitudes were expected due to peripheral habituation as the thermode position

was fixed. Greffrath et al. compared pain ratings and EP amplitudes of CHEPS-evoked noxious stimuli with variable thermode location between stimuli, similar to the study design in paper I and with fixed thermode location. They found the last three pain ratings to be reduced by 40% and 70% compared to the first pain rating for variable and fixed thermode location, respectively. Furthermore, the normalised EP amplitudes were decreased 10% and 50% across the first three stimuli. For this reason, we only recorded 15 stimuli at fixed location (paper II). They also concluded that pain ratings and EP amplitudes were reduced using fixed location as compared to variable location corresponding to a reduction of 5°C in stimulus temperature. (Greffrath et al., 2007) A recent study, published after conduction of these studies, demonstrated that latency jitter can be reduced by shortening of the stimulus duration (e.g. by stimulating with a higher baseline temperature) resulting in a more synchronised recruitment of afferents (Kramer, Haefeli, Jutzeler, Steeves, & Curt, 2013). Temperature rise time and the level of contact with the thermode are possible parameters with can affect latency jitter (Warbrick et al., 2009). Using the single-sweep analysis changes in latency jitter can be disclosed and studies suggested single-sweep analysis approach to be superior to the traditional averaging procedure (Hu et al., 2011; Hu, Mouraux, Hu, & Iannetti, 2010; Mayhew, Iannetti, Woolrich, & Wise, 2006; Warbrick et al., 2009).

EPs are often cleaned to remove noisily sweeps or details containing e.g. eye blinks, external noise, ect. Realignment of sweeps is also a way to keep information of amplitudes, but physiologically relevant information may be lost in these procedures. EPs (paper II) were thoroughly investigated in the traditional time domain before performing the single-sweep analysis in the frequency domain. We applied several manual, semi-automated and automated methods (e.g. the method by Hu et al. (Hu et al., 2010)) to the data in paper II, but they were not suitable to extract valid results. Besides requirement of data quality testing, the single-sweep analysis approach in paper II was fully automated and included all recorded sweeps, which makes the method objective, robust to noise, inter-observer independent and less time-consuming than other semi-automated and automated methods.

As written in Section 3.2.4 electrical brain activity is not only reflected in EPs but also in the spontaneous (resting state) EEG. The spontaneous EEG is obtained during a resting condition (with no stimulation involved) or tonic painful stimulation. Spontaneous EEG has been used to identify altered pain processing in chronic pain patients and during pharmacological intervention. Challenges of stimulus confounders are avoided in the resting state EEG method. However, different information is extracted and resting state EEG could be suggested a supplementary method to EP analysis. A major difference between resting state EEG and EPs is that a non-pain specific state is measured in the resting state generated by neuronal firing and reflects a mix of several brain regions working together.

Blood oxygen level-dependent signal analysis

As previously discussed habituation was present during repeated blocks of stimuli. To account for this, pain intensity ratings were included in the analysis model. However, only habituation between block was considered in the model. The pain response within each block was not totally stable as assumed using a boxcar model, and thus, the model fit was not optimal. This might influence the ability to detect a large significant drug effect. A robust difference in response to morphine treatment might be masked by a maybe larger individual habituation component. Study III was an explorative study and due to incomplete data and the low number of subject, who had all recordings necessary for a two-way repeated measures ANOVA analysis, different numbers of subjects were included for t-test analysis of morphine and placebo effect. Similar statistics have been used in other designs (Borras et al., 2004; Kim et al., 2013; Wanigasekera et al., 2012), but for a non-explorative study, it would be ideal to demonstrate a 2x2 interaction for both imaging data and the behavioral data to reveal a robust drug effect.

Using a task-related experiment as in paper III it is difficult to control for inter-subject variations in stimulus perception. This can be avoided by measuring brain activity in a resting state condition. Resting state functional MRI (fMRI) is a task-free measurement of the functional connectivity between brain areas reflecting synchronous slow frequency oscillations and has previously been used in pharmacological studies (Becerra et al., 2006; Gear et al., 2013; Khalili-Mahani et al., 2012).

Magnetic resonance spectroscopy analysis

Analysis of brain metabolite concentrations in the ACC was performed as ratios to creatine, which have been done previously (Feraco et al., 2011; Yabuki, Konno, & Kikuchi, 2013). It would also be possible to estimate absolute values of metabolite concentrations to obtain a more direct measurement of changes. However, a more comprehensive analysis is needed including e.g. tissue segmentation as the voxel of interest is a mix between white matter, grey matter and cerebral spinal fluid together with relaxation correction. For data analysis in paper IV other bias was possible, such as prior task hangover and between-days variability, which might blur the true absolute metabolite concentration levels. Thus, we used ratios. MRS was measured in the ACC, but it could be interesting to investigate MRS in these other brain regions which were found relevant in the BOLD analysis. Chemical shift imaging (allowing multi voxel spectroscopy) would be a method to investigate metabolite concentrations in several brain areas, but the signal-to-noise ratio is normally reduced considerably and the acquisition time will typically be too long for investigation the response to a pain stimulus (Jansen, Backes, Nicolay, & Kooi, 2006).

5.2. PAIN RESPONSES

Electrophysiological response

Decreased habituation to noxious stimuli might be related to increased activation of sensory CNS pathways. Decreased habituation have been reported in other chronic pain disorders, such as migraine, fibromyalgia and cardiac syndrome X (de Tommaso et al., 2011; Valeriani et al., 2003, 2005). The findings in paper I corresponds to previous findings in EEG and MRI studies supporting central neuroplastic changes in painful CP (Frøkjær et al., 2012; Frøkjær, Olesen, et al., 2011; Lelic, Olesen, Hansen, et al., 2014). Pain is complex and exists of sensory, cognitive and affective components, and therefore, involves physical and psychological aspects of the stimulus. Results from paper I revealed decreased N2/P2 amplitudes (habituation) for HV during repeated sequences of stimulation, whereas N2/P2 amplitudes were increased or unchanged (decreased habituation) for CP patients. The N2/P2 response is mainly related to insula and cingulate cortex activity (Garcia-Larrea, Frot, & Valeriani, 2003), which has a strong association with the emotional and affective components of pain. It could be interesting to investigate whether increased activity and metabolite changes in insula and ACC can be detected for pain patients using fMRI and MRS. Future MRI studies in painful CP should include such assessments.

Blood oxygen-level dependent response

Increased levels of CHEPS-induced BOLD activation were found in areas involved in pain processing; ACC, SII/IC, Th and the cerebellum (paper III). Previously studies on heat pain have shown similar activation including more or less identical activated areas, see Table 2 As discussed previously the perceived stimuli were not painful during the entire stimulation period. Innocuous and noxious heat stimulations has been investigated in previous studies (Becerra et al., 1999; Brooks et al., 2002; Moulton, Pendse, Becerra, & Borsook, 2012; Tseng, Tseng, Chao, Lin, & Hsieh, 2010) and some pain-specific areas can be activated for both innocuous and noxious stimuli and others only for noxious stimuli. Tseng et al. reported the anterior IC, ACC and Th to be activated for both innocuous and noxious stimuli among other areas (e.g. the cerebellum) and the SI, SII and posterior IC to be activated only following noxious stimuli (Tseng et al., 2010). Hence, the measured BOLD response in the ACC and SII/IC might be enhanced with a more consistent stimulus and other pain-specific areas as the SI and PFC might also be activated.

Table 2: Examples of heat pain studies in healthy volunteers induced by contact heat.

Authors	N	Paradigm	Thermode position	Results
(Quiton, Keaser, Zhuo, Gullapalli, & Greenspan, 2014)	14	Tonic	Fixed	↑ pACC, aMCC, aIC, SII ↓ SI, frontal lobe
(Moulton et al., 2012)	16	Tonic	Fixed	↑ ACC, IC, supramarginal gyrus, angular gyrus, superior parietal lobule, frontal gyrus, thalamus, primary motor cortex, SII
(Shenoy et al., 2011)	12	Phasic	Moved	↑ post-central gyrus, IC, MCC, frontal gyrus, cerebellum, thalamus
(Tran et al., 2010)	14	Tonic	Fixed	↑ pACC, IC, orbito frontal, prefrontal, thalamus, inferior parietal lobule, SI
(Tseng et al., 2010)	12	Tonic	Fixed	↑ SI, SII, IC, PMA, frontal gyrus, cerebellum, SMA, thalamus, lentiform nucleus, midbrain
(Staud, Craggs, Perlstein, Robinson, & Price, 2009)	13	Phasic	Fixed	↑ ACC, IC, thalamus, SI, SII
(Roberts et al., 2008)	10	Phasic	Moved	↑ IC, post-central gyrus, SMA, MCC, pre-central gyrus
(Brooks et al., 2002)	18	Tonic	Fixed	↑ IC, ACC, SII, cerebellum, frontal gyrus
(Apkarian, Gelnar, Krauss, & Szeverenyi, 2000)	7	Tonic	Fixed	↑ IC/SII, PM/MI, SI ↓ SMA, CC, PM/MI, posterior parietal cortex
(Becerra et al., 1999)	12	Tonic	Fixed	↑ Frontal gyrus, ACC, PCC, thalamus, motor cortex, SI, SII, SMA, IC, cerebellum

Studies are listed in descending order based on year published. N: number of subjects; ↑: increased activation; ↓: decreased activation; pACC: pregenual anterior cingulate cortex; aMCC: anterior midcingulate cortex; aIC: anterior insula cortex; SII: secondary somatosensory cortex; SI: primary somatosensory cortex; *ACC: anterior cingulate cortex; IC: insula cortex; MCC: middle cingulate cortex; PMA: premotor area; SMA: supplementary motor area; PM/MI: premotor and primary motor regions; PCC: posterior cingulate cortex; Phasic refers to brief heat pulses. Stimuli with duration of at least several seconds are here called tonic. This stimulus is typically repeated in the "on" periods of "on-off" paradigms.*

Magnetic resonance spectroscopy response

N-acetylaspartate/creatine increased in response to CHEPS stimulation. Increased brain activity reflects an increase in neuronal energy demand which is thought to reflect enhanced glutamate neurotransmission (Magistretti & Pellerin, 1999). We did not find changes in glutamate (paper IV), which is consistent with Kupers et al., who investigated metabolite changes in the rostral ACC following painful tonic heat stimulation (Kupers et al., 2009). On the other hand, other studies in acute pain found increased levels of glutamate in IC and ACC (Gussew et al., 2010; Mullins et al., 2005). Physiological mechanisms responsible for the changes in brain metabolite concentrations in response to acute pain are not well described. N-acetylaspartate is a neuronal and axonal marker and involved in neuronal metabolism (Castillo, Kwock, Scatliff, & Mukherji, 1998; Clark et al., 2006; Manji, Moore, Rajkowska, & Chen, 2000; Moffett, Ross, Arun, Madhavaroa, & Namboodiri, 2007; Tsai & Coylet, 1995). N-acetylaspartate is synthesised within the mitochondria. Reduced levels of N-acetylaspartate are correlated with a decrease in adenosine triphosphate and oxygen consumption (Manji et al., 2000; Tsai & Coylet, 1995) and N-acetylaspartate is suggested to be a reservoir of glutamate (Clark et al., 2006). Thus, an increase in N-acetylaspartate/creatine might be explained by the role of N-acetylaspartate in neuronal metabolism, and changes in N-acetylaspartate and glutamate could be related in a complex metabolic way. Thus, this could theoretically explain different observations between studies in glutamate and N-acetylaspartate, depending on the exact setup and conditions.

5.3. MORPHINES EFFECTS

Electrophysiological response

Decreased oscillations in low frequency bands (delta 0.5-4 Hz and theta 4-8 Hz) and increased oscillations in high frequency bands (beta 12-32 Hz and gamma 32-80 Hz) during heat stimulation were found after morphine treatment compared to placebo (paper II). In paper I, N2 and P2 of the EPs were appearing around 460 ms and 550 ms after the stimulus, respectively. Thus, the average peak-to-peak latency interval was 90 ms, which corresponds to a frequency around 5.5 Hz (1/0.180 s). Taken individual variability in this interval in to considerations, oscillations of the N2/P2 latency interval, which reflect a major part of the pain specific morphology, was found in the low frequency bands. Thus, decreased low frequency oscillations reflect decreased N2/P2 amplitudes of EPs. It has previously been shown that opioids decrease the N2/P2 amplitude of EPs (Chizh, Priestley, Rowbotham, & Schaffler, 2009; Malver et al., 2014). On the other hand, other studies reported unchanged amplitudes of the average EPs evoked from electrical oesophageal and rectal stimulation following morphine treatment (Lelic, Olesen, Gregersen, et al., 2014; Staahl et al., 2011), and it could be speculated that morphine reduce latency jitter, reflected by higher average amplitudes compared to placebo treatment. Opioid-induced increases in high frequency oscillations of spontaneous EEG during

morphine treatment have also been shown (Matejcek, Pokorny, Ferber, & Klee, 1988). As mentioned earlier in the discussion, the N2/P2 response is related to insula and cingulate cortex activity and other studies showed that morphine alter the dominant electrical activity and networks in the limbic system, where opioid receptor density are high (Lelic, Olesen, Brock, Staahl, & Drewes, 2012; Lelic, Olesen, Gregersen, et al., 2014; Staahl et al., 2011). Hence, changes in frequency oscillations are likely related to altered pain processing induced by morphine treatment.

Blood oxygen-level dependent response

Morphine treatment induced reduced pain-related brain activation in insular cortex, anterior cingulate cortex and inferior parietal cortex. This supports the above mentioned findings of decreased insula and cingulate cortex activity reflected in decreased frequency oscillations assessed by EEG. Pharmacological fMRI studies (both during rest and pain) reported opioids-induced changes in BOLD signal in several regions in the brain, see Table 3. These studies cannot be fully compared to the results in paper III, as they used different conditions, opioids, doses, and drugs were administered intravenously. Thus, they did not only measure the isolated analgesic effect but also activation of areas involved in sedation and reward. These side-effects are less pronounced after oral administration of morphine, and hence our findings may more specifically reflect the true analgesic effect. Becerra et al. reported the SI, thalamus, ACC, hypothalamus, periaqueductal gray, nucleus accumbens and hippocampus to be involved in the analgesic response (Becerra et al., 2006). Decreased pain response in pain-specific areas during remifentanil infusion have been reported (Wager et al., 2013; Wanigasekera et al., 2012; Wise et al., 2002). Furthermore, ACC and the IC have high density of opioids receptors (Jones et al., 1991; Petrovic et al., 2002; Willoch et al., 1999). This seems consistent with our findings of reduced brain response in the ACC and the IC after morphine treatment. A stronger effect of morphine can likely be obtained with a more painful stimulus intensity and a higher treatment dose, as indicated in a previous fMRI study in opioids showing the BOLD response to be dose-dependent (Upadhyay et al., 2012).

Table 3: Results from studies of opioid-induced changes in BOLD signal during pain and rest.

Authors	N	Opioid	Condition	Results (among others)
(Gear et al., 2013)	15	i.v. nalbuphine	Rest	↓ MFC, iOFC, pcPC, superior temporal pole, cerebellum ↑ occipital and temporal cortex, IC, Th, caudate, Hi, pons, cerebellum
(Wager et al., 2013)	21	i.v. remifentanil	Pain	53% reduction of pain signature response
(Khalili-Mahani et al., 2012)	12	i.v. morphine	Rest	functional connectivity NOIs including prefrontal regions, posterior parietal areas, medial temporal regions, primary sensory, primary motor, basal ganglia and cerebellum
(Upadhyay et al., 2012)	36	i.v and sl. buprenorphine	Pain	↓ in sensorimotor/sensory-discriminative circuitry ↑ in limbic and mesolimbic circuitry
(Wanigasekera et al., 2012)	25	i.v. remifentanil	Pain	↓ IC, ACC, basal ganglia
(Becerra et al., 2006)	8	i.v. morphine	Rest	Regions involved in analgesia: ↓ SI, Th, ACC, PAG ↑ Hy, NA, Hi
(Wise et al., 2002)	9	i.v. remifentanil	Pain	↓ IC, ACC

Studies are listed in a descending order based on year published. I.v.: intravenious; sl: sublingual; MFC: middle frontal cortex; iOFC; inferior orbitofrontal cortex; pcPC: post central parietal cortex; IC: insula cortex; Th: thalamus; Hi: hippocampus; NOIs: networks of interest; ACC: anterior cingulate cortex; PAG: periaqueductal gray; Hy: hypothalamus; NA: nucleus accumbens; Hi: hippocampus.

Magnetic resonance spectroscopy response

Concentration ratios of glutamate/creatine, myoinositol/creatine, and N-acetylaspartate/creatine were decreased during painful stimulation after morphine treatment. To my best knowledge, no existing literature describes MRS in opioid treatment in HV and more experimental studies are needed to investigate this topic further. Gao et al. found a decrease in glutamate and N-acetylaspartate and an increased level of myoinositol, and Xiang et al. reported an increase in myoinositol and aspartate levels and a decrease in glutamate levels (among other changes) after chronic morphine treatment in rats (Gao et al., 2007; Xiang et al., 2006). Otherwise changes in brain metabolite concentrations have been investigated in long-term opioids dependent subject (Haselhorst et al., 2002; Yücel et al., 2007). Yücel et al.

showed reduced N-acetylaspartate and glutamate + glutamine levels in methadone-dependent or buprenorphine-dependent subjects and Haselhorst et al. reported decreased levels of N-acetylaspartate in heroin-dependent subjects. These results are obtained from different brain areas and cannot be compared directly with results in paper IV as the studies are too different in design. But, these studies indicated that N-acetylaspartate, glutamate and myoinositol concentrations are related to the physiology of pain and opioid treatment.

CHAPTER 6. CONCLUSIONS

The pain system was modeled using CHEPS for heat stimulation in patients suffering from painful chronic pancreatitis in comparison to healthy volunteers with pain response being assessed electrophysiologically (with EEG). Decreased habituation was found for chronic pancreatitis patients and this might reflect central sensitisation, which has been demonstrated in previous studies. Thus, the method is valid to reveal altered pain processing at the electrophysiological level. To investigate other methods to extract more complementary information about central pain processing, the EEG, blood oxygen-level dependent (BOLD) and magnetic resonance spectroscopy (MRS) responses to pain were investigated in healthy volunteers. These methods showed CHEPS-induced increased activation of pain-specific areas and changes in brain metabolites, which were comparable to previous studies and understandable from a pain physiology point of view. Thus, as the pain response can be assessed by EEG, BOLD, and MRS, these methods were also used to investigate the morphine-response in healthy volunteers. Morphine decreased low-frequency oscillations in the pain evoked EEG revealing decreased N2/P2 amplitudes, which might reflect decreased activity in insula cortex and the anterior cingulate cortex. Decreased insula cortex and anterior cingulate cortex activity was confirmed using BOLD fMRI. MRS of the anterior cingulate cortex showed decreased levels of metabolite concentrations and especially decreased levels of glutamate and N-acetylaspartate may reflect decreased brain activity and neuronal metabolism. These results are in line with the high density of opioid receptors in insula and the anterior cingulate cortex. Overall, from the results presented in this thesis, this emphasise the role of insula cortex and the anterior cingulate cortex in pain processing as well as in morphine-treatment. More importantly, it can be concluded that the present methods are valid to assess the pain response and drug effect. As the mechanisms behind chronic pain and chronification are complex, knowledge obtained from the combination of more modalities and different methods will likely contribute to expanded understanding of these mechanisms and play an important role in development of new drugs and optimisation of treatment strategies of chronic pain.

CHAPTER 7. FUTURE PERSPECTIVES

The ability to assess and combine information from multiple modalities and techniques provides a strong tool in the investigation of pain processing and analgesic effects of existing drugs as well as in development of new drugs for treatment of chronic pain. In this thesis, three different measurements of the pain and morphine effect were presented. In future studies of chronic pain patients, it would be beneficial to include even more information such as pharmacogenomics, metabolomics, and structural MRI. Structural MRI can easily be obtained during the MRI session and allows information on neuroplastic changes in cortical thickness and microstructure (diffusion tensor imaging), and this can be combined with the functional information. Such structural and microstructural methods have previously been used to reveal alterations in chronic pain patients (Frøkjær et al., 2012; Frøkjær, Olesen, et al., 2011). Other EEG and MRI measurements such as resting state EEG, resting state fMRI, arterial spin labeling and multi voxel spectroscopy are also likely to provide future supplementary information and we work on developing and explore these measurements. Especially combining resting state fMRI and diffusion tensor imaging may provide a powerful tool to understand brain plasticity and reorganisation of the central nervous system in chronic pain disorders as structural and functional changes are merged. Information from multiple modalities can be included in a combined analytical model to extract characteristics (features) of the pain and treatment response and in prediction of the pain and treatment response.

We investigated pain and morphine response by EEG, BOLD and MRS in two different sessions in study II, and the results from the individual studies were interpreted separately. One approach to obtain more information from future studies is the possibility to combine acquisition of more modalities simultaneously ("hybrid-imaging" such as MR/PET, MR/CT, MR/SPECT, PET/CT ect.). Furthermore, simultaneous EEG and fMRI has been recorded in several studies, all though this is not without technical challenges (Christmann, Koeppe, Braus, Ruf, & Flor, 2007; Garreffa et al., 2004; Iannetti et al., 2005). One major advantage of combined acquisition of more modalities is the ability of measuring the same neurophysiological state.

To further explore the mechanisms behind pain and morphine response in healthy volunteers (study II), resting state EEG and resting state fMRI recordings were included in the study and the analyses are ongoing. The above mentioned EEG and MRI methods can also easily be applied in the investigation of other drugs. Ongoing analysis of the drug response in healthy volunteers is performed in a randomised, double-blinded, placebo-controlled crossover study using oxycodon (opioid) and venlafaxcin (antidepressant). Based on knowledge of the pain mechanisms behind CP pain obtained from our previous work, we use a combination of EEG and MRI based methods (resting state EEG, resting state

fMRI, MRS and structural MRI) in a randomised, double-blinded, placebo-controlled prospective clinical trial assessing the effect of a ketamine treatment approach in patients with painful chronic pancreatitis.

As an overall and optimal goal, we aim in a future perspective to understand and improve treatment in chronic pain disorders and to provide personalised treatment.

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