Tetrahydrocannabinol in Chronic Pain

Cortical Mechanisms of Pain and Analgesia

Marjan de Vries

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Cortical Mechanisms of Pain and Analgesia

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Chapter 1

General introduction and outline of the thesis

Basic concepts of pain

Pain is a subjective experience. Two individuals with exactly the same cause of pain, can feel different intensities of pain. In fact, pain can be present without a clear anatomical substrate.

The International Association for the Study of Pain (IASP) defines pain as: *"An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"*.[1] Following this definition, pain requires the integration of sensory, cognitive, and affective information. Pain research is complicated by this multidimensional and subjective character of pain. Commonly used instruments to quantify pain in individuals, such as the visual analogue scale (VAS) and numeric rating scale (NRS), assess the amount of pain, but are sensitive to various kinds of bias.[2,3]

Acute pain is an important protective function of our body, that normally disappears when the injury has subsided. However, in some situations, acute pain becomes chronic often for unclear reasons. Pain is then no longer protective, but maladaptive, resulting from abnormal functioning of the peripheral and central nervous system.
Chronic pain is defined as: "*any pain that persists beyond the anticipated time of healing*".[4] Usually pain is regarded as chronic when it lasts or recurs for more than 3 months.[5] Chronic pain of moderate to severe intensity occurs in 19% of adult Europeans, seriously affecting their daily activities, social and working lives.[6] Chronic pain can be classified in different categories including chronic visceral pain, such as chronic pancreatic pain, and chronic postsurgical pain.

Chronic pancreatic pain

Chronic pancreatitis (CP) is a major source of morbidity in European countries, with an annual incidence of approximately 6.0 per 100.000 inhabitants.[7] CP is a disease characterised by progressive destruction of the pancreatic gland, usually resulting in impairment of exocrine and endocrine function.[8] Abdominal pain is the most frequent and dominant symptom in CP, and most patients develop recurrent episodes of chronic pain during the course of their disease. The pain is typically described as a constant, severe, dull pain in the epigastrium, which often radiates to the flanks and back. However, this classical pain pattern is not universal and differs among patients in character, location and severity.[9] Currently, the main treatment methods for chronic pancreatitis are focused on correction of pancreatic insufficiency, management of complications, and pain management.[10]

The pathophysiology causing pain in CP is incompletely understood and multifactorial. Several underlying mechanisms have been suggested: 1) increased intrapancreatic

pressure within the pancreatic duct or parenchyma resulting in tissue ischemia; 2) inflammation in the pancreas; 3) extrapancreatic causes of pain such as bile duct and duodenal stenosis due to extensive pancreatic fibrosis and inflammation, and 4) maladaptive plasticity within the nervous system including alterations in peripheral nerves and central sensitisation.[11-13] Because these underlying pain mechanisms are poorly understood, treatment is challenging and often unsatisfactory.[14] Therefore, pancreatic pain carries a high burden of morbidity because of its long duration and recurrent attacks.

Chronic postsurgical pain

Chronic postsurgical pain (CPSP) is increasingly recognized as a potential adverse outcome of surgery, in particular after limb amputation, breast surgery, thoracotomy and inguinal hernia repair.[15-17]
CPSP is defined as pain developing after surgery and persisting for at least three months.[4] However, many debate this timeframe, as wound healing and postsurgical inflammation can still persist and pain complaints may decline up to over a year.[18-20]
Incidences of moderate to severe CPSP at 12 to 36 months after surgery range from 12% - 18% in European countries.[21,22] In 23% of the patients visiting chronic pain clinics, surgery is considered the cause of pain, that is most frequently (48%) located in the abdomen.[23]

Abdominal CPSP is often refractory to treatment and thought to be the result of a combination of factors. Some researchers suggest that intra-abdominal adhesions are the primary cause for this pain.[24] Adhesions are fibrous bridges that connect two (abdominal) tissues that normally freely move along each other and develop after nearly every abdominal surgery. In studies following patients with chronic postoperative pain after previous surgery, adhesions were identified as the most likely cause of pain during diagnostic laparoscopy in 57% of patients.[25] An alternative hypothesis for explaining persistent postsurgical abdominal pain is that pain results from nerve injury (i.e. neuropathic pain). Hence, neuropathic pain develops as a result of a lesion or disease affecting the somatosensory nerves system either in the peripheral or central nervous system.[26] Moreover, CPSP is associated with changes in pain sensibility originated from plasticity of the central nervous system,[27] and decreased inhibitory pain modulation.[28,29]

Neural reorganization of chronic pain

A complex neural reorganization from the periphery to the neocortex seems to occur for different types of chronic pain.[30] First, pain chronicity is associated with peripheral reorganization of afferent signalling, changing sensitivity for nociceptors, and possibly for tactile afferents.[30] Chronic pain is also associated with increased responsiveness

of nociceptive neurons in the central nervous system, termed central sensitization.[31] Changes to nociceptive signal processing in the central nervous system are typically expressed as hyperalgesia, i.e. increased pain in response to noxious stimuli, and allodynia, i.e. pain in response to a non-nociceptive stimulus. Additionally, accumulating evidence shows that the human brain undergoes extensive reorganization in chronic pain conditions.[32] Several characteristic changes in central pain processing are also observed in patients with painful CP and CPSP,[12,13] but need to be further investigated.

Pharmacological pain management

The standard extrapolated guideline for analgesic treatment follows the principles of the "pain relief ladder" provided by the World Health Organisation.[33] However, satisfactory pain relief is often lacking or incomplete, which can be attributed to the different multidimensional pain mechanisms underlying the individual chronic pain condition. Because central sensitization alters the properties of neurons in the central nervous system, the pain is frequently no longer reliably coupled to the presence of particular peripheral stimuli. Therefore, research is increasingly focused on (adjuvant) analgesics, that modify these underlying pain processes.

Cannabis-based drugs as potential analgesic

The medical use of cannabis has received increasing attention since the discovery of delta-9-tetrahydrocannabinol (THC) in 1964, which is the principal psychoactive compound of the *Cannabis sativa* plant.[34] Interest in the therapeutic potential of cannabinoids has escalated further with the discovery and cloning of the endocannabinoid system in the early 1990s. THC induces its effects by binding to two types of G-protein-coupled cannabinoid receptors, termed CB1 and CB2.[35,36] CB1 receptors are predominantly found in the brain and spinal cord, in particular highly expressed in brain regions critical for emotion processing including the amygdala, hippocampus, and anterior cingulate cortex.[37] Although CB2 receptors are also found in the central nervous system, CB2 is mainly considered a peripheral cannabinoid receptor. CB2 receptors are mostly observed on cells of the immune system, including the pancreas, and is therefore speculated to play a part in immunoregulatory responses.[38]

Patients who use medicinal cannabis these days, usually take in THC by means of smoking or vaporizing. Inhalation is known to produce a reliable pharmacokinetic profile, however it has some obvious disadvantages. Additionally, whole plant extracts of cannabis contain a complex mixture of natural cannabinoids and other chemical compounds. These may interact to provide a superior therapeutic profile, but on the other hand, may also induce unintended adverse events. To avoid adverse events, new

pharmaceutical products were developed containing either natural or synthesized THC (dronabinol is the international non-proprietary name (INN) of THC). One of these pharmaceutical products is Namisol® (Echo Pharmaceuticals, Weesp, The Netherlands), which is an oral tablet containing pure, natural THC isolated from the Cannabis sativa plant. It was developed using a novel drug delivery technology, Alitra™, to improve the absorption and bioavailability of poorly soluble lipophilic compounds. A previous phase I study of single doses THC demonstrated reliable pharmacokinetic and tolerability profiles in healthy young volunteers.[39]

Neurophysiological assessment of pain

Several techniques have been used to study the complex reorganisation of chronic pain in the central nervous system, e.g. quantitative sensory testing (QST), positron emission tomography (PET), (functional) magnetic resonance imaging ((f)MRI) and electroencephalography (EEG). Each technique, has greatly contributed to our knowledge of underlying pain mechanisms and provided new insights for the diagnoses and treatment of chronic pain. These techniques are also increasingly used to evaluate the efficacy and mechanisms of (novel) analgesics.

QST comprises a standardized sensitivity test consisting of thermal, mechanical or electrical stimuli. Calibrated stimuli are applied to capture perception and pain thresholds, thus providing information on the presence of sensory loss as well as a gain of function (e.g. hyperalgesia),[40] linked to different levels of the nervous system. QST has the disadvantage that it is a psychophysical test, reflecting the subjective report from a patient to a objective stimulus, making QST susceptible to various kind of biases and measurement errors.

Neuroimaging tools such as fMRI and PET have a high spatial resolution, but measure indirect measures of neuronal activity, resulting in a relatively poor temporal resolution. EEG directly measures the brain's electrical activity, giving high temporal resolution but low spatial resolution. Other advantages of EEG are the relatively low costs and portable and easy to apply equipment compared to neuroimaging equipment. This allows EEG-based methods more easily for clinical use.

Electroencephalography

In 1924, Hans Berger recorded the first human EEG on the surface of the scalp.[41] He observed that EEG consistently changed with the general status of the subject, e.g. from relaxation to alertness. Berger also concluded that brain activity could be seriously affected by certain pathologic conditions such as convulsive seizures.[42] Since then, EEG measurements are commonly used for both clinical and research purposes.

EEG is described as electrical activity of an oscillating type generated by brain structures

recorded from the scalp surface. Neurons are specialized cells that are able to transmit electrical signals from one cell to another and produce local current flows. EEG measures mainly the electrical currents that flow during synaptic excitations of the dendrites of many pyramidal neurons in the cerebral cortex. Only large populations of active neurons can generate electrical activity recordable on the scalp surface by amplification strategies. The EEG originates from the difference between the electric potential of a surface electrode with respect to a reference surface electrode. The peak to peak amplitude of the EEG is relatively small, normally ranging from 0.5 to 100 μV in amplitude, when measured on the scalp. EEG recordings are generally divided into two types: spontaneous EEG at a resting state and evoked EEG in response to a stimulus.

Frequency analysis of spontaneous brain activity

The spontaneous EEG, also called resting state EEG, is recorded during a state of awake rest and characterized by sinusoidal oscillations. Several signal processing techniques, such as Fast Fourier Transform (FFT), are applied to extract specific characteristics from the raw EEG signal. FFT transforms a signal from the time domain into the frequency domain. Basically, the raw EEG signal consists of multiple slow and rapid oscillations, that can be broken down in sinusoids at each different frequencies and plotted in a frequency power-spectrum. The bandwidth ranges from 1 Hz to about 80 Hz and is typically described in distinct frequency bands, such as delta (1-4 Hz), theta (4-7.5 Hz), alpha (7.5-13 Hz), beta (13-32 Hz), and gamma (32-80 Hz). The resting state EEG with eyes closed is dominated by oscillations in the alpha-band, most prominently recorded at the parietal and occipital cortex.[43]

Evoked brain potentials

Evoked potentials (EPs) involve voltage polarity changes in the EEG in response to the onset of a stimulus. EP amplitudes tend to be low compared to amplitudes of the spontaneous EEG activity components, so single EPs are difficult to recognize from the raw EEG signal. Therefore, EP recordings are averaged across epochs of EEG time-locked to a set of repeated stimuli. This signal averaging improves the signal to noise ratio, whereby the stimulus-specific activity becomes visible as an EP.[44] An EP thus reflects, with high temporal resolution, only that brain activity which is consistently associated with the stimulus processing. EPs can be elicited by stimuli of various modalities, which may be electric, auditory, visual, somatosensory, etc., resulting in different stimulus-specific EP patterns. An EP is characterised by different peaks with corresponding latencies and amplitudes. It is generally assumed that early components of EPs largely depend on the physical parameters of the stimulus, whereas late components of EPs are related to the manner in which the subject evaluates the stimulus.[45]

Aims of this thesis

The first goal of this thesis was to investigate potential neuroplastic changes in brain activity associated with chronic pain using both spontaneous and evoked EEG recordings. The second goal of this thesis was to evaluate the therapeutic potential of a novel oral tablet containing purified THC for the treatment of chronic abdominal pain. Therefore, several phase II clinical drug studies were performed in order to evaluate the efficacy, safety and tolerability of oral THC in patients with chronic abdominal pain. Beyond these clinical outcome measures of analgesia, we aimed to investigate experimental pain measures utilizing EEG in order to study potential antinociceptive effects of THC in chronic pain management.

Thesis outline

The aims of this thesis have been elaborated in observational EEG studies, phase 2 clinical drug studies using several clinical and experimental outcomes and a review. The thesis can be subdivided into three consecutive parts:

PART I: Cortical processing in chronic (postsurgical) pain

Changes in brain activity have been observed in several chronic pain conditions, suggesting that chronic pain involve changes in central pain processing mediated through mechanisms of neural plasticity. This concept of central plasticity has been further explored in the first part of this thesis utilizing different EEG techniques.

In **Chapter 2**, we described the cortical processing of painful stimuli recorded in the EEG in patients with persistent pain after breast cancer surgery compared with those patients without persistent pain after breast cancer surgery. Changes in cortical processing were recorded in the EEG utilizing pain related EPs to noxious electrical stimuli. We hypothesized that chronic pain is associated with an enhanced brain response to painful stimuli in supposed neuropathic pain.

Alterations in central pain processing were also studied in patients with painful CP, a serious form of visceral pain, which is described in **Chapter 3.** In this study, we investigated the brain's resting state activity within the alpha frequency band in chronic pain patients related to CP in order to explore novel potential EEG biomarkers for chronic pain. The clinical usefulness of EEG biomarkers for CP pain and the relation to disease progression were also addressed.

PART II: Efficacy and safety of tetrahydrocannabinol in chronic abdominal pain

The majority of clinical trials assessing the efficacy of THC for pain treatment have been focused on cancer-related pain, central neuropathic pain syndromes, and acute pain conditions. We aimed to investigate the therapeutic potential of THC in patients with

chronic abdominal pain.

Chapter 4 describes the results of a randomized, single dose, double-blinded, placebo controlled crossover study in patients with painful CP. We present the analgesic efficacy, pharmacokinetic profiles, pharmacodynamic effects and safety results of a single dose oral THC in CP patients with chronic abdominal pain subdivided into opioid and non-opioid users.

The results of a randomized, double-blind, placebo-controlled trial evaluating the analgesic efficacy, pharmacokinetics, and tolerability of oral THC in patients with chronic abdominal pain during a treatment period of 50-52 days are described in **chapter 5**. THC was administered 3 times daily during a treatment period of 50-52 days.

Chapter 6 provides an overview of clinical trials that have been conducted to investigate the analgesic efficacy of cannabis-based products with standardized THC content for chronic non-malignant pain. Furthermore, common limitations in clinical trials and a mechanism-oriented approach to evaluate the therapeutic potential of THC are discussed in this review.

PART III: Neuronal mechanisms of tetrahydrocannabinol

The experimental outcome measures of the clinical trials comparing THC and placebo are discussed in the last part of this thesis. **Chapter 7** addresses the antinociceptive effects of THC by investigating underlying pain related cortical activity in a crossover study. We investigated whether a single dose of orally administrated THC alters the resting state EEG and EPs to pain related electrical stimuli in patients with chronic pancreatic pain. Additionally, the reproducibility of EEG patterns over a two week period was evaluated within this study.

Neural correlates of THC in relation to its analgesic potency were further explored and reported in **chapter 8.** We evaluated the long term effects of THC after a treatment period of 50-52 days using similar pain related EEG indices in patients with chronic abdominal pain due to CP or surgery. We hypothesized that THC would decrease theta activity at a resting state and reduce evoked brain amplitudes. The relation between clinical pain intensities and objective EEG outcomes were also analyzed and discussed in this chapter.

Chapter 9 addresses the main findings with respect to literature and future perspectives. Part of this general discussion is a narrative on mechanism-oriented approach to pain in chronic pancreatitis.

Chapter 10 provides a summary of the studies presented in this thesis in English and Dutch.

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PART I

Chapter 2

Patients with persistent pain after breast cancer surgery show both delayed and enhanced cortical stimulus processing

Van den Broeke EN, de Vries M, van Goor H, Vissers KC, van Rijn CM, Wilder-Smith OH.
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ABSTRACT

Background: Women who undergo breast cancer surgery have a high risk of developing persistent pain. We investigated brain processing of painful stimuli using electroencephalogram (EEG) to indentify event-related potentials (ERPs) in patients with persistent pain after breast cancer treatment.

Methods: Nineteen patients (eight women with pain, eleven without persistent pain), who were treated more than 1 year previously for breast cancer (mastectomy, lumpectomy, and axillary lymph node dissection) and/or had chemoradiotherapy, were recruited and compared with eleven healthy female volunteers. A block of 20 painful electrical stimuli was applied to the calf, somatopically remote from the initially injured or painful area. Simultaneously the EEG was recorded, and a visual analogue scale (VAS) pain rating obtained.

Results: In comparison with healthy volunteers, breast cancer treatment without persistent pain is associated with accelerated stimulus processing (reduced P260 latency) and shows a tendency to be less intense (lower P260 amplitude). In comparison to patients without persistent pain, persistent pain after breast cancer treatment is associated with stimulus processing that is both delayed (ie, increased latency of the ERP positivity between 250-310 ms [P260]), and enhanced (ie, enhanced P260 amplitude).

Conclusion: These results show that treatment and persistent pain have opposite effects on cortical responsiveness.

INTRODUCTION

In recent years interest has grown in the alterations in brain processing present in patients with persistent pain. Brain imaging techniques like fMRI and PET have been used to investigate brain function by measuring the evoked response to applied somatosensory stimuli.[1,2] The results regarding altered pain processing by the brain in the context of persistent pain are highly incongruent, perhaps due to large variability between the patients regarding pain history, pain etiology, pain distribution and psychological characteristics.[1,2]

Use of a postoperative model may help overcome some of these problems, because it permits study of a homogenous patient population regarding pain etiology, pain distribution and treatment. Furthermore this model makes it possible to differentiate between the effect of treatment and the effect of pain because a comparative patient group (same treatment, but no pain) can be included for comparison.

It has been shown that women who undergo surgery for breast cancer have a high risk of developing persistent postsurgical pain.[3-6] This pain persistence is difficult to treat and is accompanied by a significantly diminished quality of life.[5,7]

The often used generic term "postmastectomy pain syndrome" in cases of persistent pain after breast cancer treatment might suggest a homogeneous disease category. But this is debatable.[8] In fact, different types of pain have been observed after breast cancer treatment, like phantom breast pain[3,9], scar pain[10], neuropathic pain[6], complex regional pain syndrome[11], pain arising from the axillary web syndrome[12] and the more recently prospectively investigated myofacial pain syndrome, which is typically observed during the first year after breast surgery including axillary lymph node dissection (ALND).[13]

The etiology of persistent pain after breast cancer treatment is probably multifactorial.[8] This is because breast cancer treatment includes different types of surgical interventions (ie, mastectomy, lumpectomy, sentinel lymph node biopsy, and ALND), and adjuvant therapies like chemotherapy, radiation and endocrine therapies. All these interventions may contribute to the development of the persistent pain, and could have their own characteristics. However, nerve damage and radiotherapy appear to be significant risk factors.[8]

A frequently observed phenomenon in persistent postsurgical pain conditions, and also in patients after breast cancer surgery, is a change in the sensitivity of tactile and pain processing. This change consists of a combination of sensory loss, particularly in the skin innervated by the possibly damaged nerves, and hypersensitivity.[4,6,8,14,15]

To our knowledge, studies investigating the evoked brain response using electroencephalography (EEG) in the context of persistent postsurgical pain are scarce.[2,16] In contrast to fMRI and PET, EEG directly measures neuronal activity; furthermore it makes it possible to study the sequential activation of different brain structures in time. The aim of this study is to investigate brain processing of painful stimuli using EEG (or more specifically, event-related potentials [ERPs]) in patients with persistent pain after breast cancer surgery. To investigate possible changes in ERPs as result of the presence of pain, these results (ie, from patients with pain) are compared with those in women without persistent pain after breast cancer surgery. In addition, we aim to investigate possible ERP changes as result of breast cancer treatment by comparing the results of the patients without pain with healthy female volunteers. Our main hypothesis is that persistent pain is associated with an enhanced brain response to painful stimuli.

MATERIALS AND METHODS

Participants

Nineteen patients (eight women with pain and eleven without pain) who had been treated for breast cancer were recruited from a clinical database of the Radboud University Nijmegen Medical Centre. Approval for the study was obtained from the Medical and Ethical Review Board Committee region Arnhem-Nijmegen, Nijmegen, The Netherlands (NL 30189.091.09). All subjects signed an informed consent form. At the moment of inclusion none had evidence for metastases or disease recurrences. All patients (with and without pain) had been operated ≥ 1 year ago at the time of participating. Patients all underwent a mastectomy or lumpectomy and ALND but no breast reconstruction. The rationale for investigating this population of patients is the high incidence of persistent pain after this type of surgery (mastectomy or lumpectomy + ALND).[3,4] Only patients who had unilateral breast cancer were included. Persistent pain was defined as pain persisting continuously or intermittently for more than 3 months after surgery.[17]

Besides patients, eleven healthy female volunteers were also recruited from the Nijmegen area. Patients as well as healthy volunteers were excluded from the study if they:

1) had undergone breast reconstruction,
2) had a psychiatric or neurological condition (for patients; neurological signs as a result of the treatment were excepted),
3) used pain medication or other medication that potentially affects brain processing like anti-depressants, anti-psychotics, anti-epileptics and benzodiazepines (hormone therapy as adjuvant therapy used by the patients excepted),

4) suffered from any pre-existing pain or pain syndrome.

Subjects were instructed not to consume caffeine-containing beverages for twelve hours before the recording session. This was to avoid the caffeine-induced theta decrease in EEG.[18]

Variables measured

Demographic and clinical characteristics

The composition of the two breast cancer surgery groups (with and without pain) was based on a standardized question (obtained via an interview by telephone) whether the patient experienced ongoing pain (yes or no) as a result of the breast cancer treatment. For confirmation, the same question was asked again on the day of measurement, together with an additional standardized question (only if the patient experienced pain) regarding pain intensity as a measure of past experienced pain load ('What is the averaged intensity of the breast treatment-related pain during the last three months on a numeric 0-10 rating scale (NRS)?').

Other demographic and clinical characteristics obtained were age, menopausal status, surgical treatment, and chemotherapy, radiation, and/or hormone therapy.

Patients who undergo ALND during breast cancer treatment are at risk for developing lymphedema.[19] Hypothetically, this could contribute to the persistence of pain. Therefore we measured limb volume differences (unaffected compared to. affected limb) as an indirect reflection of the possible presence of lymphedema. To do so, we measured the limb volume of both sides (arms) via water displacement.[20] Subjects were instructed to lower their arm slowly into a fully filled volume meter and asked to stop when the top of the volume meter came in contact with the axilla.[20] The amount of spilt water was collected in a measuring cup (ml). The volume of the opposite (control) arm was also measured. The difference in volume of spilt water between the two sides (affected and control) was calculated. This test was also performed in the healthy volunteers to test if there are normally differences in volume between the two sides.

Data about the type of pain and pain-related sensory signs in the patients with pain were collected using the Douleur Neuropathique 4 (DN4) questionnaire.[21,22] This questionnaire includes pain descriptors as well as three clinical tests reflecting altered somatosensory processing. The tests were performed by a physical therapist. For measuring hypoesthesia to touch, a brush (SENSElab, brush-05; Somedic, Horby, Sweden) was applied on different skin sites in the location of the pain. For measuring hypoesthesia to pinprick, a Semmes-Weinstein monofilament (nr. 5.07, 10.0 g) was applied to different skin areas in the location of the pain. For measuring brush evoked or increased pain within the location of pain, the same brush as for hypoesthesia was used. The effects of stimulation of the first two clinical tests (hypoesthesia to touch and pinprick) were quantified by comparing the

skin sites in the location of pain to a control site on the contralateral body site.
It is important to mention that, in this study, the DN4 questionnaire is not used as a screening or diagnostic instrument for neuropathic pain, because at present it is not validated for this purpose in this population of surgical patients. Thus we used the DN4 exclusively to collect data regarding the clinical qualitative characteristics of the pain syndrome.
Patients of both groups (with and without persistent pain) were asked if they had experienced tactile hypaesthesia or numbness since their treatment. If they did, they were asked to draw on a map the size and anatomical area of hypaesthesia.

Electrophysiological measures
A multi-channel electroencephalogram (EEG) (BrainVision, Brain Products GmbH, Waldkirch, Germany) was recorded during the experiment (band-pass 0.1-100 Hz, sample frequency 2000 Hz) with 64 active electrodes mounted in an elastic electrode cap. The electrodes were arranged according to the international 10-20 system and electrode CPz was used as common reference. Eye movements were detected by horizontal and vertical electrooculogram (EOG) recordings. Horizontal EOG was measured from the outer canthus of the left eye, and vertical EOG supra orbital to the left eye. Impedance was kept under 20 kΩ for all leads.

Painful stimulation
Subjects received painful stimulation on the calf, between the medial and lateral head of the gastrocnemius, using a concentric electrode (CE).[23] Because of its concentric design and small anode–cathode distance this stimulating electrode produces a high current density at relatively low current intensities. In this way, depolarization is more limited to the superficial layer of the dermis (where nociceptive [Aδ] fibers are present) with less recruitment of deeper lying non-nociceptive fibers. Stimulation with this electrode produces a pinprick-like painful sensation. The stimulated site was balanced across patients with regard to the affected side. In healthy subjects, balancing was according to lateral dominance.

The stimulation protocol consisted of 20 double pulses (monopolar square wave; duration 0.5 ms and double-pulse interval 5 ms) with a random inter-pair interval ranging from 7 to 10 seconds. The double pulses were delivered through the CE using a constant current stimulator (Twister®, Dr. Langer Medical GmbH, Waldkirch, Germany) and with an intensity of 150% of the individual pain threshold. This individual pain threshold was determined by an ascending sequence of increasing current intensities starting from 0 mA and in steps of 0.5 mA. This procedure stopped when the pain threshold was

achieved, as verbally reported by the subjects. This threshold determination protocol was performed twice and the mean was used in the experiment to set intensity of stimulation.

During stimulation, subjects were comfortably seated in a chair and were instructed to passively perceive the stimuli with eyes closed (as this condition is less prone to artifacts), without making any movements. A computer display was placed in front of the subject (0.5 m) together with a computer mouse. The display was used to show the visual analogue scale (VAS) (see Behavioral measure), preceded by a tone (65 dB). Participants were instructed to open their eyes after the tone and use the mouse to mark the VAS, after which they closed their eyes again.

Behavioral measure
In order to quantify the amount of pain as a result of the painful stimulation, subjects were asked to rate, at random times within a train of 5 double pulses, the amount of pain caused by the last received stimulus on a VAS. The VAS ranged from 0 cm = "no pain" to 10 cm = "unbearable pain" and was rated by the subject by moving the mouse pointer (vertical line) on a horizontal bar.

Procedure
At the beginning of the experiment, demographic and clinical characteristics were collected. Next, the individual pinprick-like pain thresholds for the double pulse stimulation were determined. Finally, subjects received the experimental painful stimulation with simultaneous recording of the EEG.

Signal analysis
Event-related potentials
The EEG was analyzed offline using the software Brain Vision Analyzer sotware (v. 2.0; Brain Products GmbH, Gliching, Germany) and Matlab (2011a; MathWorks, Natick, MA). As a first step, the continuous EEG was referenced to a common average (ie, all electrodes). Next, the EEG signal (2500 Hz) was high-pass filtered at 1 Hz and low-pass filtered at 30 Hz. Based on the onset of the stimulus, the EEG was segmented into epochs from -100 ms pre-stimulus to 1000 ms post-stimulus with a total period of 1100 ms. Bad segments containing ocular artifacts were corrected using the Gratton-Coles method.[24] Segments were also inspected for other artifacts like muscle or jaw and line noise activity and were removed if necessary. As a last step baseline correction (-100 – 0 ms) was applied to all segments.
For each subject separately, all segments were averaged to obtain an averaged subject-

specific event-related potential waveform. ERP components were defined in terms of their latency and topographic distribution. Subsequently the grand average global field power (GFP) of all subjects was calculated.[25,26] Next, we calculated the topographic voltage distribution corresponding to the ERP latencies identified in the GFP plot. Then we identified the electrode in the topographic plot which shows the maximal activity and used this electrode for subsequent analysis. To insure accurate identification of point of maximal activity we also inspected the grand average ERPs (of all electrodes) for all subjects.

Individual ERP latencies were determined in the individual GFP plot corresponding to the windows of the grand average GFP latencies.[26] The mean amplitude of each ERP component was calculated at the individual GFP-latency ± 5 ms at the electrode of maximal activity.[26] The rationale for using the mean activity instead of the more commonly used maximal peak value (baseline-to-peak) is that, the fewer trials included in the subject-specific average, the more residual noise is superimposed on the maximal peak, and thus the more the maximal peak of the subject-specific average will be determined by residual noise rather than by the peak of interest. Therefore, we calculated the mean amplitude instead of the maximal peak amplitude because the former value is more stable and representative of evoked activity.[27]

Statistical analysis

The software package Graphpad Prism 5 (Graphpad, San Diego, CA) was used for statistical analysis. Because of the small sample sizes and non-Gaussian distributions, nonparametric test statistics were used for between-group comparisons. A Kruskall-Wallis test statistic (H) was used for ratio variables. In the present study only two pairs of post-hoc comparisons were tested; healthy volunteers compared to patients without pain (effect of treatment) and patients without pain compared to patients with pain (effect of pain). The Dunn's multiple comparisons test, which corrects for the number of statistical tests, was used as post-hoc test. The effect size *r* was calculated as the Z-score divided by the square root of the total number of observations. Categorical variables were tested using the Chi-squared ($\chi 2$) test statistic ($p < 0.05$).

RESULTS

Clinical and demographical characteristics

Clinical and demographical characteristics are shown in Table 1 A-C and 2.

No statistically significant differences were observed between the three groups with respect to age and limb volume differences. Median (and interquartile ranges) age and limb volume differences scores are shown in Table 1 A-C.

Table 1 Demographic and clinical characteristics of the patients (**A**) with pain, (**B**) without pain and (**C**) healthy volunteers

(A)

Patient	**Age (years)**	**Menopausal status**	**Surgical treatment**	**Additional treatment**		
				Chemotherapy	*Radiation therapy*	*Hormone therapy*
1	52	post	Mast + ALND (II)	Yes (FEC)	Yes	Yes (TAM)
2	50	post	Mast + ALND (II)	Yes (TAC)	Yes	No
3	63	post	Mast + ALND (II)	Yes (TAC)	Yes	Yes (TAM)
4	46	post	Mast + ALND (II)	Yes (TAC)	Yes	Yes (TAM)
5	57	post	Mast + ALND (II)	Yes (FEC)	No	Yes (TAM)
6	49	post	Lump + ALND (II)	Yes (FEC)	Yes	Yes (TAM)
7	65	post	Mast + ALND (II)	Yes (TAC)	Yes	Yes (TAM)
8	52	post	Mast + ALND (II)	No	No	No
Median	52					
I-Q range	49 - 61					
(%)				87.5	75.0	75.0

Patient	**Arm volume difference (ml)** *Affected side - unaffected side*	**Location of pain**	**Intensity pain (NRS)** *Mean score of last 3 months*
1	200	Arm + chest	6
2	-50	Arm	6
3	-60	Small area arm + chest (nipple and armpit)	6
4	20	chest	3
5	170	Upperarm + chest	6
6	-40	arm	3
7	60	Small area arm + chest	4
8	-110	Armpit (upperarm + top) + chest (scar)	4
Median	-10		5
I-Q range (%)	-57 - 142		3 - 6

(B)

Patient	Age (years)	Menopausal status	Surgical treatment	Additional treatment			Arm volume difference (ml)
				Chemo therapy	*Radiation therapy*	*Hormone therapy*	*Affected side - unaffected side*
1	32	post	Mast + ALND (II)	Yes (TAC)	No	Yes (TAM)	30
2	49	post	Mast + ALND (III)	Yes (TAC)	Yes	Yes (TAM)	260
3	58	post	Mast + ALND (II)	Yes (FEC)	No	Yes (ARI)	-50
4	45	post	Mast + ALND (II)	Yes (TAC)	Yes	Yes (TAM)	-80
5	42	post	Mast + ALND (II)	Yes (FEC)	No	Yes (TAM)	0
6	53	post	Mast + ALND (II)	Yes (TAC)	Yes	Yes (TAM)	170
7	58	post	Mast + ALND (II)	Yes (TAC)	No	Yes (TAM)	100
8	56	post	Mast + ALND (III)	Yes (TAC)	Yes	Yes (TAM)	330
9	47	post	Mast + ALND (III)	Yes (TAC)	Yes	Yes (ARI)	140
10	65	post	Lump + ALND (II)	No	Yes	Yes (ARI)	200
11	68	post	Lump + ALND (II)	No	Yes	Yes (TAM) and (ARI)	100
Median	53						100
I-Q range	45 - 58						0 - 200
(%)				81.8	63.6	100.0	

(C)

Control subject	Age (years)	Menopausal status	Arm volume difference (ml)
			Positive difference between left and right side
1	63	post	60
2	40	pre	20
3	50	post	70
4	61	post	30
5	46	pre	10
6	41	pre	20
7	42	pre	80
8	56	post	30
9	62	post	40
10	60	post	70
11	61	post	190
Median	56		40
I-Q range	42 - 61		20 - 70

Abbreviations: MAST, mastectomy; LUMP, lumpectomy; ALND, axillary lymph node dissection with between brackets the level of axillary dissection I, II, or III28; TAC, docetaxal (Taxotere®) + doxorubicin (Adriamycin®) + cyclophosphamide; FEC, fluorouracil + epurobicin + cyclophosphamide; ARI, Arimidex®; TAM, tamoxifen.

Table 2 Results of the Douleur Neuropathique 4 questionnaire

	Pain characteristics					Symptoms associated with the pain			Symptoms present in pain location		
	Burning	*Painful cold*	*Electrical shocks*	*Tingling*	*Pins and needles*	*Numbness*	*Itching*	*Hypoesthesia to touch*	*Hypoesthesia to pinprick*	*pain after Brushing*
1	–	–	X	X	–	X	–	–	X	–
2	X	–	–	–	–	X	–	X	X	–
3	–	–	X	X	X	X	–	X	X	–
4	–	–	–	–	–	X	X	X	X	–
5	–	–	X	–	X	X	–	X	X	–
6	–	X	X	X	X	X	–	–	–	X
7	X	–	–	–	X	X	–	–	–	–
8	X	–	X	–	X	X	–	–	X	–
% patients	37.5%	12.5%	62.5%	37.5%	62.5%	100.0%	12.5%	62.5%	87.5%	12.5%

Notes: Shown are the individual patient characteristics as well as group percentages regarding type of pain, associated

A significant association (χ^2 (2) = 7.972, p = .019) was observed between condition (healthy volunteers and patients) and menopausal status (pre- and post-). As can be seen in Table 1 A-C, all patients (with and without pain) are postmenopausal, whereas 44% of healthy volunteers are premenopausal.

No significant associations were observed between the two patient groups (with and without pain) regarding the type of surgical intervention (mastectomy + ALND or lumpectomy + ALND) and incidences of adjuvant therapies (chemotherapy, radiation, or hormone therapy), see also Table 1 A-C for incidences. The results from obtained from the DN4 questionnaire are shown in Table 2. Figure 1 shows the topography of hypaesthesia (numbness) drawn by the patients (with and without pain).

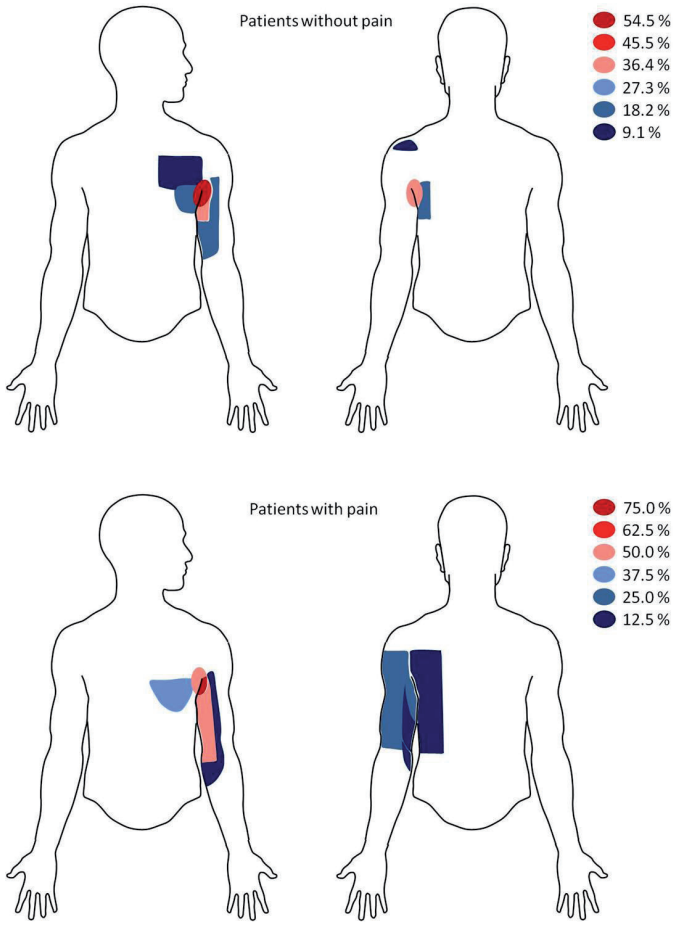


Figure 1 Area of tactile hypoesthesia (numbness).
Notes: This figure shows the topographical map of areas of tactile hypoesthesia (numbness) drawn by the patients without pain and with pain. The scale of percentages shown in the legend represents the number of patients (converted to percentages) who marked that area as hypoesthetic.

Stimulation intensity

No statistically significant differences were observed between the three groups regarding the applied stimulation intensities for noxious stimulation for ERPs. Median (and inter-quartile ranges) stimulation intensities were: healthy volunteers 3.0 (2.7 - 4.2) mA, patients without pain 3.3 (3.0 – 3.7) mA, patients with pain 3.9 (2.7 - 4.7) mA.

Behavioral tests

No statistically significant differences were observed between the three groups regarding the VAS-scores obtained during the noxious stimulation. Median (and inter-quartile ranges) VAS-scores were: healthy volunteers 4.2 (2.5 - 4.7) cm, patients without pain 3.0 (2.4 – 5.9) cm, patients with pain 2.5 (1.6 - 4.2) cm.

Event-related potentials

Based on the grand average Global Field Power (GFP) and corresponding topographic representations of all subjects (N=30) shown in Figure 2, we defined four distinctive ERP components:

1. A negative voltage between 110-180 milliseconds (ms), maximal at electrode FCz, which we label as N150,
2. A positive voltage between 190-230 ms, maximal at Cz, which we label as P200,
3. A positive voltage between 250-310 ms, maximal at FCz, which we label as P260,
4. A positive voltage between 310-380 ms, maximal at Cz, which we label as P350.

Figure 3 shows the topographic representations of the ERP components for each group at the ERP latencies.

ERP amplitude

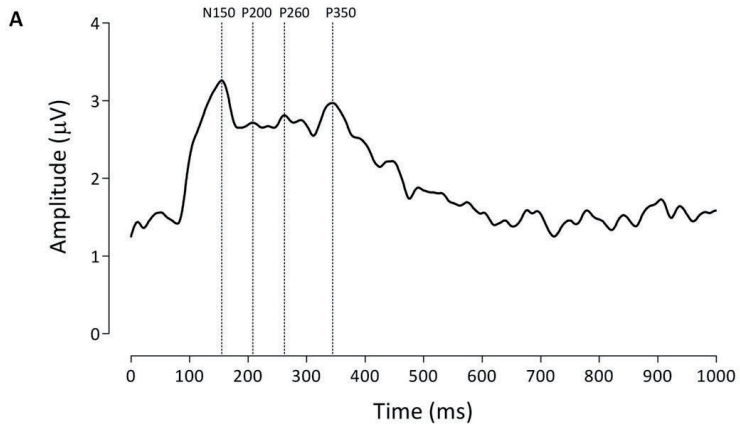
There were no statistically significant differences regarding N150, P200 and P350 between groups. Median and interquartile ranges are shown in Table 3. A statistical difference was observed for the P260 between the three groups (H (2) = 6.490, p = .039). Dunn's post-hoc tests revealed a statistically significant difference between patients with pain vs. patients without pain (p < .05; effect size r = -.49). Grand average ERPs of P260 are shown in Figure 4.

ERP latency

A statistically significant difference was observed between the three groups (H (2) = 9.367, p = .009) regarding P260 latency. Dunn's post-hoc tests revealed a statistically significant difference between patients without pain and healthy volunteers (p < .05; effect size r = .58) but also between patients with pain vs. patients without pain (p < .05; effect size r = -.56). Median and inter-quartile ranges are shown in table 3.

Table 3 Event-related potential (ERP) amplitude and latencies

	Healthy volunteers		Patients without pain		Patients with pain	
	Amplitude (μV)	*Latency (ms)*	*Amplitude (μV)*	*Latency (ms)*	*Amplitude (μV)*	*Latency (ms)*
N150 (FCz)	-2.2 (-7.0-2.4)	133.2 (128.0-159.6)	-4.6 (-6.7-1.1)	148.8 (123.2-176.4)	-3.4 (-8.1-0.8)	156.2 (146.8-161.8)
P200 (Cz)	2.7 (-1.9-4.1)	196.8 (190.0-218.4)	-1.5 (-5.0-1.5)	208.0 (196.4-224.4)	0.5 (-0.9-4.9)	203.4 (198.2-227.7)
P260 (FCz)	4.0 (2.9-6.8)	279.2 (266.8-302.8)	1.3 (-0.6-4.1)	255.6 (250.0-266.0)	5.7 (2.5-8.2)	284.4 (265-305.2)
P350 (Cz)	3.6 (2.5-7.3)	355.6 (320.8-380.0)	3.0 (0.6-5.1)	348.4 (332.0-372.4)	4.3 (3.0-8.9)	336.8 (327-351.5)



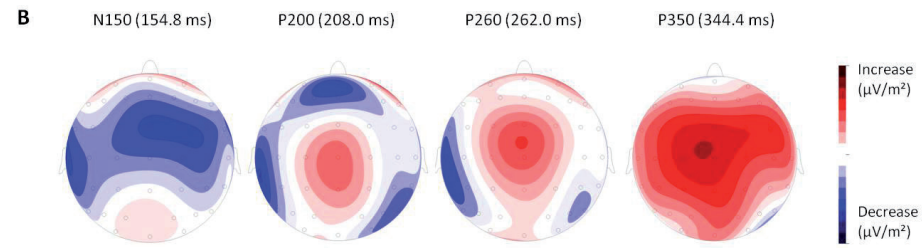


Figure 2 Grand average global field power (GFP) and corresponding topographic representations. (**A**) Grand average GFP (N = 30). The dotted lines indicate peak latency of the different event-related potential (ERP) components. Four different components can be identified: a negative voltage between 110–180 ms, maximal at FCz, labeled as N150, a positive voltage between 190–230 ms, maximal at Cz, labeled as P200, a positive voltage between 250–310 ms, maximal at FCz, labeled as P260, and a positive voltage between 310–380 ms, maximal at Cz and labeled as P350. (**B**) Topographic representations of the ERP components at the ERP latencies (N = 30). To best illustrate the maximal activity in each representation, we adjusted the scale to its maximal absolute values (for increases and decreases in voltages). As a result the scale differs between the different representations and is therefore left out.

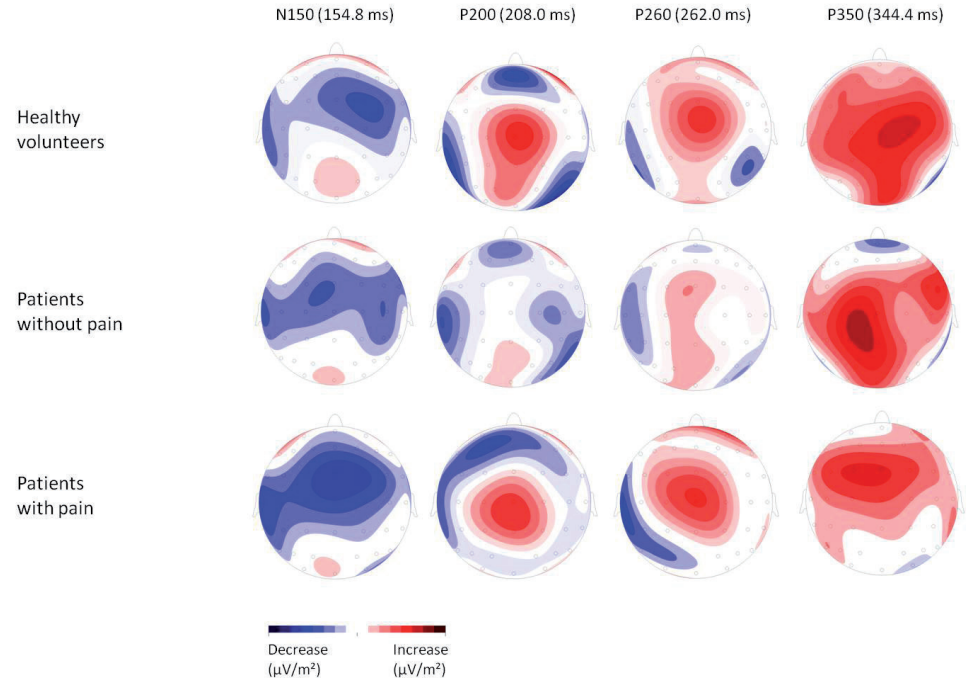


Figure 3 Group-specific topographic representations. Shown are the topographic representations of the different event-related potential (ERP) components at the different ERP latencies (Figure 2).
Notes: To best illustrate the maximal activity in each representation, we adjusted the scale to its maximal absolute values (for increases and decreases in voltages). As a result the scale differs between the different representations and is therefore left out.

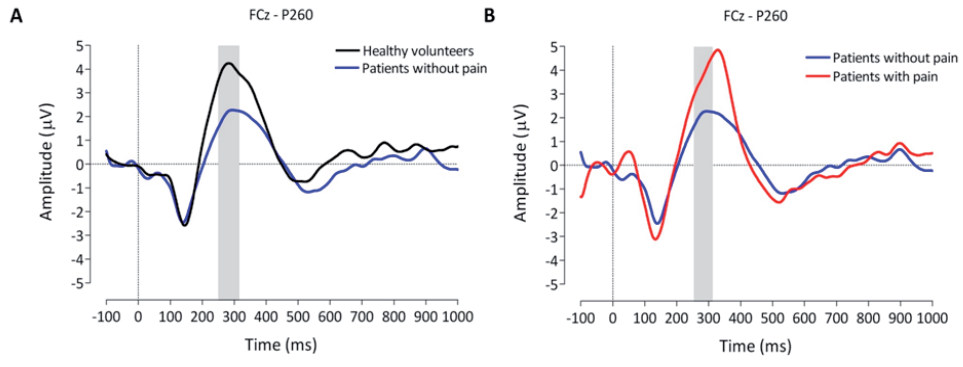


Figure 4 Event-related potential (ERP) waveforms. Grand average ERPs observed from FCz showing the P260 differences (**A**) effect of treatment, (**B**) effect of pain.
Notes: Upward deflection is positive charge and downward is negative charge. Representations of ERPs are with respect to common reference.

DISCUSSION

To our knowledge, this is the first study to investigate cortical processing by means of EEG and with this kind of stimuli in this group of patients. In comparison to patients without persistent pain, persistent pain after breast cancer treatment is associated with delayed and enhanced stimulus processing as reflected in an increased latency and enhanced amplitude of the ERP positivity between 250-310 ms (P260). Moreover, in comparison to healthy volunteers, breast cancer treatment is associated with a speeding of (reduced P260 latency) and a tendency towards a less intense (smaller P260 amplitude) stimulus processing. These results suggest that the two conditions, ie, treatment and pain persistence, have opposite effects regarding cortical responsiveness.

Breast cancer treatment and cortical processing

The comparison between patients without pain and the healthy volunteers reveals the effect of treatment on cortical processing. This comparison revealed a speeding of stimulus processing (reduced P260 latency) in patients without pain compared to the healthy volunteers. Moreover, there is a smaller late ERP amplitude (P260) in patients without pain vs. the healthy volunteers, however, not statistically significant according to the Dunn's post hoc test. This is probably due to the small sample sizes and the fact the p value has to be corrected for multiple comparisons. Indeed the effect size is r = -.45. Kreukels et al.[29] did observed a lower ERP amplitude in disease-free breast cancer survivors who were treated for breast cancer (including surgery and radiotherapy). All patients underwent surgery and radiotherapy. In this study the authors investigated the effect of different chemotherapy regimens on ERP activity in response to auditory stimuli (by using an oddball paradigm). Overall they observed a significantly reduced late ERP (i.e. P3) amplitude between patients that received chemotherapy as compared to matched control patients who had not received chemotherapy. Moreover, a shorter P3 latency was observed after chemotherapy. The authors did not find any changes in mid-latency N1 ERP amplitude or latency between the two groups (with and without chemotherapy), a finding in agreement with the present study.

Are there alternative factors that can explain the reduced brain activity? Regarding hormone therapy, Kreukels et al.[29] performed an additional sub analysis on their data in which they compared the ERP P3 amplitude between current, past and never users of tamoxifen. They found no significant difference in P3 amplitude between the three groups, suggesting that tamoxifen (and perhaps also other hormone therapy regimens) cannot explain the observed ERP reduction.

An as yet undefined pathophysiological process subsequent to amputation, e.g. deafferentation, might also change EEG activity.[30] This argument is based on the study of Karl et al.[30] Although not statistically significantly different, a lower P3 amplitude was observed in the amputees without pain compared to the healthy controls.

When we look at the clinical and demographic characteristics (Table 1) the proportion of premenopausal status between healthy women compared to the patients without pain is different. Theoretically, this could be a further factor explaining the differences in P260 amplitude between the two groups.

Persistent pain and cortical processing

The comparison between patients with and without pain reveals the effect of the presence of persistent post-surgical pain on cortical processing. Based on the results mentioned in the previous section, we suggest that breast cancer treatment (ie, chemotherapy) affects late ERP activity, ie, lower ERP amplitude and shorter latency. The larger ERP amplitude (and increased latency) seen in the patients with pain compared to the patients without pain is likely the result of the presence of pain additionally to the effect of breast cancer treatment. Therefore we conclude that persistent pain after breast cancer treatment is associated with delayed (increased P260 latency) and enhanced (larger P260 amplitude) stimulus processing.

Interestingly, Karl et al.[30], using an oddball paradigm, compared the visual P3 amplitude between upper limb amputees with and without persistent pain and healthy volunteers. Patients with pain showed significantly higher P3 amplitudes than patients without pain, but neither group were statistically different from the healthy volunteers. The latter result could be due to the small sample sizes (patients with pain N= 5, patients without pain N=5 and healthy volunteers N=10). However, the ERP findings observed in the study of Karl et al. appear to involve later ERP activity (between 300-500 ms) than in our study (between 250-310 ms). Possible explanations for the fact that in the two studies different ERP activities are affected are type of stimulus and paradigm used.

Methodological considerations

Defining (late) ERP components

The positivity around 260 ms (ie, P260) shares the same time course and topographic distribution as the previously described SP5 component (233-277 ms) evoked after painful electrical stimulation.[31] This ERP component seems to overlap with the more later positivity SP6 or pain related P2.

The positivity around 350 ms, labeled as P350, might be the pain related P2 evoked after painful electrical stimulation.[31,32] By comparing laser stimulation with electrical sural nerve

stimulation Dowman showed that this P2, evoked after painful stimulation, has similar properties as the commonly described P2, associated with selective A-delta fiber activation, and evoked after painful laser stimulation.[33-36]

However, Mouraux et al.[35] recently compared electrical intra-epidermal, electrical non-nociceptive transcutaneous and laser stimulation for their selectivity in generating A-delta fiber associated evoked brain responses. They showed that only laser and low intensity electrical intra-epidermal stimulation are able to evoke A-delta associated evoked brain responses. Additionally, they showed that intra-epidermal stimulation loses its selectivity with increasing stimulus intensity, something that occurred above intensities of 2.5 mA.[35] In the present study we used transcutaneous electrical stimulation with stimulation intensities around 3.0 mA, which tends to argue against the possibility that we selectively evoked A-delta associated brain responses.

Alternatively, the P350 could be a P3a-like component.[31,37,38] This hypothesis can be supported by the fact that:

1. A "single stimulus" paradigm as used in the present study, in which only target but no standard stimuli are delivered with long, variable and random interstimulus intervals, is able to evoke a P3a-like component,[39,40] also after painful electrical stimulation,[31] and
2. this positivity shares the same generators in the brain as the classic P3a, as is demonstrated via intracranially-recorded cortical responses evoked after painful electrical stimulation. These generators include the dorsolateral and medial prefrontal cortices, temporal-parietal junction and posterior hippocampus.[37]

Area of stimulation

In the present study, the painful stimuli were applied to a body part somatopically remote from the initially injured or painful area. We choose to do this because we wished to investigate cortical changes in pain processing (which one would expect to be generalized). For this, we need to stimulate in an area remote from the spinal segment undergoing nociceptive input due to breast cancer treatment. Our study therefore reflects only generalized but not localized effects of surgery or radiation therapy.

Sample size

An important methodological limitation of this study is the small sample size. This was the result of our opting for more strict inclusion and exclusion criteria (to avoid confounding factors), but has the advantage that the resulting patient groups are very homogenous.

Nevertheless, the ERP effects observed in the present study should be confirmed in a new future study with larger sample sizes.

Conclusions

This observational study shows that the two conditions, ie, treatment and persistent pain, have opposite effects regarding cortical responsiveness. Breast cancer treatment is associated with a speeding of and a tendency to a less intense stimulus processing. Persistent pain after breast cancer treatment is associated with delayed and enhanced stimulus processing. To our knowledge, this is the first study to investigate cortical processing by means of EEG and with this kind of stimuli in this group of patients.

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Disclosure

The authors report no conflicts of interest in this work.

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Chapter 3

Altered resting state EEG in chronic pancreatitis patients: toward a marker for chronic pain

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ABSTRACT

Objectives: Electroencephalography (EEG) may be a promising source of physiological biomarkers accompanying chronic pain. Several studies in patients with chronic neuropathic pain have reported alterations in central pain processing, manifested as slowed EEG rhythmicity and increased EEG power in the brain's resting state. We aimed to investigate novel potential markers of chronic pain in the resting state EEG of patients with chronic pancreatitis.

Participants: Resting state EEG data from 16 patients with persistent abdominal pain due to chronic pancreatitis (CP) were compared to data from healthy controls matched for age, sex and education.

Methods: The peak alpha frequency (PAF) and power amplitude in the alpha band (7.5–13 Hz) were compared between groups in four regions of interest (frontal, central, parietal, and occipital) and were correlated with pain duration.

Results: The average PAF was lowered in CP patients compared with that in healthy controls, observed as a statistically significant between-group effect (mean 9.9 versus 9.5 Hz; *P*=0.049). Exploratory post hoc analysis of average PAF per region of interest revealed a significant difference, particularly in the parietal and occipital regions. In addition, we observed a significant correlation between pain duration and PAF and showed increased shifts in PAF with longer pain durations. No significant group differences were found in peak power amplitudes.

Conclusion: CP pain is associated with alterations in spontaneous brain activity, observed as a shift toward lower PAF. This shift correlates with the duration of pain, which demonstrates that PAF has the potential to be a clinically feasible biomarker for chronic pain. These findings could be helpful for assisting diagnosis, establishing optimal treatment, and studying efficacy of new therapeutic agents in chronic pain patients.

INTRODUCTION

Diagnosis and treatment of chronic pain is challenging because, by definition, pain is a subjective experience and can be measured only by self-report.[1] Identification of physiological pain biomarkers for 1) disease severity, 2) disease progression, 3) disease prognosis, and 4) treatment effects including indication and responder identification, could help us to improve pain diagnostics and treatment. Increasing evidence supports the idea that chronic pain can be understood not only as an altered perceptual state, but also as a consequence of alterations in peripheral and central neuronal processing. Electroencephalography (EEG) can be a useful method to detect such alterations in central pain processing.[2-4]

The resting state EEG with eyes closed is dominated by oscillations in the alpha-band (7.5-13 Hz), which are widely distributed in the cerebral cortex and more prominent in the parietal and occipital regions. Resting EEG is commonly analyzed by transforming data from the time domain to the frequency domain. A measure derived from such analysis is the peak alpha frequency (PAF). The PAF is defined by two parameters: (i) the frequency at which it occurs on the frequency axis, and (ii) its amplitude on the power–density axis.

Sarnthein et al.[2] observed increased power amplitude differences in the alpha band, and a shift towards lower frequencies of the dominant peak in patients with mixed neurogenic pain syndromes. These results are supported by other resting state EEG studies investigating alterations in central pain processing in various chronic pain states.[3,5-7] Olesen et al. reported similar alterations in EEG activity in patients with chronic pancreatitis, observed as an increase in power amplitude in the θ and α frequency band.[4,8] However, they neither calculated PAF nor studied its relation to clinical pain parameters.

Chronic pancreatitis (CP) is a disease characterized by inflammation and progressive destruction of the pancreatic gland, which results in irreversible morphologic changes that typically cause pain and/or exocrine and endocrine insufficiency.[9] The most important symptom of CP is abdominal pain, present in 80-90% of patients in the time course of the disease.[10] Pancreatic pain is typically intense, long-lasting and difficult to treat.

Alterations in pancreatic nerves, including an increased number and diameter of nerve fibers and increased amount of neurotransmitters,[11,12] as well as alterations in central pain processing, including supraspinal sensitization, somatotopic reorganization and pro-nociceptive pain modulation were proposed as possible mechanisms underlying chronic pain in CP. [13-15] Altered central pain processing was demonstrated in a previous

study in CP patients using Quantitative Sensory Testing (QST), manifested as a widespread hyperalgesia (i.e. an increased pain sensitivity[16]) in distant, non-damaged tissues. This can be interpreted as a sign of spinal, supraspinal (cortical), or combined sensitization.[17] These observations support the role of central neuronal plasticity in the pain accompanying CP. If so, therapies exclusively directed at the pancreas as the nociceptive source are unlikely to be effective in achieving pain relief. Therefore, patients who may benefit from a treatment targeting central pain mechanisms need to be identified.

In the current study, we aim to investigate the brain's resting state activity within the alpha frequency band in patients with chronic pain resulting from CP in order to: (1) research novel potential EEG biomarkers, (2) investigate biomarker scalp localization, (3) study effects of disease progression on biomarkers, and (4) address the clinical usefulness of EEG biomarkers for CP pain.

METHODS

Subjects

Sixteen patients with persistent abdominal pain as a result of CP were randomly selected from the outpatient clinic of the Radboud University Nijmegen Medical Centre. CP was diagnosed based on medical history, laboratory tests and radiological findings according to the Marseille and Cambridge Classification System.[18] All patients had typical pancreatic pain, which is characterized as severe, dull epigastric pain, eventually radiating to the back. Intake of analgesics including opioids and centrally-acting medication was permitted. Patients with present alcohol use were excluded. Sixteen healthy participants were matched by age, gender and years of education. Previous studies suggest that this is an appropriate sample size to investigate the resting state EEG.[2,6,19]

Medical ethical approval was obtained for the measurements in healthy controls (Committee on Research involving Human Subjects, Region Arnhem-Nijmegen nr. 2002/008). The patients were all referred by their physician in charge for neuropsychological/ neurophysiological testing, as part of their medical follow up. The neurophysiologic testing results have already been published and revealed a decline in cognitive performance in the CP group.[20] Both patients and healthy participants gave written informed consent to use the data for scientific purposes.

EEG recording

EEG data were collected according to a standardized protocol using a Quickcap (NuAmps) with 26 scalp electrodes located according the international 10-20 electrode system (Fp1,

Fp2, F7, F3, Fz, F4, F8, FC3, FCz, FC4, T3, C3, Cz, C4, T4, CP3, CPz, CP4, T5, P3, Pz, P4, T6, O1, Oz, O2).[21] Electrooculogram (EOG) data were recorded from electrodes above and below the left eye and lateral to the outer canthi of each eye. Additional physiological data were obtained from the orbicularis oculus and the masseter muscles. Data were recorded at a sampling rate of 500 Hz and offline referenced to the mean of the signals recorded at the mastoids. The ground electrode was placed at Fpz. Electrode impedance was kept below 5 kΩ for all electrodes.

The spontaneous EEG or resting EEG was recorded during eyes closed and eyes open. Each recording lasted 2 minutes. All results presented in this study refer to the eyes closed condition to avoid artifacts and alpha activity is typically present during this condition. During the recording in eyes closed condition, participants were seated in a comfortable chair and were asked to close their eyes and relax. No further task was given.

EEG analysis

Brain Vision Analyzer 2.0 software was used for EEG analysis.

EEG data were band-pass filtered (1-120 Hz; phase shift-free Butterworth filters), and corrected for ocular artifacts according to the Gratton and Coles algorithm.[22] Each EEG recording was segmented into 12 epochs of 10 sec each. Subsequently, epochs were inspected for artifacts and rejected from further analysis if data exceeded an amplitude of 200 µV or exceeded the maximal allowed voltage step of 50 µV. This resulted in 1.7% rejection of all epochs, mainly concerning temporal electrodes. The power amplitudes of the EEG frequencies were computed using a Fast Fourier Transformation (FFT). To this end, epochs were multiplied by a Hanning window (10%), Fourier transformed and spectral distributions were averaged across all epochs for each participant and electrode separately.

Data analysis and statistics

Grand average power spectra were computed by averaging all scalp electrodes for each participant. These grand averages were averaged per group in order to obtain the overall power. Peak power amplitudes were determined as the maximum value between 7.5-13 Hz within empirically defined regions of interest (ROI). Positively skewed peak power amplitudes were log-transformed to normalize the data. A lack of lateralization, as shown in topographical distribution plots, provides the opportunity to average individual electrodes in ROI in order to obtain a more stable but targeted analysis. Hence, four horizontally arranged ROI were composed: frontal (Fp1, Fp2, F3, Fz, F4), central (FC3, FCz, FC4, C3, Cz, C4), parietal (CP3, CPz, CP4, P3, Pz, P4), and occipital (O1, Oz, O2) ROI.

Different methods can be used to quantify the variation of spectral distribution within the alpha range.[23] First, peak alpha frequency (PAF) can be measured by calculating

the frequency with the highest magnitude within the alpha range. Second, the center of gravity, rather than peak, can be measured. This gravity method has been used as a different, and possibly more stable measure of spectral distribution than the peak method.[23,24] Particularly if there are multiple peaks in the alpha range, the gravity method appears the more adequate estimate of PAF.[23] In the current study, a few participants demonstrated low-voltage EEG without clear peaks within the alpha band. The center of gravity method was assumed to be most appropriate since this method enables analysis of the entire dataset without excluding low-voltage EEG subjects from analysis. All participants demonstrated at least some peak, within 7.5-13 Hz range, assumed as the alpha frequency band, and were included for further analyses. The PAF is the weighted sum of spectral estimates, divided by alpha power, given in this equation (1).[25]

$$PAF = \Sigma(a_f \times f) / \Sigma a_f \quad (1)$$

a_f = Amplitude of frequency f

f = Frequencies within 7.5-13 Hz range (per 0.1 Hz)

For statistical analysis SPSS software for Windows v. 16.0 was used.

All variables were visually inspected and Kolmogorv Smirnoff Test was applied to test data distributions. A t-test for independent samples was applied on normally distributed data, otherwise a non-parametric Mann-Whitney U-Test was used. A General Linear Model (GLM) repeated measures ANOVA analysis was used to test whether there were statistically significant differences regarding PAF and peak power amplitudes between CP patients and healthy controls with respect to the ROI (frontal, central, parietal and occipital). Our dependent variable, the PAF, was normally distributed allowing parametric testing. Mauchly's test indicated that the assumption of sphericity had been violated. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimation. Post hoc analyses included exploratory pair-wise testing of each ROI separately using two-sided unpaired t-tests. A GLM repeated measures ANOVA was used to test whether there were significant differences between opioid and non-opioid users, and between the different etiologies of CP, with respect to the ROI. In addition, pain duration was correlated with EEG parameters using the non-parametric Spearman test. Controls did not have pain and were allocated zero scores on pain duration, and included in this analysis. In all tests the significance level was set at $p < .05$.

RESULTS

Research population

CP patients had a mean pain duration of 5.4 years, 8 patients had a history of alcohol abuse and 9 patients used opioid medication for pain relief (table 1). Matched controls did not use centrally-acting medication, and were all pain free expressed as zero scores on pain duration. No differences were observed between the CP and HC group with respect to age, gender and years of education (table 2).

Table 1. Demographic and clinical characteristics of individual patients with chronic pancreatitis.

No	Age (years)	Sex	Etiology	Pain (years)	Opioids	Other drugs
1	28	F	hereditary	6	MS Contin	PPI
2	50	M	idiopathic	5	-	PPI
3	57	F	alcohol abuse	10	Durogesic	-
4	40	M	alcohol abuse	6	Morphine	PM; NSAID
5	51	M	alcohol abuse	6	Temgesic	-
6	54	M	alcohol abuse	8	Morphine	AD
7	58	M	alcohol abuse	4	Tramadol	PM; AE
8	39	F	idiopathic	10	Durogesic/ pethidine	-
9	46	F	idiopathic	2	Oxycontin	-
10	72	M	idiopathic	6	-	PPI; NSAID
11	50	M	alcohol abuse	10	-	PM
12	48	M	biliary	4	Oxycontin	AE; BZ; PM
13	56	M	alcohol abuse	2	-	PM; PPI
14	24	F	idiopathic	2	-	-
15	52	F	alcohol abuse	5	-	AE; BZ; PM; Li
16	59	M	idiopathic	1	-	PPI
mean (SD)				5,4 (2,9)		

Notes: *Relevant drugs include antiepileptics (AE), benzodiazepines (BZ), antidepressants (AD), lithium (Li), non-steroidal anti-inflammatory drugs (NSAID), paracetamol (PM) and proton pump inhibitors (PPI).*

Table 2. Demographic and clinical characteristics of healthy controls and chronic pancreatitis patients.

	HC		CP		p
N	16		16		
Male / Female	10 / 6		10 / 6		NS
Mean (SD) age (years)	48.0	(11.27)	49.5	(11.91)	NS
Mean (SD) education (years)	11.9	(2.86)	11.8	(3.09)	NS

Abbreviations: healthy controls (HC); chronic pancreatitis patients (CP); not significant (NS)

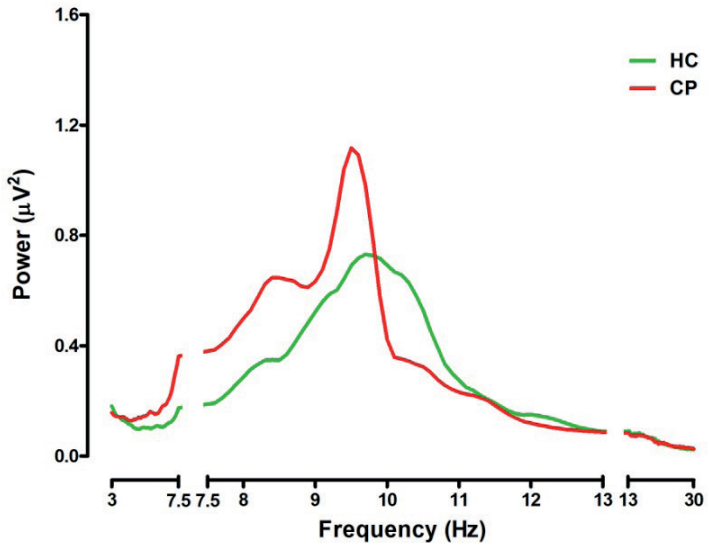

Figure 1. Grand average frequency power distributions averaged across all channels in patients with chronic pancreatitis (CP) compared to healthy controls (HC). This figure shows a shift towards lower frequencies and an increased amplitude in CP patients compared to HC.

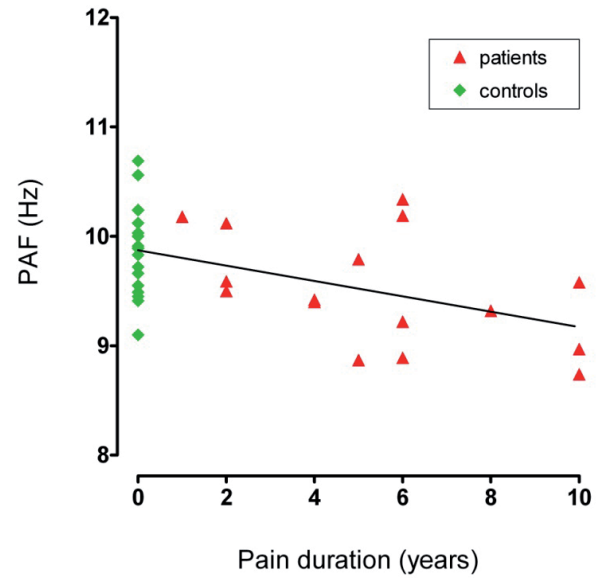

Figure 2. Individual pain durations and grand average peak alpha frequencies of both chronic pancreatitis (CP) and healthy controls (HC). HC were all pain free expressed as zero scores on pain duration. A significant correlation was found (r = -0.379; p= 0.032), indicating that an increase in pain duration is correlated with an increased shift of PAF.
Abbreviation: Peak alpha frequency (PAF)

Grand average power spectra

Absolute values of grand average power spectra amplitudes within the α-band are summarized for CP patients and HC in figure 1. No significant group differences were found in the logarithmically transformed peak power amplitudes. The corresponding PAF was significantly shifted towards lower EEG frequencies in the CP group compared to the HC group (mean ± SD: 9.9 ± .4 vs. 9.5 ± .5 Hz; 95% confidence interval of mean diff (CI) = -.68 to -.01 Hz; $P < .05$). Moreover, pain duration was significantly correlated with the grand average PAF (r = -0.379; p= 0.032), showing increased reductions in PAF with longer pain durations (figure 2).

Topographical power distributions

Differences in grand average power spectra between both groups were restricted to the frequency range between 7.5 to 10 Hz (figure 1). Thus we restricted the topographical analysis of EEG power to this part of the α-band (figure 3a-f). The topographical distribution plots showed maximum EEG alpha power in both groups as well as maximum group differences to be situated in parietal and occipital regions.

Power spectra

The average power frequency distributions were plotted separately per ROI (figure 4a-d). This figure suggests increased peak power amplitudes in CP patients compared to the HC group in each ROI, particularly parietal and occipital. However, logarithmically transformed peak power amplitudes did not differ significantly in any of the ROIs (table 3).

Table 3. Peak alpha frequency (PAF) and logarithmized peak power in healthy controls (HC) and chronic pancreatitis patients (CP).

	Mean (SD)				p
	HC		CP		
Frontal ROI					
PAF	9.7	(0.50)	9.4	(0.46)	0.190
Logarithmized peak power	-0.39	(0.98)	-0.19	(1.09)	0.586
Central ROI					
PAF	9.8	(0.42)	9.5	(0.49)	0.091
Logarithmized peak power	-0.31	(1.08)	-0.04	(0.97)	0.462
Parietal ROI					
PAF	9.9	(0.41)	9.6	(0.50)	0.037*
Logarithmized peak power	0.21	(0.97)	0.31	(1.34)	0.811
Occipital ROI					
PAF	10.0	(0.47)	9.6	(0.59)	0.019*
Logarithmized peak power	-0.24	(1.59)	0.32	(1.59)	0.332

Note: *$P<0.05$.
Abbreviations: regions of interest (ROI); standard deviation (SD).

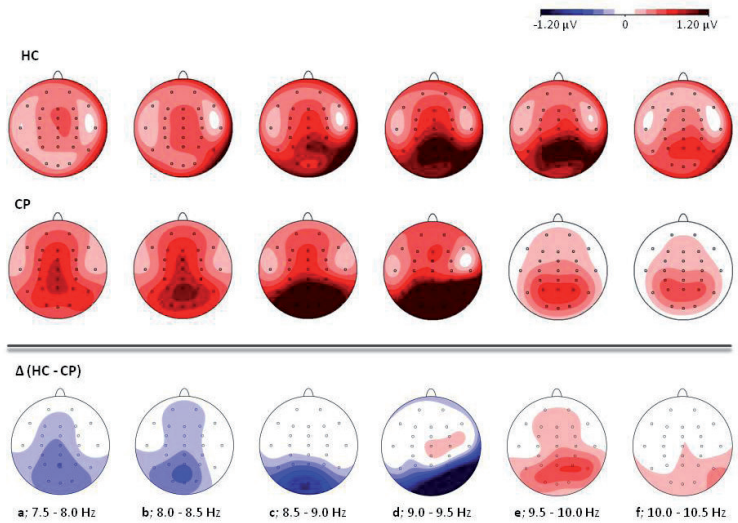

Figure 3a-f. Average topographical power distributions of the resting electroencephalogram. The average topographical distributions of EEG power showed the maximum amplitudes in the parietal-occipital regions in both chronic pancreatitis (CP) patients and healthy controls (HC). Scalp distributions are shown for frequency spectra within the alpha band.

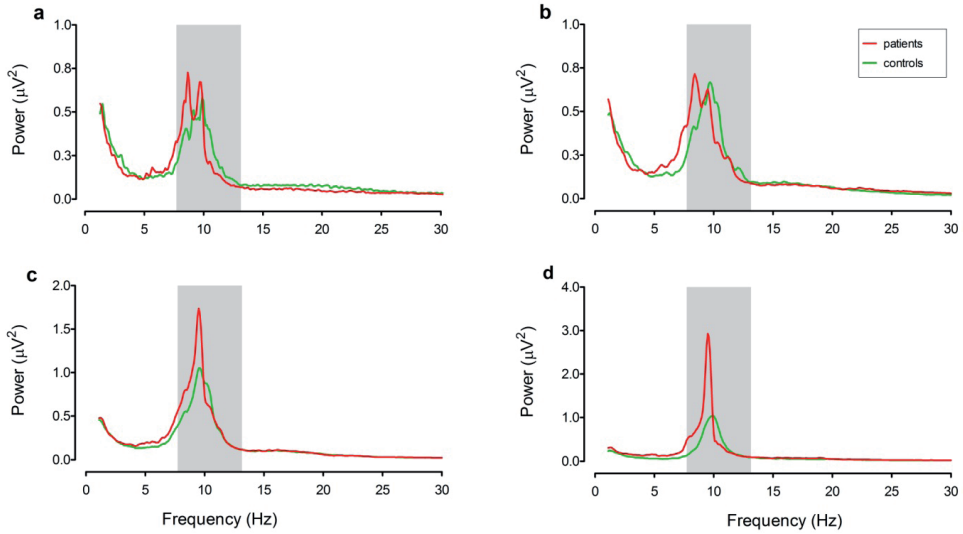

Figure 4a-d. Averaged power distributions within the frontal (a), central (b), parietal (c), and occipital (d) regions of interest (ROI) in chronic pancreatitis patients compared to healthy controls. The grey square represents the area within the alpha band.

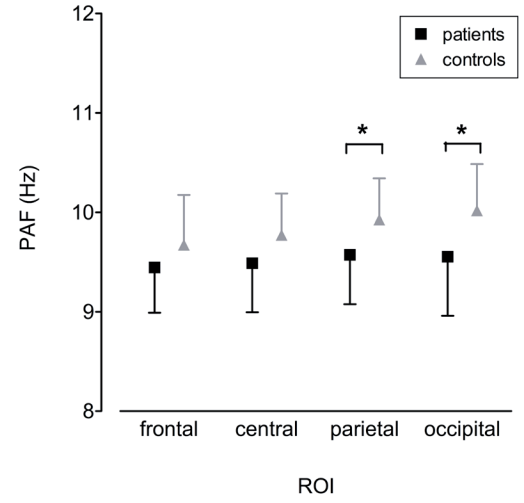

Figure 5. Peak alpha frequency in four regions of interest shown for patients with chronic pancreatitis compared with healthy controls. Red squares correspond to mean PAF in patients, green triangles correspond to mean PAF in controls, and short lines represent corresponding standard deviations. Asteriks indicate significant differences.
Abbreviations: ROI, region of interest; PAF, peak alpha frequency.

Peak alpha frequency

The mean PAF per ROI is shown in figure 5. A statistically significant between group effect was observed regarding the PAF in CP patients compared to HC (F= 4.20; p= 0.049). Within groups testing revealed a statistically significant difference of ROI (F= 11.62; p< 0.001). No significant interaction was observed between the effects of group and ROI on the PAF (F= 2.785; p= 0.085). Exploratory post hoc testing resulted in significant differences between patients and controls regarding the PAF in the parietal and occipital ROI (table 3).

The mean PAF in CP patients using opioid medication and non opioid medication were similar, 9.5 ± 0.5 Hz and 9.5 ± 0.5 Hz, respectively. Opioid use as between group factor in the RM-ANOVA indicated no significant differences regarding PAF (F= 0.015; p= 0.904) or peak power amplitudes (F= 1.593; p= 0.228). Opioid use as covariate did not modify the main between group effect. Subgroups of patients with or without alcohol abuse in history did not show significant differences regarding PAF (F= 0.063; p= 0.806) or peak power amplitudes (F= 1.984; p= 0.181).

DISCUSSION

We observed a significant shift towards lower frequencies in patients with CP compared to healthy controls, observed as a decrease in PAF over all scalp electrodes. These results are consistent with other studies investigating the brain's default state in chronic pain patients including CP reporting slowing of EEG oscillations.[2-5,7] Exploratory post hoc analysis of average PAF per ROI reveals a significant difference particularly in parietal and occipital regions. Furthermore, this study shows that longer pain durations are associated with greater declines in PAF, indicating that PAF might be a marker for disease progression.

Alpha oscillations in the resting state EEG

Continuous EEG is dominated by alpha-band oscillations (7.5-13 Hz), which are widely distributed in the cerebral cortex and recorded with larger amplitudes over posterior regions with eyes closed.[25] The exact role of alpha oscillations remains unclear, but several factors have been identified affecting the alpha activity in some way. The PAF, a primary measure of alpha activity, starts to decline with increasing age,[26] and is known to increase with cognitive processing, attentional demand and arousal.[27] Several studies found PAF to be a stable measure, showing a high intra-individual stability. [28, 29]

Spontaneous alpha oscillations in chronic pain

Multiple studies report that phasic as well as tonic painful stimuli suppress spontaneous oscillations over the cortex in healthy participants,[19,30] but only a few studies have investigated the brain default state in patients with chronic pain. Sarnthein et al.[2] reported an increased EEG power and a slowed dominant peak frequency in patients with severe neuropathic pain of various origins. Maximal differences appeared in the 7-9 Hz band in all electrodes. These results were explained by the concept of thalamocortical dysrhythmia (TCD), which is proposed as a general mechanism to explain the generation of neuropathic pain and other neurological symptoms.[2,3,31] TCD is based on diminished excitatory or increased inhibitory input of neurons in the thalamus, resulting in the presence of a persistent low-frequency, thalamocortical resonance during the awake state.[3] This mechanism has been supported by the finding that therapeutic surgical lesions in the thalamus resulted in normalization of EEG activity as well as pain relief.[2]

Two studies in patients with neuropathic pain following spinal cord injury (SCI) support the TCD theory. In both studies, peak frequency was shifted towards lower frequencies in SCI patients with pain compared to SCI patients without pain. In contrast, no differences were observed in power amplitudes.[5,7] Interestingly, this is not the case in patients with chronic low back pain, who did not demonstrate any statistically significant TCD effect. Only in a subsample of these patients with evidence for root damage was a trend for

significant effect observed. The authors of this study suggest that only patients with severe pain or neuropathic pain develop the typical TCD pattern.[32] As stated earlier, pancreatic pain is typically intense and long-lasting, and secondly, because pancreatic pain may be of neuropathic origin.[8,33]

A previous study in CP patients with pain showed slowing of EEG rhythms based on increased normalized power amplitudes in the lower frequency bands including the alpha band.[4] The present study confirms these results in a similar population of CP patients. It extends these results to additional EEG parameters, better quantifying alpha slowing using PAF, and establishes a relation to clinically relevant factors such as pain duration. Interestingly, based on our PAF, maximum differences between groups were located over the posterior regions, whereas Olesen et al.[4] reported that mainly frontal electrodes contributed to the difference based on normalized amplitude strengths. However, both studies observed slowing of EEG rhythmicity, which suggests that pancreatic pain originates from a disturbance in thalamocortical rhythmia.

Toward a biomarker for chronic pain

A simple self-evaluation of pain is not sufficient to provide insight into underlying mechanisms since multiple factors potentially affect pain experience. Therefore, development of a physiological measure reflecting underlying pain mechanisms is desirable. First, it may improve pain diagnostics through addition of a mechanism-oriented parameter reflecting central neuronal involvement in pain genesis and maintenance. Second, it may improve pain treatment by identification of patients who may benefit from a treatment targeting central pain mechanisms. Graversen et al.[34] showed that quantitative pharmaco-EEG can be used to monitor the central analgesic mechanisms of pregabalin, and suggest that this approach may be used to predict effect of treatment leading to pharmaco-diagnostic testing.

Clinical applications of EEG

Besides the fact that EEG measures different phenomena regarding brain function than fMRI or PET, EEG has several advantages: (1) PET and fMRI are based on the measurement of secondary metabolic changes in brain tissue, but not of primary electrical effects of neural excitation, (2) EEG equipment costs significantly less than neuro-imaging equipment, and (3) EEG equipment, including electrodes, a signal amplifier and a computer with EEG software, is portable and easy to apply. This enables us to record the EEG near the patient's bed. On the other hand, it usually takes a long time to apply numerous electrodes at the scalp. Thus it would be desirable to reduce the number of required electrodes in clinical practice. But which electrodes are superfluous? Our study showed the maximum alpha-band oscillations in both groups to be located in the parietal and occipital regions.

More importantly, differences between groups in PAF, the only discriminative parameter observed in our study, were located over the same posterior regions. This suggests that PAF is best measured in the parietal and occipital regions of the scalp for chronic pain diagnostics.

Methodological considerations

Future research should concentrate on limitations within the current study. First, we did not collect pain scores during or just preceding the measurements, or average pain scores over the past few months. Therefore, it was not possible for us to correlate pain intensities with EEG parameters. Second, we made a comparison of CP patients with pain vs. healthy participants to study the differential influence of chronic pain. We recruited a homogeneous group of patients, all suffering from persistent visceral pain due to diagnosed CP. Although these patients were homogeneous regarding the cause of pain, it is difficult to ascribe the observed changes in the resting EEG to just one underlying cause. Variations in pain duration as well as differences in etiology (history of alcoholism), comorbidity (e.g. exocrine and/or endocrine failure), surgical treatment history, and actual medication use may be contributing factors. Thus, it might be interesting to investigate the influence of these factors based on a third group of CP patients without pain. However, it will be challenging to find CP patients having no pain and matched by age, level of education and medication intake, which are evident factors effecting the resting EEG.

Centrally-acting medication might influence the brain resting state activity. Many patients with CP use analgesics, including opioids, for pain treatment. This presents an ethical dilemma, as patients could potentially face severe pain if their medication was discontinued. In our study, more than half of the patients used opioids at the time of measurements. A comparison between subgroups of patients with and without opioids did not reveal any significant difference. Hence, the slowed PAF observed in our study is unlikely to have been caused by centrally-acting medication.

Conclusions

The present study shows a shift of the alpha peak towards lower frequencies in CP patients with chronic pain compared to healthy controls. This shift correlates with the duration of pain, which demonstrates that PAF deserves to further study regarding its potential as a clinically useful biomarker for chronic pain. . The subdivision in four ROIs showed that this biomarker is best measured in the parieto-occipital regions of the scalp, which reduces the number of electrodes necessary; of benefit for clinical practice. Accordingly, this method appears promising in supporting diagnosis and prognosis, establishing optimal treatment and studying efficacy of new therapeutic agents in chronic pain patients.

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PART II

Chapter 4

Single dose delta-9-tetrahydrocannabinol in chronic pancreatitis patients: analgesic efficacy, pharmacokinetics and tolerability

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ABSTRACT

AIM: We aimed to assess the analgesic efficacy, pharmacokinetics, tolerability and safety of a single dose of Δ9-THC in patients with chronic abdominal pain resulting from chronic pancreatitis (CP).

METHODS: This was a randomized, single dose, double-blinded, placebo-controlled, two way crossover study in patients suffering from abdominal pain as result of CP (n=24), *post hoc* subdivided into opioid and non-opioid users. Δ9-THC (8 mg) or active placebo (5mg / 10mg diazepam) was administered orally in a double dummy design.

RESULTS: No treatment effect was shown for delta VAS pain scores after Δ9-THC compared with diazepam. Δ9-THC was well absorbed with a mean t_{max} of 123 min. No significant differences were found between Δ9-THC versus diazepam for alertness, mood, calmness or balance. Feeling anxious and heart rate were significantly increased after Δ9-THC compared with diazepam. Most frequently reported adverse events (AEs) after Δ9-THC administration were somnolence, dry mouth, dizziness and euphoric mood.

CONCLUSIONS: A single dose of Δ9-THC was not efficacious in reducing chronic pain resulting from CP, but was well tolerated with only mild or moderate AEs. The PK results in CP patients showed delayed absorption and an increased variability compared to healthy volunteers.

INTRODUCTION

Chronic pancreatitis (CP) is a disease characterized by inflammation and progressive destruction of the pancreatic gland, which results in irreversible morphologic changes that typically cause endocrine and/ or exocrine dysfunction.[1] The most important symptom of CP is abdominal pain, present in 80-90% of patients during the disease course.[2] Pancreatic pain is described by most patients as severe abdominal pain, frequently radiating to the back. The pain is typically recurrent, intense and long-lasting, and is extremely difficult to treat.[3] Initial treatment of CP consists of low fat diet and non-narcotic analgesics, which can be supplemented by oral pancreatic enzymes and proton pump inhibitors. If an acceptable level of pain relief is not obtained with these drugs, opioids are the next stage in the management of pain. Opioids have a number of well-known adverse effects including elevation of smooth muscle tone (affecting gastrointestinal motility), toxicity in the central nervous system, opioid-induced hyperalgesia and tolerance, and risk of addiction.[4,5] Alternatives to medicinal treatment exist in the form of nerve blockade, lithotripsy and surgical treatment. However, results from studies of non-medicinal treatment modalities are equivocal and these treatments are only applicable in a minority of patients. Therefore, medicinal analgesic therapy must still be considered as the first choice in the management of painful CP.[6]

Underlying pain mechanisms of CP are poorly understood and multifactorial, and therefore, treatment is often empirical and insufficient. Several intra- and extrapancreatic causes of pain have been suggested. However, most research is focused alterations in pain processing, with peripheral causes including an increase in nerve fibers and neurogenic inflammation,[7] and central causes including central sensitization and somatotopic reorganization.[8,9] Furthermore, Olesen et al. demonstrated activation of descending inhibition in early CP patients, and loss of diffuse noxious inhibitory control (DNIC) in more advanced CP patients.[10] It should be noted in this context that when opioid treatment becomes less effective the more central sensitization an individual has.[11] Thus there is a clear need for alternatives (or adjuvants) to opioid treatment in CP patients with pain, targeting changes in (central) pain processing.

Delta9tetrahydrocannabinol (Δ9-THC) is the most potent psychoactive cannabinoid from the plant *Cannabis Sativa*, and has been used to treat pain for many centuries. However, the therapeutic potential of cannabinoids in current pain management remains unclear. To date, a wide range of products containing Δ9-THC are available for medicinal purposes, including: 1) crude medicinal cannabis containing several active compounds; 2) pharmaceutical products with standardized natural or synthetic Δ9-THC content containing whole cannabis plant extract, a defined combination of Δ9-THC

and cannabidiol (CBD) or pure Δ9-THC, and 3) synthetic analogues interacting with cannabinoid receptors.[12] The pharmacokinetics (PK) of the different administration routes of herbal cannabis and cannabis-based medicines are variable and dosing is difficult to regulate. The development of pharmaceutical products for oral administration with pure and defined Δ9-THC content may offer a favorable alternative. Namisol® (Echo Pharmaceuticals, The Netherlands) is a novel formulation for oral administration, containing purified Δ9-THC isolated from the *Cannabis Sativa* plant, with a reliable PK profile as demonstrated in phase I healthy volunteer study.[13]

Δ9-THC induces pharmacological effects by binding non-selectively to cannabinoid receptors. Two cannabinoid receptors have been identified, the CB1 and CB2 receptor.[14-16] CB1 receptors are most densely present in the brain, particularly in the hippocampus, cerebellum and striatum, and occur in several areas providing targets through which cannabinoids could modulate pain. These areas include the periaqueductal gray (PAG), the rostral ventrolateral medulla, the superficial layers of the spinal dorsal horn, and the dorsal root ganglion from which they are transported to both central and peripheral terminals of primary afferent neurons.[17-19] CB2 receptors are expressed in high quantities in human immune tissues and cells, e.g. in the spleen, tonsils and leucocytes.
Apart from potential direct analgesic effects, it is suggested that cannabis might further be useful to treat pain through possible synergistic interactions with opioid analgesics or by improving the efficacy of pain treatment in patients with a tolerance to opioids.[20]

In this phase 2 study, we aimed to study the analgesic efficacy, PK, pharmacodynamics (PD) and safety of a single dose oral Δ9-THC in patients with chronic abdominal pain resulting from CP, subdivided into opioid and non-opioid users.

METHODS

This was an equally randomized (1:1 ratio), single-dose, double-blind, placebo-controlled, cross-over study to evaluate the analgesic efficacy, PK, PD, pharmacogenetics and safety of a single dose of Δ9-THC. The study population consisted of 24 subjects with CP, subdivided into daily opioid (n=12) and non-opioid users (n=12). The Medical Ethical Committee and Competent Authority approved the study (2011/114). The study was conducted according to the principles of the Declaration of Helsinki, and in accordance with the International Conference on Harmonization guidelines of Good Clinical Practice. All subjects provided oral and written consent before conduct of any protocol-related procedures. Clinicaltrials.gov identification number NCT01318369.

Study population
Eligible patients were adults (age >18 years) diagnosed with CP according to the Marseille and Cambridge Classification System. All patients had chronic abdominal pain, persistent or intermittent on a daily basis during the past 3 months, and considered their pain as severe enough for medical treatment (NRS ≥ 3). Patients in the opioid subgroup took stable doses of prescribed opioids, whereas patients in the non-opioid subgroup had not taken opioids or occasionally for pain flares in the past 2 months. The study took place at the Radboud university medical centre, the Netherlands, from October 2011 to May 2013. Patients were recruited by their physician or by advertisement.
Key exclusion criteria were: cannabis use in previous year; history of hypersensitivity to THC; BMI <18.0 or >31.2 kg/m^2; serious painful conditions other than CP; significant medical disorder or concomitant medication that may interfere with the study or may pose a risk for the patient; major psychiatric illness in history; epileptic seizure in history; diabetic neuropathy; significant exacerbation in illness within two weeks; more than 1 daily defined dose (DDD) benzodiazepines 6 hours prior to or following intake of study medication in the opioid subgroup, or more than 1 DDD benzodiazepines according to prescription in the non-opioid subgroup (1 DDD was defined as 20mg oxazepam); positive urine drug screen or alcohol test at screening or on study days; clinically relevant abnormalities in ECG or laboratory results; pregnant or breastfeeding females; intending to conceive a child; or participation in another investigational drug study within 90 days before study entry.

Randomization
Eligible patients were stratified into opioid and non-opioid users, then randomly assigned to one of two treatment sequences in a 1:1 ratio using a computer-generated list of random numbers. Patients, staff and investigator were all blinded by a double dummy design. Each study day, patients were given either a single dose Δ9-THC (Namisol® 8mg simultaneously with placebo Diazepam) or a single dose Diazepam (placebo Namisol® simultaneously with Diazepam (5mg non-opioid group/ 10mg opioid group)). Each patient subsequently received the alternative after at least a 14-day washout period. Namisol® or matching placebos were taken in three tablets (1x5mg + 2x1.5mg). The previous phase I study demonstrated that the maximal tolerable dosage with acceptable adverse events was 8 mg Namisol®. With respect to the expected THC-mediated sedative effects of cannabis, as demonstrated by frequently reported AEs such as somnolence and fatigue,[21] low dose Diazepam was used as "active placebo" to prevent unblinding of patient and investigator. A study in healthy male subjects found no central effects after a single oral dose of 2 mg Diazepam, but intermediate effects after 5 mg and highly significant effects after 10 mg Diazepam.[22] Opioid users are generally more used

to sedative (side) effects due to their regular medication use. Therefore, a dosage of 10 mg Diazepam was chosen in order to induce similar sedative effects in this subgroup. Diazepam was packaged in capsules, which were identically prepared for the placebo Diazepam. Oral administration was performed using 200 ml of water.

Study procedures

The study consisted of a screening and two treatment days, with a telephone follow-up after each study day. Screening included demographics, medical history, NRS pain score, physical examination, 12-lead electrocardiogram (ECG), standard laboratory tests, and urine drug screening in order to assess the overall eligibility of the patient. Screening was carried out a maximum of 40 days before the first day of drug administration. All patients received a pain diary to fill in five days in a row, starting on the first day after screening in order to obtain a more convenient description of the pain status of the study population.

Use of illicit drugs and use of opioids were both tested using urine drug screening tests prior to drug administration. In addition, patients were not allowed to consume alcohol within 24 hours or caffeine within 6 hours prior drug administration. Urine pregnancy tests and saliva alcohol tests were performed at the beginning of both study days.

Study days were carried out at the research department of the hospital, where each patient stayed in a separate quiet room. Patients consumed as much as they preferred from a standardized menu on the first study day, but had to consume exactly the same on the second study day. The same applied to co-medication; patients used their regular medication, including painkillers, according to prescription on both study days. Every food and medication intake was recorded.

Analgesic efficacy

A visual analogue scale (VAS) was used to quantify pain intensity. VAS scores at rest and on movement after 5 sit-ups were marked on a 10 cm line. The boundaries of these lines were "*no pain*" on the most left side and "*unbearable pain*" on the most right side. The VAS was measured predose and postdose at 0:35, 1:05, 1:40, 2:05, 3:05, 4:10, and 5:00 hours after administration of study medication.

Pharmacokinetics

Plasma concentrations of THC and its active metabolite 11-OH-THC were determined in serial venous blood samples, which were collected in 4ml EDTA tubes predose at –0:15 hours and at 0:10, 0:30, 0:45, 1:00, 1:30, 2:00, 3:00, 4:00, and 6:00 hours postdose. Immediately after collection, samples were wrapped in aluminum foil and kept on ice. Samples were centrifuged within 30 minutes at 2000 g for 10 minutes at 4°C. The handling

of THC samples was done avoiding direct light. The separated plasma was divided into primary and backup samples, and stored at –80°C until bioanalysis. Bioanalysis (Analytisch Biochemisch Laboratorium b.v., Assen, the Netherlands) was performed using a validated liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) assay method according to good laboratory practice procedures. The lower limit of quantification for THC and 11-OH-THC was 0.100 ng ml^{-1}.

Non-compartmental analysis to determine plasma PK parameters of the active compounds, THC and 11-OH-THC, was performed using the WinNonlin modeling and analysis software (version 2.1 a; Pharsight Inc., Apex, NC). The maximum plasma concentration (C_{max}), the time to reach C_{max} (T_{max}), and the AUC from 0 up to the last measurement (AUC_{0-6h}, using the linear log trapezoidal rule) were calculated from the individual plasma concentration-versus-time profiles. The terminal half-life ($t_{1/2}$) was calculated only if there were two or more points (excluding C_{max}) in the elimination phase of the plasma concentration–time curve with $r^2 > 0.80$. For that reason, five patients were excluded from this part of the analysis for both THC and 11-OH-THC. Subsequently, the areas under the plasma concentration curves extrapolated to infinity (AUC_{inf}) were calculated using the linear log trapezoidal rule and extrapolation to zero.

Pharmacogenetics

Genotyping of cytochrome P450 enzymes CYP2C9 and CYP2C19 was performed in order to investigate the effect of genetic polymorphisms on the pharmacokinetics of Δ9-THC.[23,24] Two variants in genetic CYP2C9 polymorphisms (CYP2C9*2 and CYP2C9*3) and three variants in genetic CYP2C19 polymorphisms (CYP2C19*2, CYP2C19*3 and CYP2C19*17) were genotyped. To this end, saliva from 21 participating subjects was collected from which DNA extraction and genotyping was done.

Pharmacodynamics

Predose and postdose at 0:35, 1:05, 2:05, 3:05, and 5:00 hours, drug effects on mood and behavior were explored with a set of 16 individual Bond & Lader visual analogue scales. Three main factors were calculated as described by Bond and Lader: alertness (from nine scores), mood (from five scores), and calmness (from two scores).[25] Potential subjective psychotomimetic (psychedelic) effects were evaluated using the Bowdle questionnaire. The Bowdle questionnaire consists of thirteen visual analogue lines ranging from 'not at all' to 'extremely' quantifying psychedelic effects.[26] Subjects were asked to fill in both questionnaires predose and postdose at 1:05, 2:05, 3:05, and 5:00 hours after drug administration.

Left-right (roll) and anterior-posterior (pitch) postural oscillations were measured using a gyroscope-based measurement system (SwayStar™, Balance International Innovations

GmbH, Switzerland), which was attached to the waist of the patient. Patients stood, without shoes, as still as possible in a standardized base of support with their arms hanging at both sides of their body. Body sway was measured predose and at 1:25, 2:25, 3:25, and 5:30 hours postdose for one minute with eyes open and one minute with eyes closed. During the task with eyes open patients were asked to fixate at one point. The computerized measures used for analysis reflect the 90% range roll and pitch excursion in degrees from the centre of gravity.

Safety and Tolerability

Safety and tolerability were evaluated using spontaneously reported adverse events (AEs) recorded at study days until follow-up, measurements of vital functions, ECG and laboratory tests. Blood pressure and heart rate were measured at screening and on both treatment days (predose and repeatedly postdose). ECG was recorded at screening, predose and at the end of each treatment day. Hematology, blood chemistry, and urinalysis were performed at screening and at the end of the study.

Statistical Methods

This was an exploratory study for which no sample size calculation was performed. Patients withdrawn prior to the first study day were replaced in order to have a total number of 24 evaluable patients for the analysis. The placebo treatment was considered as equal between opioid and non-opioid users, despite the distinction in dose treatment across both groups. For statistical analysis SPSS software for Windows v.20 was used. All statistical tests were performed two-tailed, and the limit for statistical significance was set at $P<0.05$.

Differences between Δ9-THC versus diazepam in VAS scores at rest at time point 2:05H were the primary outcome of this study. This was based on the assumption that C_{max} is reached within two hours after medication intake. Differences between both treatments were statistically analyzed using a linear mixed model analysis with two fixed factors (period and treatment) and a random subject effect (random intercept). A period * treatment interaction was absent. The effect of treatment (Δ9-THC vs. placebo) was exploratory post hoc evaluated for both subgroups (opioid vs. non-opioid).

Statistics of repeated measures data were analysed using the area under the curve (AUC) of difference with baseline as summary measure. The AUC was computed using the trapezoid rule, $\Delta X*(Y1+Y2)/2$, repeatedly for each adjacent pair of points defining the curve from zero until the last measurement. Differences between Δ9-THC versus diazepam were statistically analysed using a linear mixed model analysis. Opioid users and non-opioid users were compared in a subgroup analysis. The pharmacokinetics of patients with genetic polymorphisms were compared observationally.

RESULTS

Twenty-five patients were enrolled according to the flowchart in figure 1. One patient was not treated because of a positive drug screening on the first study day and was replaced. Two patients in the opioid subgroup were lost to cross over after the first study day, one female patient due to mild AEs and one male patient after withdrawal of consent. Consequently, 24 patients received a single dose Δ9-THC, and 22 patients received a single dose Diazepam.

Patient demographics and baseline characteristics are described in table 1. The mean age at screening was 52 years, mean BMI was 23.0 kg/m^2, and 9 of 24 patients were female. Patients reported a mean NRS at screening of 6.0, whereas the mean VAS reported in the pain diary was 3.9. The average abdominal pain duration was 8.3 years at screening.

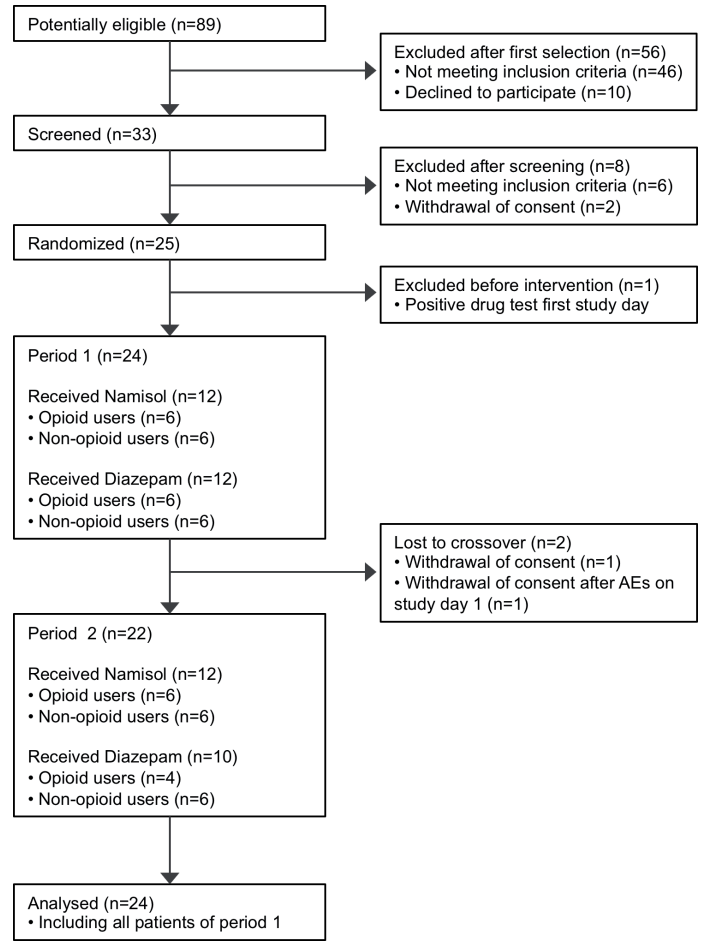

Figure 1: Participant flowchart

Table 1: Baseline demographics and disease characteristics

	Sex (M/F)	Age (years)	BMI (kg/m²)	Etiology CP	Pain screen (NRS)	Pain diary (VAS)	Pain duration (years)	Concomitant medication
Opioid subgroup								
1	M	54	25,7	Post ERCP	6	4,2	5	SOPI, PCM, AC
2	M	48	26,9	Idiopathic	4	4,5	2	SOPI, PCM, AC
3	M	46	26	Idiopathic	7	2,2	21	SOPI, AC, PE
4	F	61	26,6	Idiopathic	5	5,2	15	SOPI, PE
5	M	44	18,8	Neoplasm	3	4,5	0	SOPI, PE
6	M	42	22,5	Alcohol	6	5,1	14	WOPI, PCM, PE
7	M	45	22	Idiopathic	6	7,2	4	SOPI, WOPI, PCM
8	F	42	21,5	Hereditary	6	4,9	13	SOPI, PCM
9	M	52	22,2	Alcohol	5	4,4	1	SOPI, NSAID, PCM
10	M	50	26,2	Idiopathic	8	2,5	2	SOPI, PE
11	F	34	19,5	Idiopathic	4	4,0	11	SOPI, PCM
12	F	52	19,2	Idiopathic	8	4,6	8	SOPI, AC
mean (SD)	**8/4**	**47,5 (7,0)**	**23,1 (3,1)**		**5,7 (1,6)**	**4,4 (1,3)**	**8,0 (6,7)**	
Non-opioid subgroup								
13	F	52	26,2	Idiopathic	8	6,9	11	PCM, AC
14	M	69	26,2	Hereditary	6	5,1	4	-
15	M	56	20,6	Neoplasm	8	4,0	8	AC, PE
16	M	71	23,6	Idiopathic	5	2,1	6	PCM, PE
17	M	51	26,3	Idiopathic	7	4,7	3	NSAID, PCM, PE
18	M	53	24,2	Idiopathic	3	2,5	9	PE
19	M	39	18,4	Idiopathic	7	2,5	6	NSAID, PCM, PE
20	F	54	18,1	Idiopathic	6	3,0	22	PCM, PE
21	F	57	23,8	Idiopathic	6	1,0	6	PE
22	M	44	18,5	Alcohol	9	2,1	6	PCM, PE
23	F	62	23,3	Alcohol	5	3,2	15	PE
24	F	65	26,3	Idiopathic	5	2,7	7	PCM
mean (SD)	**7/5**	**56,1 (9,5)**	**23,0 (3,2)**		**6,3 (1,7)**	**3,3 (1,6)**	**8,6 (5,3)**	
Total mean (SD)	**15/9**	**51.8 (9.3)**	**23.0 (3.1)**		**6.0 (1.6)**	**3.9 (1.5)**	**8.3 (5.9)**	

SOPI Strong opioids including pethidine; WOPI Weak opioids including tramadol en codein; NSAID Non-steroidal anti-inflammatory drugs including diclofenac and ibuprofen; PCM Paracetamol; AC Anticonvulsants including pregabalin and gabapentin; AD Antidepressants; PA Pancreatic enzymes

Analgesic efficacy

Primary linear mixed model analysis at time point 2:05H showed no treatment effect of Δ9-THC compared with Diazepam on delta VAS pain at rest (mean diff Δ9-THC - diazepam -.17; 95% CI diff [-.95 to .61]; p=.65). Figure 2 shows the VAS pain at rest and on movement compared to baseline from 0:35H until 5:00H after administration of Δ9-THC as well as diazepam. The AUC VAS pain at rest (mean diff 18.37; 95% CI diff [-60.49 to 97.23]; p=.63) and AUC VAS pain on movement (mean diff -18.14; 95% CI diff [-168.31 to 132.03]; p=.80) after Δ9-THC were both not significantly decreased compared with diazepam. These parameters were similar for opioid vs. non-opioid users.

Pharmacokinetics

Mean plasma concentration-versus-time curves of THC and 11-OH-THC are shown in figure 3 and table 2 summarizes the PK of THC and its active metabolite 11-OH-THC. The PK parameters were similar between opioid and non-opioid users. One patient demonstrated a clearly enhanced C_{max} compared to the rest of the population, which could not be explained by genetic polymorphism.

Table 2: Pharmacokinetic parameters of THC and 11-OH-THC

		THC		11-OH-THC	
		Mean	**SD**	**Mean**	**SD**
Cmax	Group (n=24)	4,01	3,39	4,38	1,50
(ng/mL)	Opioid (n=12)	4,44	4,40	4,51	1,62
	Non-opioid (n=12)	3,58	2,08	4,25	1,44
Tmax	Group (n=24)	122,80	87,99	135,70	77,50
(min)	Opioid (n=12)	126,60	90,49	142,10	86,66
	Non-opioid (n=12)	119,10	89,26	129,30	70,44
$AUC_{0\text{-Last}}$	Group (n=24)	477,50	381,80	764,90	241,30
(ng*min/mL)	Opioid (n=12)	507,90	506,70	777,70	298,10
	Non-opioid (n=12)	447,20	214,70	752,20	180,50
$AUC_{0\text{-inf}}$	Group (n=24)	532,20	442,50	920,70	316,40
(observed)	Opioid (n=11)	577,70	571,10	954,00	400,00
(ng*min/mL)	Non-opioid (n=8)	469,70	173,20	883,70	205,90
$T_{1/2term}$	Group (n=24)	67,12	20,37	110,10	26,57
(min)	Opioid (n=11)	67,89	19,71	111,70	29,51
	Non-opioid (n=8)	66,05	22,57	108,40	24,55

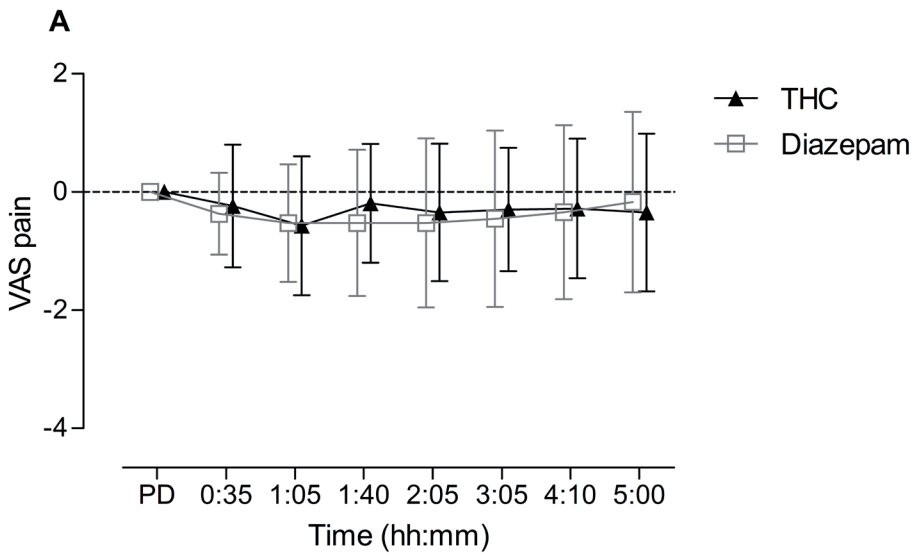

Figure 2: VAS pain. Differences (mean and SD) in VAS pain compared to baseline were shown for Δ9-THC and diazepam measured at rest (A) and on movement (B) in patients with pancreatic pain (n=24). Abbreviation: PD= predose, maximal 1 hour prior drug administration.

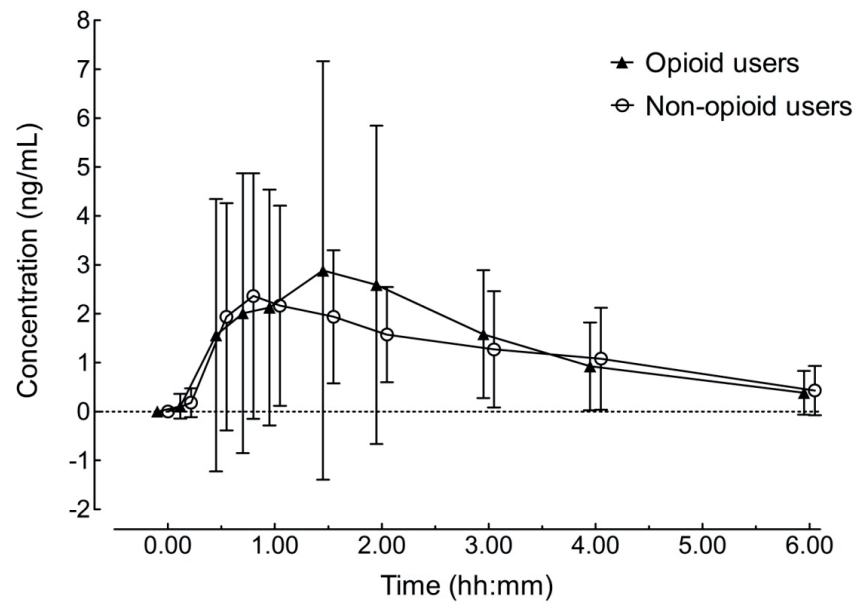

Figure 3: Mean plasma concentration-time curves of THC (A) and 11-OH-THC (B) after a single dose of Δ9-THC in CP patients subdivided in opioid (n=12) and non-opioid (n=12) users. Error bars represent standard deviation (SD).

Pharmacogenetics

Several genetic polymorphisms were observed. Two patients were heterozygote carriers of CYP2C9*2 (C>T) and four patients were heterozygote carriers of CYP2C9*3 (A>C). One patient was found to be AA homozygote and four patients GA heterozygote for CYP2C19*2 (G>A). No CYP2C19*3 (G>A) polymorphisms were observed. Genetic polymorphisms in CYP2C19*17 (C>T) were found for five subjects who were heterozygote CT carriers. Genetic CYP2C9 and CYP2C19 polymorphisms did not evidently effect the pharmacokinetics of Δ9-THC.

Pharmacodynamics

Figure 4 shows the effects of Δ9-THC and diazepam for alertness, mood and calmness obtained by the VAS Bond and Lader questionnaire. No significant differences were found between Δ9-THC vs. diazepam. Feeling anxious obtained by the VAS Bowdle questionnaire was significantly increased after Δ9-THC compared with diazepam (mean diff 166,92; 95% CI diff [10,86 to 322,97]; p=.037).

Overall 10 body sway measurements (4% of all measurements), from which 6 in the eyes closed condition and 8 after Δ9-THC administration, could not be conducted due to adverse events at that particular moment. There were no group differences in balance outcomes in both the eyes open and eyes closed condition between Δ9-THC and diazepam. However, balance performance was considerably disturbed in certain individuals after both Δ9-THC and diazepam. These individuals were found in both subgroups. Heart rate was significantly enhanced after Δ9-THC compared to diazepam (at time point 1:40H mean diff -5.5 BPM; 95% CI diff [-9.0 to -1.9]; p=.004). In one patient, heart rate in rest was measured above 100 BPM after Δ9-THC intake. Δ9-THC and diazepam did not affect diastolic or systolic blood pressure. Alterations in heart rate were not associated with PK parameters such as C_{max} and AUC_{inf}. All pharmacodynamic parameters were similar for opioid vs. non-opioid users and did not affect the treatment effect.

Safety and Tolerability

All related, probably related and possibly related AEs are presented in table 3. Overall, there was a higher frequency of AEs following Δ9-THC administration compared to diazepam (54 AEs in 24 patients vs. 36 AEs in 22 patients, respectively), although fewer patients reported at least one AE after Δ9-THC administration compared to diazepam (71% vs. 91% respectively). The most frequently reported AEs after Δ9-THC administration were somnolence, dry mouth, dizziness, and euphoric mood. Somnolence, dizziness, and fatigue were most commonly related or possibly related to diazepam administration. All AEs were mild or moderate, and equally divided between opioid and non-opioid

Table 3: Summary of adverse events

Adverse Event	Diazepam (n=22)		Δ9-THC (n=24)	
	N	%	N	%
General				
Fatigue	8	36%	7	29%
Nervous system symptoms				
Somnolence	11	50%	8	33%
Dizziness	6	27%	4	17%
Headache	3	14%	2	8%
Balance disorder	0	0%	2	8%
Amnesia	0	0%	1	4%
Paraesthesia	1	5%	2	8%
Depressed level of conciousness	1	5%	0	0%
Psychiatric symptoms				
Confusional state	0	0%	2	8%
Indifference	0	0%	1	4%
Euphoric mood	2	9%	4	17%
Derealisation	0	0%	1	4%
Disorientation	0	0%	1	4%
Tension	0	0%	1	4%
Gastro-intestinal system symptoms				
Nausea	1	5%	3	13%
Vomiting	0	0%	1	4%
Steatorrhoea	0	0%	1	4%
Constipation	1	5%	0	0%
Abdominal discomfort	0	0%	1	4%
Dry Mouth	0	0%	5	21%
Throat irritation	0	0%	1	4%
Vision symptoms				
Visual impairment	1	5%	3	13%
Cardiac symptoms				
Heart rate increased	1	5%	1	4%
Eye symptoms				
Dry eye	0	0%	1	4%
Photophobia	0	0%	1	4%
TOTAAL	**36**		**54**	

users. The number of AEs was not associated with PK parameters such as C_{max} and AUC_{inf}. However, the subject showing the highest C_{max} also had the greatest number of AEs. There

were no serious AEs during the study. One patient was withdrawn after administrating Δ9-THC on the first study day due to somnolence, dizziness, increased heart rate, nausea, paraesthesia, and feelings of tension. There were no clinically relevant changes in vital signs, ECG parameters, or safety laboratory parameters (hematology, biochemistry, and urinalysis).

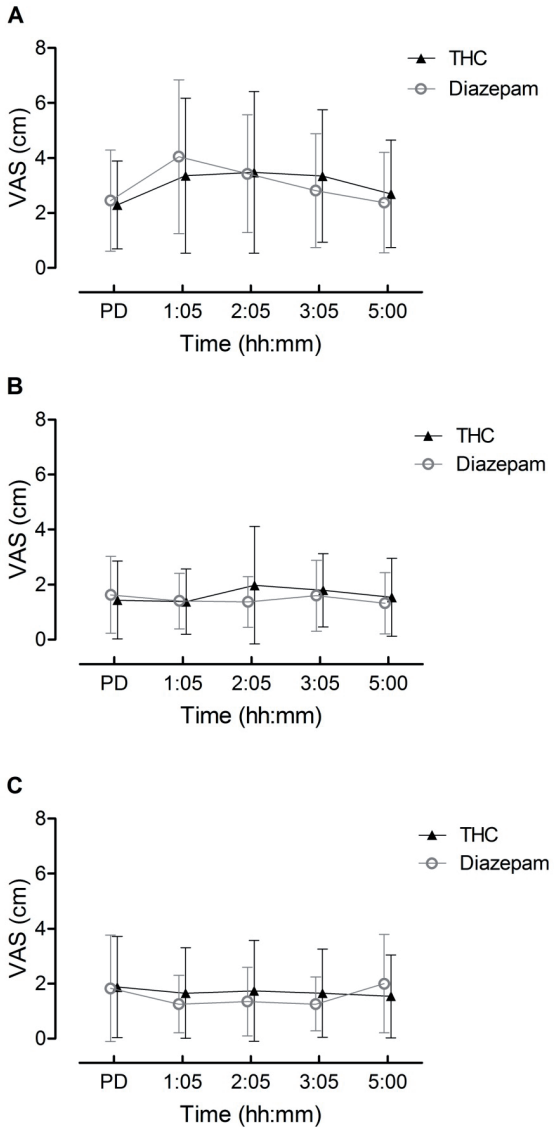

Figure 4: VAS Bond and Lader questionnaire. Mean scores for alertness (A), mood (B), and calmness (C) were shown for Δ9-THC and diazepam in CP patients (n=24). Error bars represent standard deviation (SD). Abbreviation: PD= predose.

DISCUSSION

Our study investigated the analgesic efficacy, pharmacokinetics, pharmacodynamics and safety of a single dose Δ9-THC in patients with chronic abdominal pain related to CP. We demonstrated in an exploratory study, that a single dose of 8 mg Δ9-THC is not efficacious in reducing chronic pancreatic pain compared to the active placebo diazepam. Δ9-THC was absorbed with an average T_{max} of 123 minutes, which was similar for opioid and non-opioid users, but slower than observed in a previous study in healthy subjects.[13] We observed a small, but significant increase in feeling anxious after Δ9-THC compared to diazepam. Other pharmacodynamic outcomes did not differ between Δ9-THC and diazepam. A single dose of Δ9-THC was well tolerated resulting in mild to moderate AEs.

Analgesic efficacy

Several RCTs investigated the analgesic efficacy of different products containing THC in various pain states.[12,27-30] In a majority of these studies, THC treatment resulted in pain reduction in chronic pain, whereas the data in acute pain were less conclusive. Most studies in chronic non-malignant pain conditions demonstrated analgesic efficacy in chronic non-malignant pain using a single dose or treatment periods of 2 to 15 weeks.[20,31-42] The majority of studies with cannabis-based medicines were conducted in patients suffering from central neuropathic pain in multiple sclerosis. Ours is the first study in patients with chronic abdominal pain resulting from CP, which is generally recognized as difficult to treat and associated with high opioid use.

Narang et al. demonstrated that patients who received a single dose THC experienced decreased pain intensity compared with placebo in patients taking opioids for chronic non-malignant pain of various origin (e.g. low back, lower extremity, cervical, and abdominal/pelvic pain), suggesting that THC may have an additive effect on pain relief.[20] Preclinical evidence also suggests that THC may act synergistically with opioids.[43,44] However, in the present study we did not observe any analgesic effect of Δ9-THC compared to diazepam nor a difference between opioid users and non-opioid users. Although pain was decreased after Δ9-THC administration, the same effect was observed after diazepam administration. As for diazepam no analgesic efficacy is described and is used in other pain studies as active placebo,[45] it is assumed that the pain relief after diazepam is a placebo effect. It is well known that placebo and nocebo effects are present in chronic pain populations.[46]

Several explanations for the lack of analgesic effect in our study can be proposed:

1) a single dose of Δ9-THC is insufficient to achieve adequate exposure duration. THC is lipophilic and will diffuse to the fatty tissues immediately. The question is whether the THC concentration at target site is sufficient to modulate pain.

Therefore, long-term treatment studies are necessary to achieve sufficient exposure duration and evaluate the efficacy of Δ9-THC.

2) the dosage of 8 mg Δ9-THC is inadequate for each individual patient. The dosage should be adjusted for individual patients according to genetic, mechanistic, and other patient-related factors that potentially influence the PK and clinical effects.[47,48]
3) Δ9-THC is effective only in certain types of pain, e.g. chronic vs. acute, or visceral vs. neuropathic. It is difficult to specify responders, because the working mechanism of how THC potentially modulates pain is unclear. It should be noted that several previous clinical trials demonstrated analgesic efficacy in chronic pain, particularly in multiple sclerosis, whereas the data in acute pain were less conclusive.[12]
4) sensitization of nociceptive pathways (e.g. central sensitization) and alterations in central cognitive and autonomic processing, which are all associated with chronic pancreatic pain,[49-51] impedes analgesic efficacy in this particular research population.
5) THC in general is ineffective for pain relief. However, the absence of a significant pain relief in current study, after only one single dose, does not give evidence that supports this suggestion.

Pharmacokinetics

The mean plasma concentration curves demonstrate that THC was generally well absorbed and further metabolized to 11-OH-THC in this group of CP patients. However, it should be noted that, according to the mean plasma concentration curve of THC, the time to reach maximal THC concentration was 45-90 min, whereas the computed mean T_{max} of THC was 119-127 min. This phenomenon can be explained by the observation that subjects with an early T_{max} have a much higher C_{max} compared to those subjects with a late T_{max} which show a relatively low C_{max}. The previously mentioned phase I study reported a time to reach maximal THC concentration of 39–56 min, but these subjects were young, healthy and fasted before Δ9-THC administration.[13] Thus, the absorption of Δ9-THC was delayed in a subgroup of CP patients, resulting in an increased variability.

CP is associated with malabsorption,[52,53] which potentially affects drug absorption and could explain the inter-individual PK variation in patients with CP.[54] Drug absorption in CP patients might further be affected by alterations in gastrointestinal intraluminal pH, gastrointestinal motility, bacterial overgrowth and changed pancreatic gland secretion.[54] In addition, bowel dysfunction is a common adverse effect of prolonged opioid use,[55] which may affect the absorption of drugs as well. Therefore, the role of these factors in modulating the pharmacokinetic profile of THC should be further studied.

Pharmacogenetics

We aimed to evaluate the effects of CYP2C9 and CYP2C19 polymorphism on the pharmacokinetics of Δ9-THC, which is subsequently relevant for its efficacy and adverse effects. Sachse-Seeboth et al. found that the homozygous CYP2C9*3 variant affected the pharmacokinetics of THC, resulting in a three folded area under the plasma concentration curve of THC, as well as a trend towards increased sedation after oral administration of THC.[23] In the current study, we did not observe significant differences between wild-type subjects and subjects with homozygous or heterozygous CYP polymorphisms. This can be explained by the small number of subjects with a genetic variant. However, it cannot be precluded that genetic polymorphisms may have contributed to the inter-individual variation in the pharmacokinetics of Δ9-THC.

Pharmacodynamics

Several psychological outcomes such as alertness, feelings of unreality, control of thoughts, feeling high, and feeling drowsy seem to be affected after administration of both Δ9-THC and diazepam. Feeling anxious was the only outcome with a significant difference between Δ9-THC and diazepam, which is not surprising considering the anxiolytic properties of diazepam. Similar results were observed for the body sway measurements. Balance disturbances were found in several individuals after both Δ9-THC as well as diazepam. After 1:40 hours postdose, heart rate was significantly enhanced with 5.5 beats per minute after Δ9-THC compared to diazepam. This is in line with previous studies and for most patients not clinically relevant.[13]

Adverse effects

Δ9-THC was generally well tolerated resulting in only mild to moderate adverse events, which were very similar compared to those observed in healthy volunteers.[13] However, we observed an inter-individual variation with certain subjects experiencing no single side effect while others experienced several side effects at the same time. This could not be explained by subgroups of (non)opioid users or pharmacogenetic polymorphisms, and could not be associated with pharmacokinetic parameters such as C_{max} or AUC_{last}. However, side effects of THC are considered to be dose-related,[13] and therefore, adverse events should be avoidable by adjusting the dosage or by adequate dosage titration.

Methodological considerations

The similarities in the pharmacodynamics of Δ9-THC compared to diazepam clearly demonstrate that we succeeded in adequate blinding of subjects by giving the impression of an active psychotropic drug in both periods. Additionally, with respect to the sedative effects of THC, diazepam was used to control for indirect pain relief

through the sedative effects on the experienced pain. Diazepam is more often chosen as active placebo for THC and other central working analgesics.[45,56] However, it should be mentioned that the role of GABA in mediating the transmission and perception of pain is not evidently clear. GABAergic neurons are widely distributed throughout the central nervous system, including regions of the spinal cord dorsal horn known to be important for transmitting pain impulses to the brain.[57] GABA receptor agonists demonstrate antinociceptive properties in a variety of pain models in animal studies,[58] and showed possible anti-hyperalgesic effects in experimental human pain models.[59] However, benzodiazepines largely lack clear analgesic efficacy in humans,[57,60] and diazepam is thus unlikely to affect the primary outcome. The comparison with diazepam, however, may have complicated the evaluation of PD effects of Δ9-THC. Several psychedelic outcomes such as alertness, feelings of unreality, control of thoughts, feeling high, feeling drowsy, and feeling anxious were affected after administration of both drugs.

Conclusion

This study demonstrates that a single dose of 8 mg Δ9-THC was not efficacious in achieving pain relief. At this dose, Δ9-THC was generally well tolerated with mostly mild AEs. The PK results in CP patients showed delayed absorption and an increased variability compared to healthy volunteers, most probably due to underlying pathology and concomitant medication use. Further long-term treatment studies are necessary to evaluate the efficacy and tolerability of Δ9-THC in chronic pancreatitis and other chronic visceral pain conditions.

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Conflict of interest

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: HvG received a grant from the European Union, the European Fund for Regional Development (EFRO, 'Here is an investment in your future') for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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Chapter 5

Tetrahydrocannabinol Does Not Reduce Pain in Patients With Chronic Abdominal Pain in a Phase 2 Placebo-controlled Study

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ABSTRACT

BACKGROUND & AIMS: Delta9tetrahydrocannabinol (THC) is the most abundant cannabinoid from the plant *Cannabis sativa*. There is only equivocal evidence that THC has analgesic effects. We performed a phase 2 controlled trial to evaluate the analgesic efficacy, pharmacokinetics, safety, and tolerability of an oral tablet containing purified THC in patients with chronic abdominal pain.

METHODS: Sixty-five patients with chronic abdominal pain for 3 months or more (numeric rating scale scores of 3 or more) after surgery or due to chronic pancreatitis were randomly assigned to groups given the THC tablet or identical matching placebos for 50–52 days. Subjects in the THC group were given the tablet first in a step-up phase (3 mg, 3 times daily for 5 days and then 5 mg, 3 times daily for 5 days) followed by a stable dose phase (8 mg, 3 times daily until day 50–52). Preceding and during the entire study period, patients were asked to continue taking their medications (including analgesics) according prescription. Patients reported any additional pain medications in a diary. Efficacy and safety assessments were conducted preceding medication intake (day 1), after 15 days, and at 50–52 days. Plasma samples were collected on study days 1, 15, and 50–52; mean plasma concentration curves of THC and 11-OH-THC were plotted. The primary endpoint was pain relief, measured by a visual analogue scale of the mean pain (VAS mean scores), based on information from patient diaries. Secondary endpoints included pain and quality of life (determined from patient questionnaires), pharmacokinetics, and safety.

RESULTS: At days 50–52, VAS mean scores did not differ significantly between the THC and placebo groups ($F(1, 46) = .016$; $P = .901$). Between the start and end of the study, VAS mean scores decreased by 1.6 points (40%) in the THC group compared to 1.9 points (37%) in the placebo group. No differences were observed in secondary outcomes. Oral THC was generally well absorbed. Seven patients in the THC group stopped taking the tablets due to adverse events, compared with 2 patients in the placebo group. All (possibly) related adverse events were mild or moderate.

CONCLUSIONS: In a phase 2 study, we found no difference between a THC tablet and a placebo tablet in reducing pain measures in patients with chronic abdominal pain. THC, administered 3 times daily, was safe and well tolerated during a 50–52 day treatment period.

Clinicaltrials.gov no: NCT01562483 and NCT01551511.

INTRODUCTION

Chronic abdominal pain remains a major clinical challenge. Two typical chronic abdominal pain etiologies of visceral origin are chronic pancreatitis (CP) and postsurgical pain (PSP). Approximately 80–90% of CP patients suffer from chronic abdominal pain during the course of their illness.[1,2] Incidences of painful post abdominal surgery adhesion development vary in literature from 45 to 90%.[3-5] Intra-abdominal adhesions are believed to be the most likely cause of PSP.[4] CP and PSP are both associated with an increased responsiveness of nociceptive pathways in the central nervous system, termed central sensitization.[6-8] Central sensitization produces pain hypersensitivity by changing the sensory response in the central nervous system, and is associated with the development and maintenance of chronic pain.[7] Because central sensitization alters the properties of neurons in the central nervous system, the pain is frequently no longer reliably coupled to the presence of particular peripheral stimuli. Therefore, pharmacologic treatment options that produce analgesia by targeting these changes in the central nervous system are required.[8]

The introduction of cannabinoids offers an interesting alternative for chronic pain management. Delta-9-tetrahydrocannabinol (THC) is the principal psychoactive compound of the *Cannabis sativa* plant,[9] and interacts with two cannabinoid receptors, termed CB1 and CB2. CB1 receptors are predominantly found in the brain and spinal cord, while CB2 receptors are located primarily in the periphery, including the immune system.[10] CB1 receptors are also highly expressed in regions critical for emotion processing including the amygdala, hippocampus, and anterior cingulate cortex.[11] Brain activity within this emotion-related circuitry was found to be increased in patients with chronic pain.[12,13] Hence, it was suggested that cannabinoids may modulate pain perception by disturbing the connectivity within this circuit. This was demonstrated by Lee et al., who observed that THC reduced the functional connectivity between the amygdala and the primary somatosensory cortex (S1) during pain processing.[14] Further research indicated that THC does not selectively affect these limbic regions, but rather interferes with sensory processing, which in turn reduces sensory-limbic connectivity, leading to deactivation of affective regions.[15] Thus it may be expected that THC interferes, although not selectively, with the affective components of pain.

The majority of clinical trials on the efficacy of THC for pain treatment has been focused on cancer related pain, central neuropathic pain syndromes, and acute pain conditions.[16-18] We aimed to investigate the efficacy, pharmacokinetics and safety of a novel cannabinoid-based product, an oral tablet containing purified natural THC, in patients with chronic abdominal pain.

METHODS

Study design

This phase II study used an equally randomized (allocation ratio 1:1), double-blind, placebo-controlled, parallel design. The study initially started as two clinical trials in (1) patients with painful CP and (2) patients with chronic abdominal PSP. Integration into one study was necessary due to a disappointing recruitment rate. Initial trials used identical study designs, treatment schemes and outcome parameters. Integration was supported by an independent statistician, who reviewed blinded interim data. The medical ethical committee approved both initial studies as well as the protocol amendment concerning study integration prior to study closure. The study was conducted according to the principles of the Declaration of Helsinki, and in accordance with the International Conference on Harmonization guidelines of Good Clinical Practice. All subjects provided oral and written consent before conduct of any protocol-related procedures. All authors had access to the study data and had reviewed and approved the final manuscript. Clinicaltrials.gov identification numbers NCT01562483 and NCT01551511.

Study population

Adult patients (age >18 years) suffering from abdominal pain developed after a surgical procedure or resulting from chronic pancreatitis were eligible for participation, if they had persistent or intermittent abdominal pain (on a daily basis for at least 3 months) severe enough for medical treatment (average NRS ≥ 3).[19] Key exclusion criteria were: daily cannabis use in past three years; history of hypersensitivity to THC; serious painful conditions other than PSP or CP; significant medical disorder or concomitant medication that may interfere with the study or may pose a risk for the patient; major psychiatric illness in history; epileptic seizure in history; affected sensory input such as diabetic neuropathy; BMI >36.0 kg/m^2; significant exacerbation in illness within two weeks; positive urine drug screen or alcohol test at screening or on study days; clinically relevant abnormalities in ECG or laboratory results; pregnant or breastfeeding females; intending to conceive a child; or participation in another investigational drug study within 90 days before study entry. Preceding and during the entire study period, patients were asked to take their co-medication, including analgesics, according prescription. Patients reported additional pain medication (taken as needed) in a diary. The study was executed at the Radboud university medical center, the Netherlands. Patients were recruited by their physician or via advertisements.

Randomization and study treatment

Tablets with standardized Δ9-THC content (Namisol®, Echo Pharmaceuticals, Weesp, the Netherlands) or identical matching placebos were administered orally during a 50-52 days add-on treatment. The study treatment consisted of two phases (supplementary figure 1): a step-up phase (day 1-5: 3 mg TID; day 6-10: 5 mg TID), and a stable dose phase (day 11-52: 8 mg TID). It was permitted to taper the dosage to 5 mg TID, when 8 mg was not tolerated. Independent pharmacists dispensed either active or placebo tablets according to a computer-generated randomization list stratified for opioid and non-opioid users using separate lists. Treatment allocation was strictly concealed from participants, investigators, and all other study personnel involved in the study until end of study and database lock.

Study procedures

Efficacy and safety assessments were conducted preceding medication intake on day 1 (visit 2), after 15 treatment days (visit 3) and 50-52 treatment days (visit 4). Several phone calls were performed by the investigators during and after the treatment period (day 4-5, 9-10, 21-23, 28-30, 38-40 and 59-61) in order to evaluate the tolerability, safety and compliance.

Additional study procedures in supplementary material.

Primary efficacy outcome

The primary endpoint was change in pain intensity at the end of study treatment versus baseline of THC compared with placebo. A visual analogue scale was used in order to quantify the mean (VAS_{mean}), minimal (VAS_{min}) and maximal (VAS_{max}) pain intensity in a daily diary, starting five days preceding first medication intake until the end of study treatment. The boundaries of these 10 cm lines were 0 for no pain and 10 for unbearable pain.

Statistics primary outcome

VAS_{mean} pain was analyzed by an Analysis of Covariance (ANCOVA) of the VAS_{mean} at day 50-52 (last day of diary) between placebo and THC that incorporates VAS_{mean} at baseline (mean day -5 to -1 pre-treatment) as covariate in the analyses. Possible moderating variables such as subpopulation (pancreatitis/postsurgical) and opiate user (y/n) were evaluated by observing potential interactions and post hoc subgroup analyses.

Secondary outcomes and statistics are fully described in supplementary material.

RESULTS

A total of 69 patients were assessed for eligibility during screening, of whom 65 were included and randomized (figure 1). Sixty-two patients started study medication, of whom 21 (8 CP/ 13 PSP) patients in the THC arm and 29 (15 CP/ 14 PSP) patients in the placebo arm were included in the modified intention to treat efficacy analysis. For the safety analysis, 30 (12 CP/ 18 PSP) patients were included in the THC arm and 32 (15 CP/ 17 PSP) patients in the placebo arm. Patient characteristics are shown in table 1. Eligible patients were recruited from October 2012 to July 2014, and stopped due to poor recruitment.

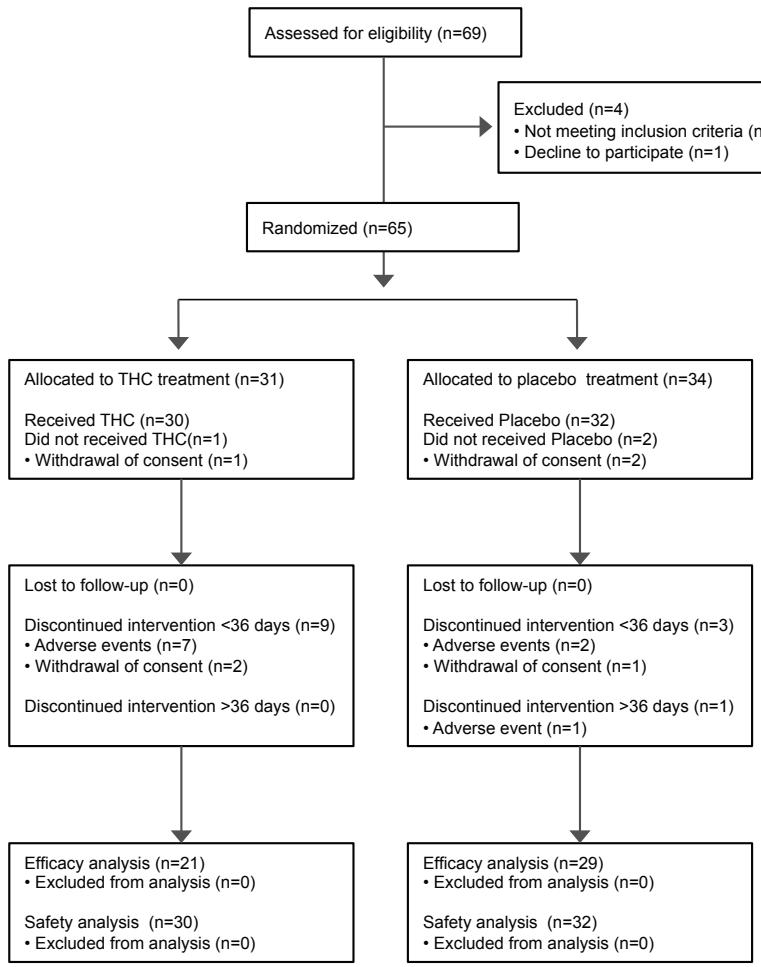

Figure 1: Participant flowchart.

Table 1: Demographic and clinical characteristics.

	CP (n=23)		PSP (n=27)	
	THC	Placebo	THC	Placebo
Gender (male/female)	7/1	11/4	2/11	5/9
Age (years)	53.9 (7.5)	53.9 (10.3)	52.2 (11.3)	51.9 (8.2)
BMI (kg/m2)	24.2 (5.0)	24.3 (3.8)	27.0 (4.5)	26.4 (3.5)
Ethnicity				
Caucasian	8	14	12	14
Mixed Afro-Caucasian	0	0	1	0
Asian	0	1	0	0
NRS pain at screening	5.3 (1.7)	5.9 (1.6)	6.9 (1.0)	7.0 (0.8)
Concomitant medication				
None	0	0	0	2
PCM	3	12	12	10
NSAID	3	2	5	1
Weak opioids	3	6	5	7
Strong opioids	7	11	4	4
Antiepileptics	3	4	1	3
Smoking status				
Current smoker	6	6	4	6
Past smoker	1	6	1	5
No smoker	1	3	8	3
Etiology CP				
Alcohol	6	3		
Hereditary	0	1		
Idiopathic	2	7		
Neoplasm	0	2		
Other	0	2		

Continuous data are expressed as mean (SD) and categorical data as numbers (n). Weak opioids were defined as codeine and tramadol. Strong opioids were defined as opioid-based therapies such as oxycontin, fentanyl and morphine.
Abbreviations: *PCM=paracetamol, NSAID= non-steroidal anti-inflammatory drugs.*

Efficacy

For patients in the efficacy analyses, mean (SD) VAS_{mean} pain scores at baseline were 4.0 (1.9) and 5.2 (1.8) for THC and placebo respectively, and for patients in the safety analysis, including drop-outs, 4.3 (1.9) and 5.2 (1.9) points respectively. VAS_{mean} pain scores during THC and placebo treatment are shown in figure 2. Primary efficacy analysis of the average

VAS pain at the last day of diary did not reveal significant difference between THC and placebo treatment (95% CI of diff [-1.31, 1.16], F(1, 46) =.016, p =.901). Mean VAS pain scores were reduced on average of 1.6 points (40%) in the THC arm compared to 1.9 points (37%) in the placebo arm. Parallel results were observed for minimal and maximal reported VAS pain. Subgroup analyses of CP (95% CI of diff [-2.23, 1.78], F(1, 19) =.056, p =.816) and PSP (95% CI of diff [-1.87, 1.70], F(1, 24) =.010, p=.922) patients revealed similar results and did not affect these outcomes as covariate. VAS pain outcomes are presented in table 2.

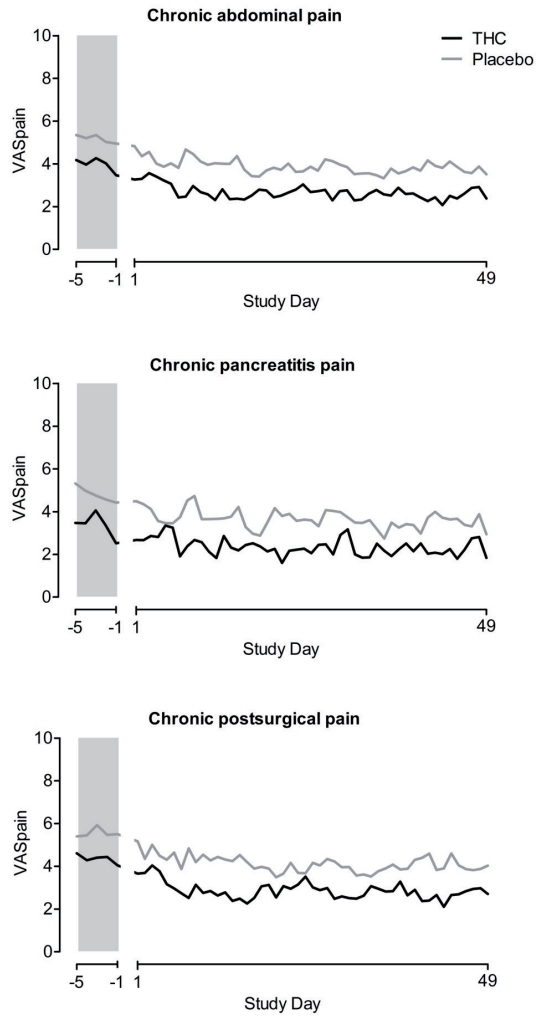

Figure 2: Mean VAS pain at baseline (day -5 to -1) and during study treatment (day 1 to 49) for THC and placebo in patients with chronic abdominal pain (n=50), subdivided in chronic pancreatitis (n=23) and postsurgical pain (n=27). VASpain scores are shown until day 49, which is the last day of diary for most patients. Grey bars represent baseline period.

Table 2: VAS pain scores

		Mean VAS pain		Minimal VAS pain		Maximal VAS pain	
		Mean	SD	Mean	SD	Mean	SD
Chronic abdominal pain (n=50 modified ITT analysis)							
THC	Baseline	4.0	1.85	2,79	1,53	4,61	2,39
	Last day	2.4	2.28	1,75	1,97	4,20	2,78
	Mean last 5 days	2.9	2.13	1,85	1,76	4,61	2,39
	Diff (last day minus baseline)	-1.6	1.78	-0,96	1,77	-0,40	0,85
Placebo	Baseline	5.2	1.75	3,03	1,85	5,66	2,24
	Last day	3.5	2.42	2,54	1,98	5,44	2,63
	Mean last 5 days	3.8	2.20	2,61	1,75	5,66	2,24
	Diff (last day minus baseline)	-1.9	2.18	-0,87	1,14	-0,12	1,50
Chronic abdominal pain (n=62 including drop-outs)							
THC	Baseline (including drop-outs)	4.3	1.93	3,28	1,98	4,61	2,39
Placebo	Baseline (including drop-outs)	5.2	1.89	3,12	2,52	5,66	2,24
Chronic Pancreatitis (n=23)							
THC	Baseline	3.4	2.32	1,84	1,41	4,64	2,64
	Last day	1.7	2.56	1,26	1,65	4,03	3,22
	Mean last 5 days	3.1	2.81	1,46	1,71	4,64	2,64
	Diff (last day minus baseline)	-1.7	1.61	-0,70	0,77	-0,57	0,94
Placebo	Baseline	4.9	1.94	2,80	2,23	5,58	2,23
	Last day	3.1	2.23	2,25	1,95	4,98	3,06
	Mean last 5 days	3.6	2.09	2,31	1,75	5,58	2,23
	Diff (last day minus baseline)	-2.1	2.28	-1,01	1,31	-0,40	1,76
Postsurgical pain (n=27)							
THC	Baseline	4.4	1.48	3,26	1,40	4,59	2,34
	Last day	2.8	2.08	2,01	2,14	4,28	2,65
	Mean last 5 days	2.8	1.70	2,04	1,82	4,59	2,34
	Diff (last day minus baseline)	-1.5	1.94	-1,07	2,08	-0,30	0,82
Placebo	Baseline	5.6	1.54	3,28	1,37	5,74	2,34
	Last day	3.9	2.61	2,82	2,03	5,88	2,18
	Mean last 5 days	3.9	2.37	2,89	1,78	5,74	2,34
	Diff (last day minus baseline)	-1.7	2.16	-0,74	0,99	0,13	1,22

Secondary efficacy outcomes

No statistically significant differences were observed in pain related questionnaires such as the patient global impression of change, pain catastrophizing or pain related anxiety. Measures of depression and generalized anxiety, quality of life, treatment satisfaction did also not change after THC treatment compared with placebo. For the domain pain of the SF-36 a trend was observed in favor of THC ($F(1, 47) = 4.023$; $p = .051$). Additionally, no differences were observed in subjective feelings corresponding to alertness, mood and calmness nor for psychedelic effects including difficulties in controlling thoughts, feeling high and feeling drowsy for THC compared with placebo.

No statistically significant differences between THC and placebo were observed for appetite level. Subjects in the THC group gained on average 0.8 kg in weight and patients in the placebo group lost on average 0.4 kg during study treatment (NS ($F(1, 47) = 1.711$; $p = .197$)). Balance disturbances were shown in several individuals, but did not statistically increase during THC treatment compared with placebo.

Table 3: Pharmacokinetic parameters of THC and 11-OH-THC after 50-52 days oral dosing of 8 mg or 5 mg TID THC in patients with chronic abdominal pain

	THC 8 mg TID			THC 5 mg TID		
	N	Mean	SD	N	Mean	SD
THC						
C_{max} (ng/mL)	14	5,21	2,51	5	4,35	2,65
t_{max} (h)	14	1,43	1,52	5	1,78	1,72
$AUC_{0\text{-}Last}$ (ng*h/mL)	14	9,89	3,23	5	8,62	2,96
$AUC_{0\text{-}tau}$ (ng*h/mL)	13	11,01	3,42	3	10,56	2,55
$t_{1/2term}$ (h)	13	3,10	1,27	3	3,32	1,89
11-OH-THC						
C_{max} (ng/mL)	14	6,89	2,97	5	5,50	1,54
t_{max} (h)	14	1,58	1,31	5	2,22	1,32
$AUC_{0\text{-}Last}$ (ng*h/mL)	14	19,32	8,44	5	19,03	6,25
$AUC_{0\text{-}tau}$ (ng*h/mL)	12	20,15	8,37	3	22,13	8,04
$t_{1/2term}$ (h)	12	2,82	0,75	3	4,52	2,41

$AUC_{0\text{-}inf}$, $AUC_{0\text{-}tau}$, $t_{1/2term}$ and λ_z were calculated only if there were two or more points (excluding C_{max}) in the elimination phase of the plasma concentration–time curve with $r^2 > 0.80$.

Pharmacokinetics

PK samples on day 50-52 time-locked after medication intake were analysed for 19 (8 CP/ 11 PSP) subjects resulting in 14 PK profiles of 8 mg and 5 PK profiles of 5mg THC. Mean THC plasma concentration curves of THC and 11-OH-THC were plotted (supplementary figure 2). Evaluation of the pharmacokinetics at an individual patient level revealed that some patients demonstrate a relatively late t_{max} accompanied with a relatively low C_{max}, which cannot be observed in the plasma concentration curves. Table 3 summarizes the calculated PK parameters of THC and 11-OH-THC. The t_{max} of THC was 1.4 hours in patients on 8mg TID compared with 1.8 hours in patients on 5mg TID Namisol® regimen, and the $t_{1/2term}$ was 3.1 hour and 3.3 hour respectively. Mean (± SD) trough levels for THC were 0.70 (± .59) ng/mL on day 15 and 0.57 (± .32) ng/mL on day 50-52. One patient demonstrated predose concentration levels below the lower limit of quantification on day 15.

Safety

Seven patients administering THC discontinued study treatment due to AEs compared with 2 patients in the placebo group. These patients did not tolerate a dosage of 5 mg TID THC and withdrew due to mild to moderate AEs. Another 5 patients in the THC arm, compared with 2 patients in the placebo arm, tapered their dosage to 5 mg TID.

A summary of (possibly) related AEs are presented in table 4. Five patients experienced serious AEs during the study treatment that were all considered not to be related to the study drug. Further AEs were mild or moderate. All subjects fully recovered from AEs. There were no clinically relevant changes in vital signs, ECG parameters, or safety laboratory parameters (hematology, biochemistry, and urinalysis).

Treatment compliance

A mean (± SD) of 97% (± 4%) of all placebo study medication was taken correctly compared with 98% (± 2%) in the THC treatment arm. There were no patients with a poor compliance (<75%), as measured by the amount of medication returned to the hospital after the treatment period. One subject appeared to be not compliant according PK predose levels on day 15, but demonstrated regular trough levels on day 50.

Table 4: Summary of (possibly) related adverse events occurring in ≥10% patients treated with THC or placebo included in the safety analyses (n=62). All (possibly) related adverse events were mild to moderate.

	THC (n=30)		Placebo (n=32)	
Averse events (PT term MedDRA)	N	%	N	%
General				
Decreased appetite	6	20%	1	3%
Increased appetite	7	23%	6	19%
Nervous system disorders				
Amnesia	4	13%	1	3%
Balance disorder	3	10%	4	13%
Disturbance in attention	4	13%		
Dizziness	24	80%	11	34%
Dysgeusia	3	10%	1	3%
Headache	14	47%	18	56%
Somnolence	15	50%	11	34%
Psychiatric disorders				
Confusional state	3	10%	3	9%
Depressed mood	3	10%	2	6%
Euphoric mood	4	13%	2	6%
Irritability	2	7%	2	6%
Sluggishness	3	10%		
Gastro-intestinal system disorders				
Abdominal pain	3	10%		
Constipation	4	13%	5	16%
Diarrhoea	3	10%	2	6%
Dry Mouth	9	30%	2	6%
Nausea	13	43%	5	16%
Skin and subcutaneous tissue disorders				
Hyperhidrosis	8	27%	5	16%
Rash			5	16%
Musculoskeletal and connective tissue disorders				
Tremor	1	3%	4	13%
Vision disorders				
Visual impairment	4	13%	1	3%

DISCUSSION

This is the first exploratory study to evaluate the analgesic efficacy, pharmacokinetics and tolerability of THC, 1) using an oral tablet with improved bioavailability and optimal blinding potential, 2) in patients with chronic abdominal pain, 3) during a relatively long-lasting treatment period of 50 days.
Contrary to our hypothesis, THC did not show a beneficial effect on chronic abdominal pain compared with placebo. Similar results were observed for minimal and maximal reported VAS pain, indicating that THC does not affect background pain or pain peaks. It should be mentioned that, despite the randomization procedure, patients in the THC group demonstrated pain of 1.2 points lower intensity at baseline than patients in the placebo group. In addition to the primary outcome, several questionnaires were used to evaluate a wide range of secondary efficacy outcomes during and after the THC treatment period. No differences were observed in pain related questionnaires or measures of depression and anxiety, quality of life and treatment satisfaction.

There are many reasons why clinical trials may fail to demonstrate analgesic efficacy on the primary endpoint. In first instance this could be related to insufficient analgesic potency of the investigational drug, but it may also be related to 1) an impaired bioavailability, 2) a large placebo response, 3) indirect analgesic effects, or 4) an inadequate study design. The absorption of orally administrated drugs might be affected particularly in patients with gastrointestinal deficits.[20] In the present study, mean plasma concentration curves of patients on both 5 mg as well as 8 mg TID treatment regimen demonstrate that THC was generally well absorbed and further metabolized into 11-OH-THC. The t_{max} of THC was 1.4 hour in patients on 8mg TID compared with 1.8 hour in patients on 5mg TID THC regimen. This delay in absorption in patients on 5mg TID THC was accompanied with an enhanced $t_{1/2term}$ duration, which overall resulted in comparable $AUC_{0\text{-}tau}$ between the two treatment regimens. It should be mentioned that the PK sampling until 6 hours postdose was too short for two patients on 5mg TID THC in order to obtain all elimination parameters. So these parameters are probably an underestimation. However, the reliable pharmacokinetic profiles observed in our study population do not explain the lack of observed efficacy.

A large placebo response of 37% pain reduction was observed in current study, which is common in chronic visceral pain studies. A meta-analysis including 8.364 patients with irritable bowel syndrome allocated to placebo observed a pooled placebo response of 37.5%.[21] However, a previous RCT of our study group also observed a high reduction of average pain score by 24% in the placebo arm, but this did not prevent proof of

superiority of pregabalin over placebo using a very similar study design in patients with CP.[22] Underlying mechanisms of the placebo effect can be derived from psychological and neurobiological viewpoints. Two well supported mechanisms from a psychological point of view are expectancy and conditioning.[23] Factors that influence the magnitude of the placebo response in RCTs include type of active medication, randomization ratio, and the number of planned face-to-face visits, thereby supporting the expectancy hypothesis.[24] High expectations toward treatment efficacy of THC might have contributed to the substantial placebo response as observed in the present study.

The lack of observed analgesic efficacy can also be considered from a mechanistic point of view. Two major mechanisms are currently proposed to underlie chronic pain and its development: 1) sensitization of nociceptive processing (central sensitization/ hyperalgesia), and 2) alterations in central cognitive and autonomic processing.[8,13] Consequently, the focus of treatment options for chronic pain has been shifting away from targeting the anatomical site to targeting changes in the peripheral and central nervous system. The anti-hyperalgesic potential of THC is not clearly demonstrated in human and should be further evaluated using measurements such as quantitative sensory testing or EEG.
Patients with persistent pain demonstrated increased brain activity in areas considered to mediate emotion including the perigenual anterior cingulate cortex, the medial prefrontal cortex, and parts of the amygdala.[13] Thus, the representation of pain in the brain shifts over time to areas implicated in cognitive function, particularly emotion.[25] The frontal-limbic distribution of cannabinoid receptors in the brain suggests that cannabis may preferentially target the affective qualities of pain. A study conducted by Lee et al. demonstrated that dronabinol reduced the reported unpleasantness, but not the intensity of ongoing pain and hyperalgesia.[14] This suggests a shift in central nervous system function from nociceptive to cognitive, affective and autonomic sensitization in patients moving from acute to chronic pain. Therefore, an agent targeting particular brain areas related to the cognitive emotional feature of chronic pain, such as THC, might be efficacious in our chronic pain population, but might be better measured using affective outcomes of pain.

In general, THC was well tolerated resulting in only mild to moderate (possibly) related adverse events, which were similar to previous studies in CP patients and healthy volunteers[42].[26,27] The considerable number of AEs reported in the placebo group as well as the withdrawal of patients because of AEs, despite being in the placebo arm, indicate that AEs were partly determined by nonpharmacological effects.[28,29] This so called nocebo effect induces negative effects due to negative expectations. Cannabis

is a generally well known product, particularly as recreational drug to induce desired psychotropic effects such as euphoria, relaxation, and perceptual alterations. Therefore, it is plausible that patients in this study were influenced by expectations, which may have influenced the occurrence of AEs.

A major limitation of the present study is the small sample size, which is insufficiently large to allow subgroup analyses. However, considering the confidence intervals of the effect, it is doubtful that an increased sample size would have been resulted in significant differences.
Furthermore, the present study comprises a heterogeneous patient population regarding etiology and anatomical site of the pain. However, all patients suffered from chronic abdominal pain, which is associated with central sensitization and alterations in central cognitive and autonomic processing.[8,13] The presence of central sensitization in chronic pain patients supports the choice of treatments that reduce pain by normalizing hyperexcitable central neural activity, which makes the initial pain etiology or peripheral stimulus and past or currently received pain treatments less important. These variables and other patient characteristics might have contributed to inter-individual differences in treatment effects – while on the other hand enhancing the generalizability of the study.
Additionally, it should be mentioned that most patients already had received different pain treatments including analgesics, which failed to provide a satisfactory level of pain relief. Thus, this study included a selection of patients who did not respond to registered analgesics with a proven efficacy.

In summary, we conclude that THC treatment showed acceptable safety and tolerability profiles during a 50-52 day add-on treatment period, but did not significantly reduce pain scores or secondary efficacy outcomes in patients with chronic abdominal pain compared to placebo. Further research should evaluate the the effects of THC on secondary and tertiary central pain processing.

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Supplementary material: Methods

Study procedures

Potential participating patients were screened for eligibility within 7-35 days prior to start of study treatment (visit 1). Screening included demographics, medical history, concomitant medication, smoking habits, physical examination, 12-lead electrocardiogram (ECG), standard laboratory blood tests (hematology, biochemistry, virology) and urine screening tests (urinalysis, drug screening and pregnancy test). Furthermore, all patients received a diary to report pain scores, add-on analgesics and adverse events.

Study days were carried out at the clinical research center of the Radboudumc, where each patient stayed in a separate quiet room.

Secondary efficacy outcomes

Pain related questionnaires included the patient global impression of change (PGIC)[1] evaluated on day 15 and 50-52, pain catastrophizing scale (PCS)[2, 3] evaluated on day 1, 15 and 50-52, and pain anxiety symptom scale (PASS)[4] evaluated on day 1 and 50-52. The hospital anxiety and depression scale (HADS)[5], and quality of life questionnaire (RAND SF-36)[6] were filled out at day 1 and 50-52. Treatment satisfaction (TSQM v. II)[7] and the patient appetite level (AppLe) were evaluated at the last study visit. The AppLe was a modification of the PGIC to evaluate any change in appetite in the last week and compared to before the study period.

Drug effects on alertness, mood, and calmness were explored using the Bond & Lader questionnaire, and potential subjective psychotomimetic (psychedelic) effects were evaluated using the Bowdle questionnaire.[8, 9] Both questionnaires were filled out on day 1, 4-5, 9-10, 15, and 50-52.

Left-right (roll) and anterior-posterior (pitch) postural movements were measured using a gyroscope-based measurement system (SwayStar™, Balance International Innovations GmbH, Switzerland), which was attached to the waist of the patient. Patients stood, without shoes, as still as possible in a standardized base of support with their arms hanging at both sides of their body. Body sway was measured for one minute with eyes open, one minute with eyes closed and for 30 seconds with eyes open standing on one leg of preference. Patients were asked to fixate at one point during the tasks with eyes open. The computerized measures used for analysis reflect the total angular area and 90% range roll and pitch excursion in degrees from the centre of gravity.

Safety and Tolerability

Safety and tolerability were evaluated using spontaneously reported adverse events (AEs) and measurements of vital functions, ECG and laboratory tests. AEs were recorded in a daily diary, at study visits and phone calls up to 2 weeks after study drug discontinuation. Blood pressure and heart rate were measured at screening and on both study days. ECG, hematology, blood chemistry, and urinalysis were performed at screening and at the end of the study.

Pharmacokinetics

Plasma concentrations of THC and its active metabolite 11-OH-THC were determined predose on day 1, 15 and 50-52 to confirm a baseline state, determine trough levels and test the compliance. The PK sampling on day 50-52 was extended with 7 additional samples time-locked after medication intake at 0:30, 1:00, 2:00, 3:00, 4:00, 5:00, and 5:55 hours postdose. Blood samples were collected in 4ml EDTA tubes and immediately after collection wrapped in aluminum foil and kept on ice. Samples were centrifuged within 30 minutes at 2000 g for 10 minutes at 4°C. The handling of THC samples was done avoiding direct light. The separated plasma was divided into primary and backup samples, and stored at –80°C until bioanalysis. Bioanalysis (Analytisch Biochemisch Laboratorium b.v., Assen, the Netherlands) was performed using a validated liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) assay method according to good laboratory practice procedures. The lower limit of quantification for THC and 11-OH-THC was 0.100 ng ml^{-1}.

Statistical analysis

The primary outcome of this study was change in pain intensity, measured by the VAS_{mean} in a daily diary, between THC and placebo treatment. VAS_{mean} pain was analyzed by an Analysis of Covariance (ANCOVA) of the VAS_{mean} at day 50-52 (last day of diary) between placebo and THC that incorporates VAS_{mean} at baseline (mean day -5 to -1 pre-treatment) as covariate in the analyses. Possible moderating variables such as subpopulation (pancreatitis/postsurgical) and opiate user (y/n) were evaluated by observing potential interactions and post hoc subgroup analyses. Secondary efficacy outcomes were analyzed in a similar manner. All participants who received the study medication for at least 36 days were included in the efficacy analyses according to the intention to treat principle. Dropouts before day 36 were replaced and data of dropouts were excluded from further analyses for efficacy. Safety analyses was performed on all randomized subjects who received at least one dose of THC or placebo.

For statistical analysis SPSS software for Windows v.20 was used. All statistical tests were performed two-tailed, and the limit for statistical significance was set at $P<0.05$. The initial

study in CP patients was powered (α = 0.05, power = 0.80) to detect a decrease of at least 1.0 VAS_{mean} pain in the THC group compared with placebo, resulting in 34 patients per group. Variances in pain scores were extrapolated from a similar study with pregabalin.[10] No information was available to estimate the SD in the initial PSP study, therefore, same numbers were adopted for this study. Input variances for the integrated study were considered to be too unreliable to conduct a sample size calculation. Therefore, no sample size calculation was performed for this early phase 2 clinical trial.

Non-compartmental analysis to determine plasma PK parameters of the active compounds, THC and 11-OH-THC, was performed using the WinNonlin modeling and analysis software (version 2.1 a; Pharsight Inc., Apex, NC). The maximum plasma concentration (C_{max}), the time to reach C_{max} (T_{max}), and the AUC from 0 up to the last measurement (AUC_{0-last}, using the linear log trapezoidal rule) were calculated from the individual plasma concentration-versus-time profiles. The terminal half-life ($t_{1/2\ term}$) was calculated only if there were two or more points (excluding C_{max}) in the elimination phase of the plasma concentration–time curve with r^2>0.80. For that reason, one patient was excluded from this part of the analysis for THC and two patients for 11-OH-THC. Subsequently, the areas under the plasma concentration curves extrapolated to the end of the dosing period (AUC_{tau}) were calculated using the linear log trapezoidal rule and extrapolation to 8 hours.

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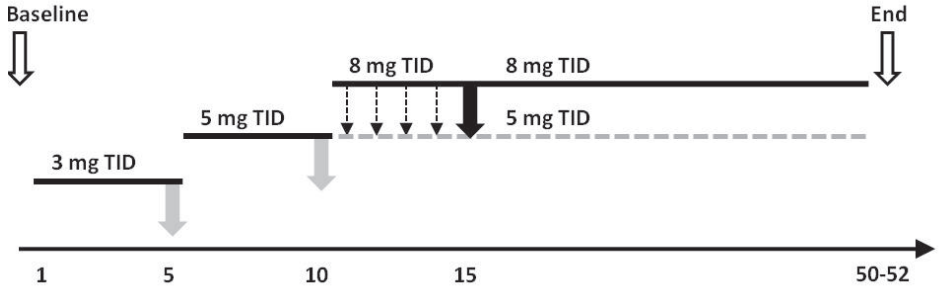
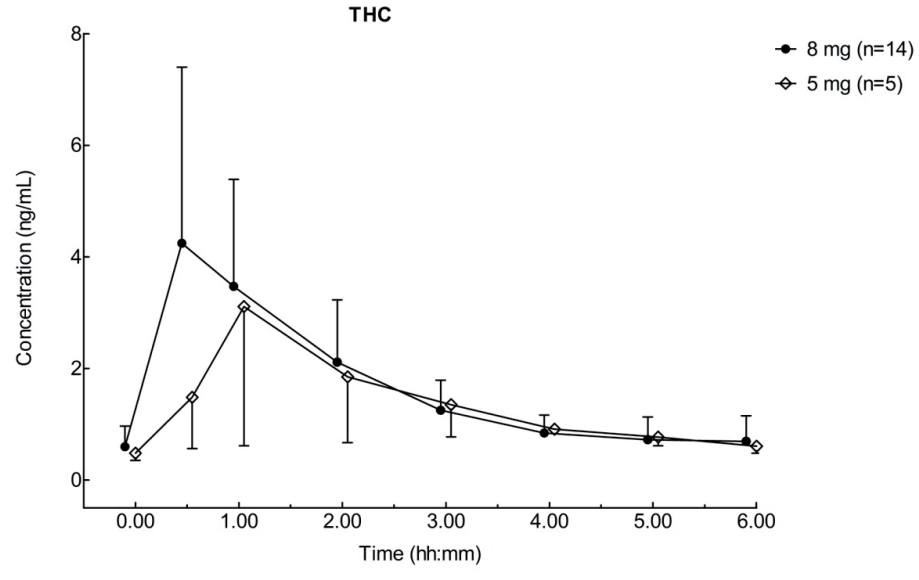


Supplementary material: figure 1. After baseline measurements, patients administrated 3 mg TID THC or placebo from day 1 to 5. On day 5, tolerability was evaluated. The dosage of day 6 to 10 was increased to 5 mg TID or, when not tolerated, the patient was withdrawn. On day 10, the tolerability was evaluated again. From day 11 to 15, the dosage was further increased to 8 mg TID. This dosage could be tapered to 5 mg TID, when 8 mg appeared to induce unacceptable adverse events (dotted arrows). At day 15 the tolerability was evaluated again. If tolerable, patients proceeded with 8 mg TID, but if not, the dosage was reduced to 5 mg TID.
Grey filled arrows represent decision points I en II: increased dosage or withdrawal. Black filled arrow represents decision point III: continue 8 mg TID, taper to 5 mg TID, or withdrawal. Dotted line represents the permitted dose adjustment of minimal 5 mg TID.



Supplementary material: figure 2. Mean (unilateral SD error bars) plasma concentration curves of THC and 11-OH-THC obtained after 50-52 treatment days in chronic abdominal pain subjects taking 5 mg versus 8 mg TID THC.

Chapter 6

Dronabinol and chronic pain:

importance of mechanistic considerations

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ABSTRACT

INTRODUCTION: Although medicinal cannabis has been used for many centuries, the therapeutic potential of delta-9-tetrahydrocannabinol (Δ9-THC; international non-proprietary name = dronabinol) in current pain management remains unclear. Several pharmaceutical products with defined natural or synthesized Δ9-THC content have been developed, resulting in increasing numbers of clinical trials investigating the analgesic efficacy of dronabinol in various pain conditions. Different underlying pain mechanisms, including sensitization of nociceptive sensory pathways and alterations in cognitive and autonomic processing, might explain the varying analgesic effects of dronabinol in chronic pain states.

AREAS COVERED: The pharmacokinetics, pharmacodynamics and mechanisms of action of products with a defined dronabinol content are summarized. Additionally, randomized clinical trials investigating the analgesic efficacy of pharmaceutical cannabis based products are reviewed for the treatment of chronic nonmalignant pain.

EXPERT OPINION: We suggest a mechanism-based approach beyond measurement of subjective pain relief to evaluate the therapeutic potential of dronabinol in chronic pain management. Development of objective mechanistic diagnostic biomarkers reflecting altered sensory and cognitive processing in the brain is essential to evaluate dronabinol induced analgesia, and to permit identification of responders and/or non-responders to dronabinol treatment.

INTRODUCTION

Although medicinal cannabis has been used for thousands of years, the therapeutic potential of cannabinoids in current pain management is still unclear. Evidence supporting the analgesic efficacy of cannabinoids face several difficulties. First, there is limited level one evidence of randomized clinical trials (RCTs), which is generally accepted as the most reliable evidence of whether a treatment is effective. Second, chronic pain patients are a heterogeneous group comprising many different pain syndromes and underlying mechanisms.[1] Pain is influenced by several aspects other than nociceptive input and altered nociceptive processing, such as cognitive, emotional and social factors, all contributing to the entire pain experience. Thus acute pain differs from chronic pain and cancer related pain differs from non-malignant pain, which makes a comparison between these pain populations complicated. Third, a broad range of cannabis based products are used including herbal crude with distinct administration forms and undefined absorption as well as pharmaceutical products with a known bioavailability.

The International Association for the Study of Pain (IASP) defines pain as chronic if it persists beyond the normal tissue healing time, which is usually three to six months.[2] Chronic pain is associated with an abnormal state of responsiveness or increased gain of the nociceptive pathways in the central nervous system, termed central sensitization,[3] as well as with alterations in cognitive functioning. Changes to nociceptive signal processing in the central nervous system are typically expressed as hyperalgesia, i.e. increased pain in response to noxious stimuli, and allodynia, i.e. pain in response to a non-nociceptive stimulus. Because central sensitization alters the properties of neurons in the central nervous system, the pain is frequently no longer reliably coupled to the presence of particular peripheral stimuli. Cognitive neuroplasticity is manifest as shifts in brain activity in patients with chronic pain from sensory representation areas to areas related to cognitive function and considered to mediate emotion.[4]

Opioids are frequently prescribed for chronic pain. However, opioids often do not provide effective or satisfactory pain relief in chronic pain conditions. Additionally, adverse consequences of prolonged opioid use, including addiction, tolerance and opioid induced hyperalgesia, call for alternatives in medical pain treatment. The discovery and cloning of the endocannabinoid system in the early 1990s increased scientific interest in the therapeutic potential of cannabinoids. New pharmaceutical products were developed containing either natural or synthesized delta-9-tetrahydrocannabinol (Δ9-THC), which is the principal psychoactive compound of the *Cannabis sativa* plant. Dronabinol is the international non-proprietary name (INN) of Δ9-THC. To date, a wide

range of products containing Δ9-THC are described in literature: 1) crude herbal cannabis for recreational use containing undefined concentrations of active compounds; 2) crude medicinal cannabis containing estimated concentrations of active compounds used for medical purposes; 3) synthetic analogues interacting with cannabinoid (CB) receptors, and 4) pharmaceutical products with standardized natural or synthetic Δ9-THC content containing whole cannabis plant extract, a defined combination of Δ9-THC and cannabidiol (CBD), or pure Δ9-THC. CBD is another non-psychotropic constituent of cannabis, which has very low affinity for cannabinoid CB1 and CB2 receptors.[5] It may act as a high potency antagonist of cannabinoid receptor agonists and an inverse agonist at the CB2 receptor.[6]

For many patients the only way they can use cannabis as a medicine is to obtain material that is of variable quality, composition and purity and is illicit. They commonly smoke herbal cannabis, which has some obvious disadvantages. Most important is that smoking of herbal cannabis results in high plasma levels after inhalation that cause immediate side effects and makes it difficult to administer accurate therapeutic dosages. Additionally, the smoke produced contains irritants and carcinogens, and additionally, patients do not wish to smoke medicines or do not know how to do so. The development of pharmaceutical products for oral or sublingual administration with defined Δ9-THC content offers a favorable alternative. Psychological and physiologic effects after intake of oral dronabinol preparations were similar compared to whole plant drug cannabis.[7,8] However, the effects of dronabinol in whole plant cannabis may be modulated by other cannabinoids, mainly CBD, and other cannabis constituents.[9,10] This was demonstrated by a study in patients with intractable cancer-related pain where a combination of THC and CBD showed a more promising efficacy profile than the THC extract alone.[11] Potential interactions between phytocannabinoids and cannabis terpenoids are extensively reviewed by Russo et al.[9,10]

Several randomized clinical trials have been conducted to investigate the analgesic efficacy of cannabis-based products with standardized Δ9-THC content. The aim of this review is to provide an overview of these trials, offer a brief description of the endocannabinoid system, and the describe the pharmacokinetics and potential side effects of dronabinol in the treatment of chronic non-malignant pain. Common limitations in current clinical trials and challenges are discussed in the expert opinion section of this review. Furthermore, we propose a mechanism-based approach to evaluate the therapeutic potential of Δ9-THC in chronic pain management, not only for subjective pain relief but also in reducing pain sensitivity.

OVERVIEW OF DRONABINOL CHEMISTRY

The endocannabinoid system

The endocannabinoid system consists of two cannabinoid receptors, the CB1 and CB2 receptors,[12,13] and the endogenous ligands for these receptors, such as anandamide and 2-arachidonoylglycerol (2-AG).[14,15] The distribution of CB receptors and the role of endocannabinoid-hydrolyzing enzymes within pain modulatory circuits has recently been reviewed.[16]

THC induces pharmacological effects by binding non-selectively to G protein coupled CB receptors. CB1 receptors are expressed most densely on neurons in the brain, spinal cord and peripheral nervous system, but are also expressed by some non-neuronal cells in many peripheral organs and tissues.[17,18] In the central nervous system, CB1 receptors are most expressed in the cerebral cortex, basal ganglia, cerebellum, hippocampus, periaqueductal grey (PAG), rostral ventromedial medulla, certain nuclei of the thalamus, amygdala, and dorsal primary afferent spinal cord region.[19-21] CB1 receptor expression appears to be sparse or absent in the vital centers of the brainstem. In contrast, CB2 receptors are most densely expressed in the peripheral nervous system and in human immune cells, e.g. resident in the spleen, tonsils and leucocytes.[22,23] Previous studies suggested that CB2 receptors are also expressed in the central nervous system,[24-26] but many investigators were not able to detect neuronal CB2 receptors in healthy brains.[27-29] Although the expression of CB2 receptors in neurons has remained controversial, it is now well accepted that CB2 receptors are expressed in brain microglia during neuroinflammation.[30]

Thus cannabinoid receptors occur in high density in many areas related to pain. They densely populate the PAG and the rostral ventrolateral medulla, which are important brain areas involved in descending pain modulation. They are also concentrated in the superficial layers of the spinal dorsal horn, and they are found in the dorsal root ganglion, and peripheral terminals of primary afferent neurons.[20,31] Putative analgesic effects of cannabinoids may therefore be produced by both central mechanisms, e.g. via activation of descending modulatory pathways,[32] and peripheral mechanisms, e.g. by inhibiting release of neurotransmitters from nociceptive primary afferents.[33] In addition, a recent study investigated the effect of oral THC on the affective qualities of pain. They concluded that amygdala activity contributed to the dissociative effect of dronabinol on pain perception.[34]

Pharmacokinetics

The pharmacokinetic profile of Δ9-THC varies with route of administration and formulation.[35,36] After inhalation of the smoke of a cannabis cigarette, Δ9-THC reaches a

maximum plasma concentration within minutes. Psychotropic effects commence within seconds to a few minutes, reach a maximum after 15–30 minutes, and taper off within 2–3 hours.[35] Bioavailability after oral ingestion of cannabinoids is low compared to inhalation and variable among different formulations. Maximal plasma concentrations after oral administration are usually reached after 60-120 min,[37,38] and in some subjects after up to 6 hours.[37] Therefore, the onset of pharmacodynamic effects is delayed compared to inhalation, but the duration is prolonged because of continued slow absorption from the gut.[39] Once absorbed, dronabinol and metabolites are rapidly distributed to all other tissues at rates dependent on the blood flow. Because they are extremely lipid soluble, cannabinoids accumulate in fatty tissues.[40] The pharmacokinetics following sublingual administration is similar to that after oral administration. In addition, kinetics of cannabinoids are much the same for females and males,[39] as well as for frequent and infrequent users.[41]

Metabolism

Cannabinoids are metabolized in the liver by microsomal hydroxylation and oxidation catalysed by enzymes of the cytochrome P-450 complex.[42,43] Hydroxylation results in 11-hydroxy-THC (11-OH-THC), which is possibly more potent than THC itself and may be responsible for some of the effects of cannabis. Further oxidation takes place to produce 11-nor-9-carboxy-THC (THC-COOH), which is an inactive metabolite. First-pass metabolism in the liver further reduces the oral bioavailability of dronabinol, i.e. much of the dronabinol is initially metabolized in the liver before it reaches the sites of action.[35]
The excretion of THC and metabolites is slow due to rediffusion of THC from body fat and other tissues into the blood.[44] Most of the absorbed THC is excreted as metabolites in faeces (more than 55%) and in urine (approximately 20%).
Pharmacokinetic interactions may occur due to metabolic interference with the cytochrome P450 subsystem in the liver. CYP450 inhibition by THC may lead to delayed elimination of other medications metabolized by the same pathway, which can lead to raised plasma levels of the medications in question. However, studies of THC inhibition and induction of major human CYP-450 isoforms generally reflect a low risk of clinically significant drug interactions with most use, but specific human data are lacking.[45,46]

Potential side effects

Cannabis and individual cannabinoid receptor agonists such as Δ9-THC have very similar, although not identical, side effects.[47] These side effects depend on the dose and route of administration, composition of the product and treatment indication. A systematic review studying the safety of medical cannabinoids found an increased risk of nonserious adverse events compared to placebo, but no difference in serious adverse

events. Additionally, the risk associated with long-term use of cannabinoids is poorly qualified in current clinical and observational studies.[48] Common side effects for THC products in general include dizziness, somnolence, lethargy, abnormal feeling, dry mouth, nausea and increased appetite.[49,50]
However, most adverse effects appear to be dose-related, and can be avoided by adequate dose titrating. Moreover, some side effects could be classified as potentially beneficial (e.g. euphoric mood, somnolence, increased appetite), and are not necessarily harmful. Sleep parameters were in fact significantly improved in several RCTs performed with a THC/CBD combination spray in chronic pain.[51]

Pharmaceutical formulations

To date, four pharmaceutical preparations with defined dronabinol content have been developed and/or are available for selected indications in certain countries.

- *Cannador* (IKF-Berlin, Germany) is an oral capsule, containing whole cannabis plant extract with standardized Δ9-THC and CBD content in approximately a 2:1 ratio. This product is no longer under investigation in clinical studies and the project to bring it on the market has been stopped.
- *Marinol* (Solvay Pharmaceuticals, Belgium) is an oral capsule containing synthetic Δ9-THC.
- *Namisol* (Echo Pharmaceuticals, The Netherlands) is an oral tablet containing pure, natural Δ9-THC isolated from the *Cannabis sativa* plant. To date, only phase I results are published.
- *Sativex* (GW Pharmaceuticals, UK) is a whole cannabis based oromucosal spray, containing primarily natural THC and CBD in a standardized 1:1 ratio, and minor cannabinoids and terpenoids.

Parallel to the development of pharmaceutical cannabis based medicines, governments of several countries set up programs to supply quality-controlled herbal cannabis (Bedrocan, The Netherlands). Furthermore, Nabilone (Valeant Pharmaceuticals International, USA), which is a synthetic analogue of Δ9-THC for oral administration, was not included in this review because this agent has different pharmacokinetic and pharmacodynamic properties.

THERAPEUTIC POTENTIAL OF DRONABINOL IN CHRONIC PAIN MANAGEMENT

Animal studies using either acute or chronic pain models have demonstrated significant analgesic and antihyperalgesic effects of cannabinoids. However, the role of cannabinoids in human analgesia or antihyperalgesia is less well documented.[52] An increasing number of randomized controlled trials have investigated the analgesic efficacy of different products containing dronabinol in various pain states. These trials have been evaluated in a few good quality reviews.[1,50,53-58] In summary, products containing dronabinol demonstrated analgesic efficacy in a majority of studies in chronic pain, whereas the data in acute pain were less conclusive. No significant difference was found in the summed pain intensity difference over 6 hours, nor in the time to rescue analgesia, between Δ9-THC and placebo in patients undergoing elective abdominal hysterectomy.[59] A more promising effect in acute pain was found by Holdcroft et al., who demonstrated a dose-response effect for decreasing pain intensity at rest in acute postoperative pain.[60] However, (weak) analgesic effects of cannabinoids in acute human experimental pain and acute postoperative pain models were also accompanied by hyperalgesic effects, suggesting cannabinoid-induced sensitization,[61] particular at higher doses.[62] The analgesic effects of cannabinoids in chronic pain states appear more promising, with significant pain reduction being documented in the majority of clinical studies.[63-75]

Randomized controlled trials in chronic non-malignant pain

An overview of randomized controlled trials with standardized dronabinol products in human chronic non-malignant pain treatment is provided in table 1. The majority of these studies were conducted in patients suffering from central neuropathic pain in multiple sclerosis (MS).

Dronabinol reduced spontaneous pain intensity as measured with a numerical rating scale (NRS) over a treatment period of 3 weeks,[72] and improved overall pain ratings associated with spasm over a treatment period of 15 weeks.[75] Additionally, dronabinol improved median radiating pain intensity and pressure pain thresholds in MS patients.[72]

Five randomized controlled trials (RCTs) compared the analgesic efficacy of the oromucosal THC/CBD combination spray with placebo in patients with MS-related pain. Rog et al. demonstrated efficacy for up to 4 weeks,[70] whereas a 6 week treatment in a subsample of MS patients with pain did not show a significant between group effect, but only within group effects in both the THC/CBD and placebo group.[73] A recent study in 339 patients with MS-related neuropathic pain failed to show a significant difference in the number of responders between THC/CBD spray compared to placebo. The responder analysis at week 14 of phase A of this study showed a large proportion of responders to

THC/CBD treatment, with 50% of patients on THC/CBD spray classed as responders at the 30% level, compared to a similarly large number of 45% placebo responders. Phase B of this study demonstrated an increased time to treatment failure in the THC/CBD spray group compared to placebo.[65] Two crossover studies in patients with neuropathic symptoms, mainly MS-related, reported significant reductions in VAS pain in favor of products containing dronabinol.[67,74]

In other neuropathic pain conditions, such as peripheral neuropathic pain with allodynia, The THC/CBD combination spray produced statistically significant improvements in pain levels, dynamic and punctate allodynia.[68] A significant pain reduction was also reported in patients with central neuropathic pain due to brachial plexus avulsion.[63] However, a significant pain reduction was observed within the THC/CBD spray group but not between the THC/CBD and placebo groups in patients with painful diabetic peripheral neuropathy.[71] In patients with rheumatoid arthritis, which is an inflammatory rather than a neuropathic pain syndrome, morning pain at rest and on movement were improved with THC/CBD spray compared to placebo.[64]

A pilot study that compared the effectiveness of dronabinol with that of an active control, diphenhydramine, in patients with pain below the level of spinal cord injury found no significant difference in pain intensity ratings.[69] The efficacy of dronabinol as an adjuvant treatment to opioid therapy for chronic pain patients was assessed by Narang et al.[66] Patients who received dronabinol experienced decreased pain intensity and increased satisfaction compared with placebo. In an extended open-label titrated trial of dronabinol as add-on medication to patients on stable doses of opioids, titrated dronabinol contributed to significant relief of pain compared with baseline. Thus, the use of dronabinol was found to result in additional analgesia among patients taking opioids for chronic non-malignant pain.

Table 1. Randomized controlled trials of products with defined Δ9-THC content in chronic pain treatment

Author (date)	Product	Indication	Number (n)	design	Treatment (duration)	Efficacy
Narang et al. (2008)[66]	Synthetic Δ9-THC	Chronic pain on opioid treatment	30	Crossover	Phase I: single dose RCT Phase II: 4 weeks open label extension	Significant pain reduction
Svendsen et al. (2004)[72]	Synthetic Δ9-THC	Central neuropathic pain in multiple sclerosis	24	Crossover	15-21 days treatment periods	Significant reduction of NRS pain intensity
Rintala et al. (2010)[69]	Dronabinol	Central Neuropathic Pain After Spinal Cord Injury	7	Crossover	56 days treatment periods	Dronabinol was no more effective than diphenhydramine for pain relief
Langford et al. (2013)[65]	Oromucosal THC/ CBD	Central neuropathic pain in multiple sclerosis	339	Parallel	Phase I: 14 weeks Phase II: open-label plus 4-week randomized-withdrawal extension	No difference in number of responders Significant increased time to treatment failure in Sativex group
Selvarajah et al. (2010)[71]	Oromucosal THC/ CBD	Painful diabetic peripheral neuropathy	30	Parallel	12-weeks	significant improvement in pain scores within groups, between groups not significant
Nurmikko et al. (2007)[68]	Oromucosal THC/ CBD	Neuropathic pain of peripheral origin with allodynia	125	Parallel	5 weeks plus open-label extension	Significant reduction of NRS pain scores
Blake et al. (2006)[64]	Oromucosal THC/ CBD	Rheumatoid arthritis (RA)	58	Parallel	5 weeks	statistically significant improvements in pain on movement and pain at rest
Rog et al. (2005)[70]	Oromucosal THC/ CBD	Central neuropathic pain in multiple sclerosis	66	Parallel	5 weeks	Significant reduction of NRS pain intensity
Berman et al. (2004)[63]	Oromucosal THC/ CBD, THC	Central neuropathic pain from brachial plexus avulsion	48	Crossover	2 week treatment periods	Significant pain reductions, but not two points reduction

Continued **table 1**

Wade et al. (2004)[73]	Oromucosal THC/ CBD	Pain in multiple sclerosis	160 37 with pain	Parallel	6 weeks	No difference in VAS pain between groups, within groups both decreased
Notcutt et al. (2004)[67]	Oromucosal THC/ CBD, THC, CBD	Chronic pain, mainly neuropathic (MS)	24	Crossover	2 weeks open plus 8 x 1 week treatment periods	Extracts containing THC effective in relieving VAS pain
Wade et al. (2003)[74]	Oromucosal THC/ CBD, THC, CBD	Neurogenic symptoms in MS/spinal cord injury/ brachial plexus injury/ limb amputation	24 12 with pain	Crossover	2 week treatment periods	Significant reduction in VAS pain with CBD and THC
Zajicek et al. (2003)[75]	Oral THC/ CBD	Pain due to spasm in MS	667 419 with pain	Parallel	15 weeks	Pain associated with spasm improved

EXPERT OPINION

The introduction of cannabinoid medicines offers an interesting alternative approach in the area of chronic pain management, particularly for cases in which currently available pharmacologic treatments are not sufficient. Scientific literature on clinical research regarding medicinal cannabinoids lags far behind the extensive anecdotal experiences of both patients and their physicians. The majority of clinical trials in patients with chronic non-malignant pain summarized in this review reported improvement in pain scores in favor of products containing dronabinol. However, analgesic effects were generally weak and placebo effects were considerable in the comparative arm. In addition, the number of available studies for any one specific cannabinoid preparation, as well as other study factors such as various pain conditions, limited study population size and treatment duration, preclude the recommendation of any one specific cannabinoid drug for the treatment of any one chronic pain condition. Underlying pain mechanisms, including plasticity of nociceptive and cognitive pain processing, may explain the varying analgesic effects of dronabinol in particular chronic pain states. To date, regulatory authorities still assess the therapeutic potential of new analgesics based primarily on the patient's subjective pain experience. Hence, we suggest a mechanism-based approach beyond the measurement of subjective pain relief for future research, to evaluate the therapeutic potential of dronabinol in chronic pain management.

Underlying mechanisms of chronic pain

Two major mechanisms are currently proposed to underlie chronic pain and its development: 1) sensitization of nociceptive pathways (central sensitization), and 2) alterations in central cognitive and autonomic processing.[4,76]

Preclinical and clinical evidence suggests that persistent pain is correlated with synaptic plasticity through an increase in excitability and synaptic efficacy of neurons in central nociceptive pathways,[77] and reduced function in inhibitory pathways resulting in a decreased inhibitory efficiency.[78] Identification of the presence of such central sensitization in chronic pain patients enables a mechanism-based approach to the diagnosis and treatment of pain, by choosing treatments that reduce pain experience by normalizing hyperexcitable central neural activity,[76] or augment descending inhibition.[78]

A recent prospective study demonstrated a divergence over time in brain signatures between subjects with subacute back pain that recovered within a year versus those in whom pain persisted.[4] All subjects initially exhibited acute pain-specific brain activity, including in the insula, anterior cingulate cortex, and thalamus. Patients with persistent pain demonstrated increasing brain activity in areas considered to mediate emotion including the perigenual anterior cingulate cortex, the medial prefrontal cortex, and

parts of the amygdale.[4] Thus, the representation of pain in the brain shifts over time from the classical acute pain matrix to areas implicated in cognitive function, particularly emotion.

The frontal-limbic distribution of cannabinoid receptors in the brain suggests that cannabis may preferentially target the affective qualities of pain. An earlier mentioned study conducted by Lee et al. demonstrated that dronabinol reduced the reported unpleasantness, but not the intensity of ongoing pain and hyperalgesia.[34] This reduction in the unpleasantness of hyperalgesia was positively correlated with right amygdala activity. Dronabinol also reduced functional connectivity between the amygdala and primary sensorimotor areas during the ongoing pain state. This suggests that dronabinol may target preferentially the affective qualities of pain.[34]

The shift in central nervous system function from nociceptive to cognitive, affective and autonomic sensitization in patients moving from acute to chronic pain is increasingly well established. This indicates that the effects of pain on the brain are not uniform but differ according to degree of chronicity, and hence explain varying effects of dronabinol in different pain conditions and stages. Thus, an agent targeting particular brain areas related to the cognitive emotional feature of chronic pain, such as dronabinol, might be more efficacious in patients with evidence of this particular form of supraspinal neuroplasticity.

Individual treatment tailoring and responder identification

Most analgesics are only effective in a subset of patients and many have adverse effects.[51] The concept of personalized medicine is based on optimizing medication types and dosages for individual patients according to genetic, mechanistic, and other patient-related factors.[79,80] This mechanism-based approach may help to prevent a long undesirable trial and error process of finding an appropriate therapy for the individual patient [81]. RCTs are the current gold standard for demonstrating analgesic efficacy at the group level in patients with a specific diagnosis of chronic pain.[80] While the strict sample selection criteria, protocol standardization and controlled nature of RCTs are ideal for conclusively demonstrating analgesic efficacy and side effects in the average patient, they are less ideal at identifying individual patients likely to experience good analgesia with low side effects.[80]

Several non-invasive techniques, such as quantitative sensory testing (QST), conditioned pain modulation (CPM) and encephalography (EEG), have the potential to identify patients with a specific pattern of abnormalities in central pain processing, and thus to predict treatment outcome of specific analgesic therapy in individual patients suffering from a chronic pain disorder.[81-83]

QST provides information on sensory function at the peripheral and central level of the

nervous system by measuring pain thresholds to different external stimuli of controlled intensity.[84] The effect of pregabalin was associated with pre-treatment sensitivity to electric tetanic QST. These results were reported as first evidence that QST predicts the analgesic efficacy of pregabalin in patients with painful chronic pancreatitis.[81,85] Similar findings were reported using EEG measurements, where changes in spectral indices caused by slowing of brain oscillations were identified as a biomarker for the central analgesic effect of pregabalin.[86]

Hence, important goals for future research would be to develop objective diagnostic tests for efficient screening for defined types of altered pain-related activity in the brain, to evaluate dronabinol-induced effects regarding the reversal of such defined brain activity abnormalities, and to consequently identify responders and/or non-responders to dronabinol treatment.

Interactions between cannabinoids and opioids

Opioids have been and continue to be regularly prescribed in chronic pain treatment, but opioid therapy is controversial due to concerns regarding long-term efficacy and adverse events including addiction.[87] In addition, accumulating evidence suggests that in some patients chronic opioid exposure may actually worsen the perception of pain. This phenomenon, termed opioid-induced hyperalgesia, is an undesirable effect, in that opioid therapy enhances or exacerbates pre-existing pain, while it is originally prescribed as an analgesic.[88,89]

The existence of multiple mechanisms underlying chronic pain, including this opioid-induced hyperalgesia, may explain a limited analgesic efficacy of pharmacologic agents as monotherapy.[90] Additionally, dose-related drug side effects, such as somnolence and dizziness, may limit the tolerability of higher, more efficacious doses of single analgesic drugs. Combining drugs with different pharmacological mechanisms may result in greater efficacy by simultaneous and beneficial effects on multiple pain mechanisms.[91]

Multimodal analgesic practice is well established in acute pain management and to a lesser extent in chronic pain. However, preclinical studies demonstrate that cannabinoids act synergistically with opioids.[92-101] Administration of low doses of THC in conjunction with low doses of opioids seems to be an alternative regimen that reduces the need to escalate opioid dose while increasing opioid potency.[92] Additionally, Narang et al. reported additional analgesia among patients taking opioids for chronic non-cancer pain with dronabinol intake.[66]

Thus dronabinol may be useful in pain treatment solely, and also in combination with opioids if it has synergistic interactions with opioid analgesics and if its use improves the efficacy of pain treatment in patients with a tolerance to opioids. Future research should study the bidirectional interactions between opioids and cannabinoids and their potent

effects on pain modulation mechanisms, and investigate the efficacy of novel analgesic combination regimens comprising cannabinoids and opioids to treat chronic pain, particularly if opioid resistant.

Declaration of Interest

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Article highlights

- Several pharmaceutical products with standardized Δ9-THC content have been developed containing whole cannabis plant extract, a defined combination of Δ9-THC and cannabidiol, or pure Δ9-THC.
- The therapeutic potential of Δ9-THC to treat chronic non-malignant pain is promising.
- Chronic non-malignant pain is associated with an abnormal state of responsiveness or increased gain of the nociceptive pathways in the central nervous system and with alterations in cognitive functioning.
- Sensitization of nociceptive sensory pathways and alterations in cognitive and autonomic processing might explain the varying analgesic effects of Δ9-THC.
- Cannabinoids may preferentially target the affective qualities of pain.
- Δ9-THC may have synergistic interactions with opioid analgesics.
- Several non-invasive techniques, such as quantitative sensory testing, conditioned pain modulation and encephalography, have the potential to identify patients with a specific pattern of abnormalities in central pain processing, and to predict treatment outcome.


PART III

Chapter 7

Single dose tetrahydrocannabinol does not alter pain related cortical processing in patients with chronic pancreatitis pain

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Submitted

ABSTRACT

INTRODUCTION: How tetrahydrocannabinol (THC) can exert an effect on chronic pain is largely unknown maintaining the debate of therapeutic efficacy. Chronic pain is associated with synaptic plasticity through an increased excitability and synaptic efficacy of neurons in central nociceptive pathways (i.e. central sensitization). In this study, we evaluated the underlying neural mechanisms of THC by investigating pain related cortical activity in patients with chronic pancreatic pain.

METHODS: Twenty-four patients with chronic abdominal pain due to chronic pancreatitis (CP) participated in this randomized, single-dose, double-blind, placebo-controlled, cross-over study. Patients, stratified in opioid and non-opioid users, administered a single dose of THC (8 mg) or Diazepam (5mg non-opioid group/ 10mg opioid group) with a 14-day washout period between study days. Predose until 5h postdose, two types of cortical activity were recorded: spontaneous brain activity in a resting state and evoked potentials (EPs) to noxious electrical stimuli.

RESULTS: Test-retest reliability of all resting state alpha EEG indices was excellent, and evoked EEG parameters showed fair to good agreement. Grand average power spectra of the spontaneous EEG did not change over time following THC compared with diazepam. EP components demonstrated no significant treatment effects for N1 peak amplitude ($F(1, 17) = .43$; $p = .52$), N1 latency ($F(1, 17) = .10$; $p = .76$), P3 amplitude ($F(1, 17) = 1.78$; $p = .20$) and P3 latency ($F(1, 17) = 2.79$; $p = .11$). A significant negative correlation ($r = -.55$; $p < .05$) was observed between changes in VAS pain scores and peak alpha power.

CONCLUSION: A single dose of THC did not affect alpha indices of the resting state EEG nor EPs to pain related electrical stimuli in CP patients with chronic abdominal pain. Changes in pancreatic pain were negatively correlated with changes in peak alpha power, indicating that individual treatment responses were associated with enlarged peak power amplitude in the resting state EEG. The test-retest reliability results warrant the use of these EEG parameters for further research.

INTRODUCTION

The role of cannabinoids such as Δ9-tetrahydrocannabinol (THC) in chronic pain management remains unclear.[1] THC is the main psychoactive compound of the *Cannabis sativa* plant. Supposed analgesic effects of THC might be produced by targeting brain areas related to central pain processing and pain perception.[2,3] However, evidence from clinical trials regarding the analgesic efficacy remains equivocal.

Clinical trials generally evaluate the effectiveness of potential analgesics by means of subjective measures of pain experience. However, chronic pain is not only an altered perceptual state, but is also associated with synaptic plasticity through an increased excitability and synaptic efficacy of neurons in central nociceptive pathways (i.e. central sensitization).[4] Central sensitization is a form of synaptic plasticity, which constitutes an abnormal perceptual response to a normal sensory input and results in a spread of sensitivity.[5] The presence of central sensitization in chronic pain patients asks for a treatment that results in pain relief by targeting the hyperexcitable central neural activity.[6] Electroencephalography (EEG) may be a useful method to detect alterations in central pain processing and to study the underlying neural mechanisms of analgesics, such as THC, in patients associated with a spread of increased pain sensitivity.[7,8]

Electrical brain activity recorded in the EEG reflects the summed synaptic potentials of many activated neurons located in the cerebral cortex. EEG recordings are commonly divided into two types: resting state and evoked EEG. The spontaneous EEG is recorded during a state of awake rest and characterized by oscillations in distinct frequency bands, such as delta (1-4 Hz), theta (4-7.5 Hz), alpha (7.5-13 Hz), beta (13-32 Hz), and gamma (32-80 Hz). The resting state EEG with eyes closed is dominated by oscillations in the alpha-band, particularly at the parietal and occipital cortex.[9] Several alterations in the spontaneous brain activity of chronic pain patients are observed including a shift of peak alpha or theta frequency towards lower frequencies and/or a reduction in alpha or theta power.[10-12] Besides the brains´ resting state, one could also study possible alterations in cortical processing by recording evoked potentials (EPs) in the EEG. EPs involve voltage polarity changes in the EEG, time-locked to the onset of a stimulus and averaged across trials. Early components of EPs depend largely on the physical parameters of the stimulus, whereas later components of EPs are related to the manner in which the subject evaluates the stimulus.[13] Alterations in stimulus processing are associated with chronic pain, although these changes in both EP amplitudes and latencies are inconsistent.[14,15] This is likely the consequence of a large variability in stimulation methods, analyzing techniques and study populations, making it difficult to draw clear conclusions.

Current study is part of a larger study investigating the analgesic efficacy, pharmacokinetics and safety of a single dose THC in patients with chronic abdominal pain resulting from chronic pancreatitis (CP). Patients reported pain relief following both THC and diazepam administration, but no significant differences were observed in subjective pain measures between both study treatments. THC was generally well absorbed with an average T_{max} of 123 minutes resulting in reliable pharmacokinetic profiles.[16] Diazepam was used as active placebo to prevent unblinding and to control for indirect pain relief through the sedative effects of THC.[16] The pathogenesis of pancreatic pain is poorly understood, but neural plasticity that results in peripheral and central sensitization seems to play an important role in chronic pain due to CP.[17] In this study, we evaluated the anti-nociceptive effects of THC by investigating underlying pain related cortical activity. We hypothesized that clinically effective analgesics can modify or reverse those changes resulting from central sensitization. Hence, we investigated whether a single dose of orally administrated THC alters 1) the resting state EEG and 2) EPs to pain related electrical stimuli in CP patients with chronic abdominal pain. Additionally, individual changes in subjective pain scores were correlated with EEG indices to assess whether EEG changes were linked to underlying analgesic responses and not caused by confounding factors such as sedation and other adverse effects.

METHODS

This was an equally randomized (1:1 ratio), single-dose, double-blind, placebo-controlled, cross-over study. The primary analysis concerning the analgesic efficacy, pharmacokinetics and safety have been reported elsewhere.[16] The Medical Ethical Committee approved the study (2011/114). The study was conducted according to the principles of the Declaration of Helsinki, and the International Conference on Harmonization guidelines of Good Clinical Practice. All subjects provided written consent. Clinicaltrials.gov identification number NCT01318369.

Subjects

Twenty-four patients with CP participated in the study. All patients had chronic abdominal pain, persistent or intermittent on a daily basis during the past 3 months, and considered their pain as severe enough for medical treatment (NRS ≥ 3). Patients in the opioid subgroup (n=12) took stable doses of prescribed opioids, and patients in the non-opioid subgroup (n=12) had not or occasionally taken opioids in the past 2 months. Key exclusion criteria were: cannabis use in previous year; history of hypersensitivity to THC; BMI <18.0 or >31.2 kg/m^2; serious painful conditions other than CP; significant

medical disorder or concomitant medication that may interfere with the study or may pose a risk for the patient; major psychiatric illness in history; epileptic seizure in history; diabetic neuropathy; significant exacerbation in illness within two weeks; positive urine drug screen or alcohol test at screening or on study days.

Study procedures

Eligible patients were stratified in opioid and non-opioid users and randomly assigned into one of two treatment sequences using a computer-generated list of random numbers. Patients administrated a single dose Δ9-THC (8 mg) or Diazepam (5mg non-opioid group/ 10mg opioid group) with a 14-day washout period between both study days. With respect to the expected THC-mediated sedative effects of cannabis, Diazepam was used as "active placebo" to prevent unblinding of patient and investigator by inducing sedative effects. Patients, staff and investigator were all blinded in a double dummy design. Oral tablets with standardized Δ9-THC content (Namisol®, Echo Pharmaceuticals, Weesp, the Netherlands) have demonstrated reliable bioavailability.[16]

Patients were not allowed to use illicit drugs, consume alcohol within 24 hours or caffeine within 6 hours prior drug administration. Therefore, urine drug screening tests and saliva alcohol tests were conducted on both study days. Food intake on the second study day was identical with the first study day. Patients used their prescribed medication, including analgesics, on both study days. Study days were carried out at the clinical research center of the Radboudumc, where each patient stayed in a separate quiet room.

EEG recording

Two types of cortical activity were recorded in the EEG: Spontaneous brain activity in a resting state and EPs to noxious electrical stimuli. Both EEG measurements were consecutively conducted predose and time-locked at 1:10, 2:10, 3:10, and 5:05 hours after administration of study medication. The resting state EEG was recorded for 1 minute with eyes closed, followed by the ERP stimulation block. EEG was recorded using a multi-channel ActiCAP system (BrainVision, Brain Products GmbH, Germany) of 32 active electrodes. Electrodes were positioned according to the international 10-20 system. The ground electrode was placed at the forehead and the reference electrode in FCz position. Eye movements were detected by horizontal and vertical electrooculogram (EOG) recordings. Horizontal EOG was measured at the outer canthus of both eyes and vertical EOG above and below the left eye. Electrode impedances were maintained under 20 kΩ to ensure an optimal signal-to-noise ratio. EEG was recorded with a sampling rate of 2000 Hz. During the measurements, patients were sitting in a comfortable chair and no further task was given.

Stimulation protocol

Pain related EPs were extracted from the EEG by averaging repetitive stimulus responses within a stimulus block. A concentric surface electrode was attached to the non-dominant lower arm 10 cm distal from the cubital fossa. This concentric electrode delivers electrical stimuli which are limited to the superficial layer of the dermis, and therefore activates mainly nociceptive A-delta fibers.[18] The stimulus produces a pinprick-like pain sensation that is typical for A-delta fiber mediated pain, and has been used in previous studies.[19-21] The individual pain threshold was determined by increasing the stimulus amplitude (0,1 mA/sec), starting at zero until the pain threshold was achieved. This procedure was repeated for a second time. The stimulus amplitude was adjusted to 150% of the mean individual pain threshold. Patients received 20 painful electric doubled stimuli (pulse width of 2 ms; fixed inter-stimulus interval of 5 ms) delivered with a random inter-pair interval of 7-10 sec. Triggers were communicated to an electric constant-current stimulator (Digitimer, model DS7A) using Presentation software (version 14.9) and directly positioned into the EEG recording. Experienced stimulus intensities were measured using a visual analogue scale (VAS_{stim}) from 0 cm (no pain) to 10cm (unbearable pain). VAS_{stim} were obtained at a random moment within a train of five doubled pulses, resulting in a total of 4 VAS scores within each stimulation block.

Signal analysis

EEG data were offline processed using Brain Vision Analyzer 2.0 software. The spontaneous EEG recordings were down-sampled to 500 Hz, high-pass filtered at 1 Hz, low-pass filtered at 80 Hz and a notch filter was applied at 50 Hz. The EEG recordings were then segmented into 12 epochs of 5 sec. Ocular correction was performed according to the Gratton and Coles algorithm.[22] Epochs were inspected for artifacts and semi-automatic rejected from further analysis if data exceeded an amplitude of 200 μV or exceeded the maximal allowed voltage step of 50 μV. Less than 5% of all epochs were rejected for further analysis. The power amplitudes of the EEG frequencies were computed using a Fast Fourier Transformation (FFT). To this end, epochs were multiplied by a Hanning window, Fourier transformed and spectral distributions were averaged across all epochs for each participant and electrode separately. Grand average power spectra were computed by averaging all scalp electrodes per treatment for each participant. Alpha indices were calculated for each electrode and measurement separately and averaged to create four regions of interest (ROI): frontal (Fp1, Fp2, F7, F3, Fz, F4, F8), central (FC5, FC1, FC2, FC6, C3, Cz, C4), parietal (CP5, CP1, CP2, CP6, P7, P3, Pz, P4, P8), and occipital (O1, Oz, O2). The amplitude of the peak alpha power (PAP) was determined as the maximum value between 7.5-13 Hz and the peak alpha frequency (PAF) as the corresponding frequency within this alpha band of interest. Additionally, the gravity alpha frequency

(GAF) was calculated within the 7.5-13 Hz range as more stable alternative compared to the peak method. The GAF is the weighted sum of spectral estimates divided by alpha power.[23]

EEG recordings to extract EPs were down-sampled to 500Hz and re-referenced using the averaged recordings from all scalp electrodes. EEG data were high-pass filtered at 1 Hz, low-pass filtered at 80 Hz and a notch filter was applied at 50 Hz. The EEG was then segmented into -500 to 1000ms epochs relative to the onset of the stimulus, and corrected for ocular artifacts according to the Gratton and Coles algorithm.[22] Subsequently, epochs were inspected for artifacts and rejected from further analysis if data exceeded an amplitude of 200 μV or exceeded the maximal allowed voltage step of 50 μV. Less than 5% of all segments were removed from each dataset. After baseline correction (-100 to 0 ms) all epochs within a stimulus block were averaged for each subject individually. The grand average EP for each block and group was calculated.
Based on morphology and latency of the grand average EP, analyzed from the Cz electrode, two distinct peaks (N100 and P300) were defined. The N100 was defined as the largest negative amplitude value between 80 and 180 ms, and the P300 as the largest positive value between 180 and 400 ms. The maximum amplitude and corresponding latency of these peaks were calculated for each individual grand average EP.

Statistical analysis

All data were analyzed using SPSS software for Windows, version 22. Initially, data were examined descriptively using means, SD, and graphs. Intra-individual stability of EEG over recording sessions was evaluated for the alpha indices and EP components using the intraclass correlation coefficient (ICC) and 95% confidence interval for the relative reliability, and 95% limits of agreement according to Bland-Altman, that contains 95% of differences between repeated measurements limits, for absolute reliability. The ICC parameter ranges from 0 to 1, with values closest to 1 indicating the highest reproducibility. An ICC less than 0.4 was considered poor agreement; 0.4 to 0.59, fair agreement; 0.6 to 0.75, good agreement; and greater than 0.75, excellent agreement.[24]
Differences between THC and diazepam within the resting state EEG were statistically analyzed using a repeated measures Analysis of Variance (ANOVA) with treatment (2; THC, diazepam), ROI (4; frontal, central, parietal, occipital), and the repeated measurements (5; predose, 1:10, 2:10, 3:10, and 5:10 hours postdose) as within subject factors, order (2) and opioid user (2) as between subject factors, and VAS_{pain} at baseline as covariate. Repeated measures ANOVA were conducted for statistics of N1 and P3 peak amplitudes and latencies with treatment (2; THC, diazepam), and the repeated measurements (5; predose, 1:10, 2:10, 3:10, and 5:10 hours postdose) as within subject factors, order (2) and

opioid user (2) as between subject factors, and VAS_{pain} at baseline as covariate. Degrees of freedom were adjusted using Greenhouse-Geisser correction for within-subject factors with more than two levels. In order to evaluate the reproducibility of the baseline EEG, 95% limits of agreement and Pearson's correlation coefficients were calculated. Correlations were tested using either parametric Pearson or non-parametric Spearman test based on data distributions. All statistical tests were performed two-tailed, and the limit for statistical significance was set at $P<0.05$.

RESULTS

Twenty-five patients were included. One patient dropped-out prior the first study day and was replaced. Two patients in the opioid subgroup were lost to cross over after the first study day. EEG analysis were performed on 22 fully evaluable patients. Patient demographics and baseline characteristics are described in table 1. Baseline VAS pain scores measured on both study days (THC vs. diazepam) correlated significantly ($r = 0.79$, $p< 0.0001$).

Resting state EEG

Overall, reliability of alpha indices obtained from the baseline EEG recordings was excellent with ICC=0.95 (95% CI, 0.89-0.98) for PAP, ICC=0.84 (95% CI, 0.65-0.93) for PAF, and ICC=0.90 (95% CI, 0.78-0.96) for GAF, corresponding with 95% limits of agreement of -1.47 to 2.12 μV/Hz, -0.99 to 1.14 Hz, and -0.21 to 0.22 Hz respectively, reflecting the reproducibility of the EEG.

Grand average power spectra of the spontaneous EEG did not change over time after THC compared with diazepam administration (figure 1 A-E). Although THC seems to produce a small increase in α-activity starting 2:10H hours postdose according the grand average power spectra, this could not be demonstrated in alpha power indices. Alpha indices calculated per ROI measured at time point 2:10H, which is close to the time to reach maximum plasma concentration (T_{max}) of THC, are shown in table 2. There were no treatment effects for PAP ($F(1, 18) =2.92$, $p =.10$), PAF ($F(1, 18) =1.90$, $p =.16$), or GAF ($F(1, 18) =.74$, $p =.40$). Additionally, no repeated measurements, opioid or order effects were observed, only significant region effects.

Electrical stimulation

Intensities of pain related electrical stimulation ranged from 0.9 to 5.9 mA (mean ± SD: 2.9 ± 2.0). No treatment effect (NS ($F(1, 42) =0.07$; $p =.7887$)) was observed between THC and diazepam concerning the VAS_{stim} in response to noxious electrical stimuli (figure 2).

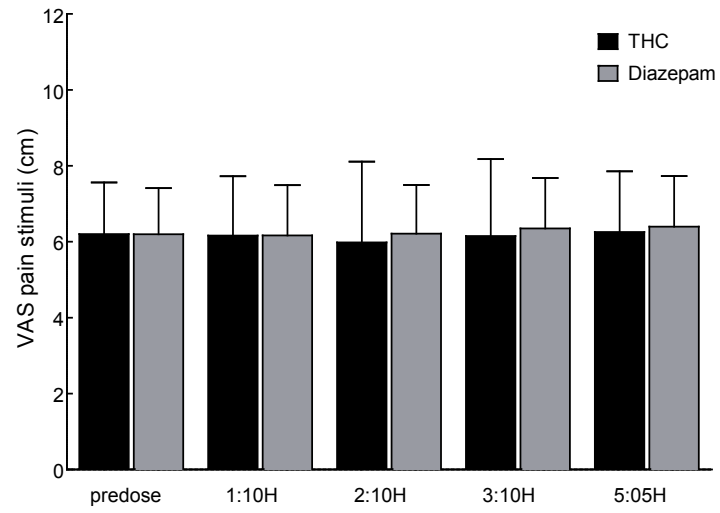

Figure 2: Average (SD) visual analogue scale (VAS_{stim}) scores of pain related electrical stimuli measured predose, and postdose at 1:10, 2:10, 3:10, and 5:05 hours after tetrahydrocannabinol (THC) compared with diazepam.

Table 1: patient characteristics

	Opioid (n=10)	Non-opioid (n=12)	Total group (n=22)
Gender (male/ female), n	7 / 3	7 / 5	14/8
Age (years), mean (sd)	49.4 (5.6)	56.1 (9.5)	53.1 (8.5)
Mean VAS pain diary, mean (sd)	4.4 (1.4)	3.3 (1.6)	3.8 (1.6)
Baseline VAS pain THC, mean (sd)	3.3 (1.7)	2.7 (2.2)	3.0 (1.9)
Baseline VAS pain diazepam, mean (sd)	3.2 (1.5)	2.3 (2.0)	2.7 (1.8)
Pain duration (years), mean (sd)	7.1 (7.0)	8.6 (5.3)	7.9 (6.0)
Concomitant medication, n			
None	0	1	1
PCM	5	7	12
NSAID	1	2	3
Weak opioids	1	0	1
Strong opioids	10	0	10
Antiepileptics	4	2	6
Antidepressants	0	0	0
Etiology CP, n			
Alcohol	1	2	3
Hereditary	1	1	2
Idiopathic	6	8	14
Neoplasm	1	1	2
Other	1	0	1

Table 2: Alpha indices of the resting state EEG at time point 2:10H (first time point following T_{max} at 122.8 min) calculated per region of interest (ROI).

ROI	Alpha index	THC		Diazepam	
		mean	SD	mean	SD
Frontal	PAP (μV/Hz)	3.17	1.85	5.07	2.68
	PAF (Hz)	8.45	0.77	8.72	0.90
	GAF (Hz)	9.82	0.17	9.91	0.19
Central	PAP (μV/Hz)	2.81	1.65	2.87	1.23
	PAF (Hz)	8.50	0.71	8.50	0.89
	GAF (Hz)	9.86	0.18	9.93	0.24
Parietal	PAP (μV/Hz)	8.11	6.39	2.56	1.25
	PAF (Hz)	8.86	0.98	8.73	1.00
	GAF (Hz)	9.74	0.26	9.77	0.32
Occipital	PAP (μV/Hz)	12.41	9.77	7.13	5.11
	PAF (Hz)	8.86	1.02	8.79	0.91
	GAF (Hz)	9.73	0.31	9.73	0.35

Abbreviations: peak alpha power (PAP), peak alpha frequency (PAF), gravity alpha frequency (GAF), time to reach maximum plasma concentration (T_{max}), tetrahydrocannabinol (THC)

Evoked Potentials

Baseline noxious electrical stimulation yielded typical EPs at the central site on the scalp (Cz electrode). EPs at Cz and corresponding scalp topographies of the N1 and P3 components, mapped at their peak latencies, were similar for both predose measurements (Figure 3A-E). The reliability was fair for N1 latency (ICC, 0.40; 95% CI, 0.00-0.70) and good for N1 peak power (ICC, 0.75; 95% CI, 0.48-0.89), P3 latency (ICC, 0.67; 95% CI, 0.35-0.85), and P3 peak power (ICC, 0.71; 95% CI, 0.41-0.87). Based on these scalp topographies demonstrating most prominent activity at the vertex, further EP analyses were performed at electrode Cz.

Grand average EPs resulting from noxious electrical stimulation measured pre- and postdose after THC or diazepam are shown in figure 4. Statistical analysis of early EP components at electrode Cz demonstrated no significant treatment effects for N1 peak amplitude (F(1, 17) =.43; p =.52) or N1 latency (F(1, 17) =.10; p =.76), in addition to no significant repeated effects for both N1 peak amplitude (F(1, 17) =1.00; p =.35) or N1 latency (F(1, 17) =.00; p =.98). Furthermore, there were no significant effects of treatment observed at electrode Cz for P3 amplitude (F(1, 17) =1.78; p =.20) and P3 latency (F(1,

17) =2.79; p =.11), nor any repeated effect for P3 amplitude (F(1, 17) =.94; p =.36) and P3 latency (F(1, 17) =1.38; p =.27). No order, no electrode and no opioid effect was observed in the ANOVAs.

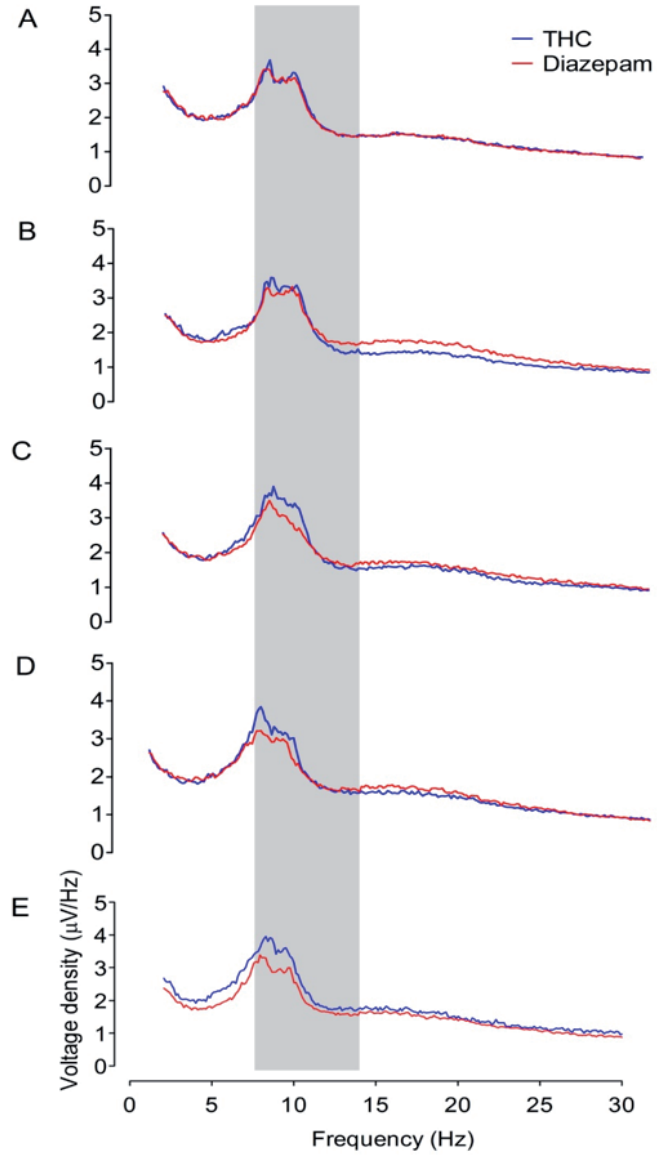

Figure 1 A-E: Grand average frequency power distributions averaged over all electrodes measured predose (A), and postdose at 1:10 (B), 2:10 (C), 3:10 (D), and 5:05 (E) hours after THC compared with diazepam. The grey square represents the area within the α-band.

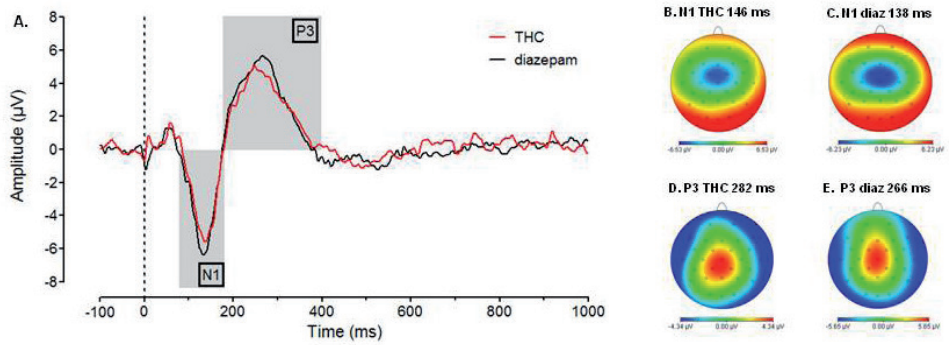

Figure 3 A-E: Baseline evoked potentials. (A) Pain related grand average evoked potentials at Cz recorded predose in the THC and diazepam condition. (B-E) Corresponding group mean scalp distributions of neural activity for the N1 and P3 components (mapped at their peak latencies) recorded baseline in the THC and diazepam condition.

Clinical pain and EEG activity

Post hoc analysis of overall alpha indices of EEG in rest did not demonstrate any significant correlation with reported pain intensity at baseline (figure 5). Postdose changes close to Tmax of THC, demonstrated a significant negative correlation between VAS pain scores and PAP, indicating that individual treatment response is associated with enlarged peak power amplitude. No statistical correlations were observed between changes in reported pain intensity and PAF or GAF.

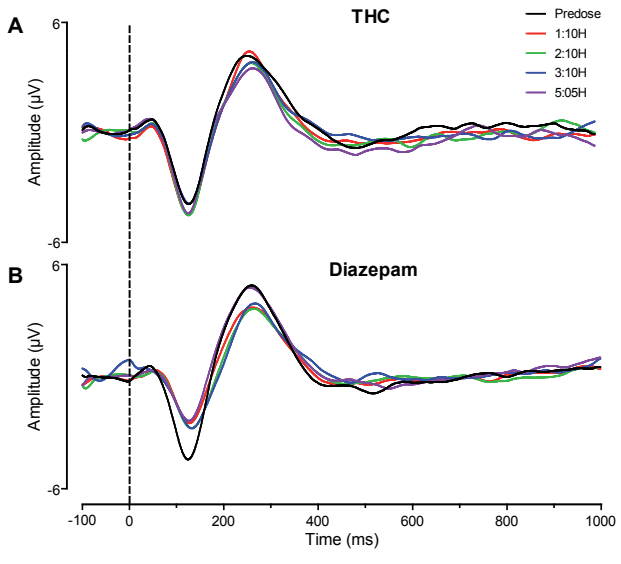

Figure 4 A-B: Pain related grand average evoked potentials at electrode Cz recorded predose and postdose at 1:10, 2:10, 3:10, and 5:05 hours after (panel A) tetrahydrocannabinol (THC) and (panel B) diazepam. Lines are smoothened for clarity.

Neither latency nor amplitude of early as well as late EP components measured baseline were associated with predose pain. Additionally, no correlation was observed between changes in EP components and changes in pain reported intensity.

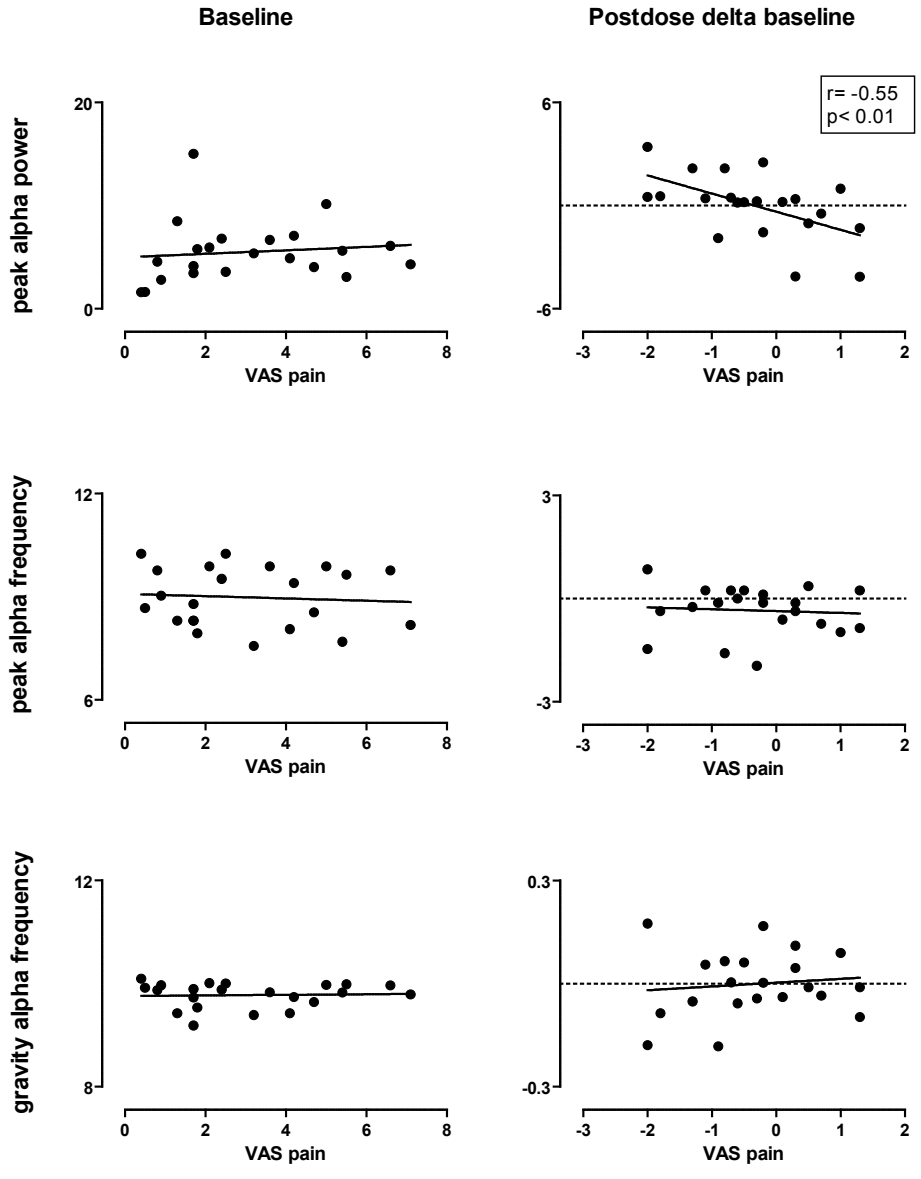

Figure 5: Scatter plots of reported pain intensity and alpha indices of EEG in rest. Left panels show baseline measurements. Right panels show postdose delta scores at time point 2:10H (EEG) and 2:05H (VAS) after tetrahydrocannabinol (THC) minus baseline. Top row: peak alpha power (μV/Hz), middle row: peak alpha frequency (Hz) and bottom row: gravity alpha frequency (Hz). Each dot represents a single patient (n=22). The line represents the best linear fit to the data from the entire group.

DISCUSSION

Our primary purpose was to investigate whether THC alters cortical brain activity in patients with chronic abdominal pain due to CP. Alpha indices obtained from the spontaneous EEG, that have shown to be related to several chronic pain populations in previous studies.[10,11,25-29] as well as pain related EPs were studied over time following THC administration and compared with diazepam. We primarily demonstrated a good test-retest reliability of all resting state alpha EEG indices in CP patients. The intra-subject variability of evoked EEG parameters showed fair to good agreement, supporting the use of these EEG parameters for further research. However, no changes were observed in the resting state EEG nor for pain related EPs following THC administration compared to diazepam. Behavioral VAS scores of electrical transcutaneous nociceptive stimulation maintained stable over time, indicating that acute evoked pain was not affected by THC. These results are in line with the subjective experience of pancreatic pain, where no analgesic effect of THC could be observed.[16] Pancreatic pain was decreased after both THC as well as diazepam administration, which was assumed to be a placebo response, since no analgesic efficacy is described for diazepam.[30] Further analysis of subjective measures of individual clinical pain scores in relation to objective measures of EEG parameters at baseline did not reveal any correlation. One significant negative correlation was found between change in VAS pain scores and change in PAP, which may indicate that individual treatment response is associated with enlarged peak power amplitude.

Resting state EEG and chronic pain

Chronic pain is related to changes in brain activity reflected in the resting state EEG. Pinheiro et al. reported in a systematic review a general trend towards increased alpha and theta power among several studies determining EEG patterns in the presence of chronic pain.[14] Changes in the resting EEG might be explained by the concept of thalamocortical dysrhythmia (TCD). TCD is based on reduced excitatory or increased inhibitory input of neurons in the thalamus, resulting in the presence of a persistent low-frequency thalamocortical spiking, causing the increased power at low frequencies.[25,31,32] Affirmatively, Sarnthein et al. observed an enhanced alpha power amplitude and a shift towards lower frequencies of the dominant peak in patients with mixed neurogenic pain syndromes.[25] Two studies in patients with neuropathic pain following spinal cord injury demonstrated a shift in peak frequency towards lower frequencies in patients with pain compared those without pain, but no differences were observed in power amplitudes.[26,27] Oppositely, Van den Broeke et al. observed a larger overall alpha amplitude in patients with chronic pain after breast cancer treatment, but no slowing.[29]

Interestingly, no changes in resting EEG were shown in patients with chronic low back pain. It was suggested that only patients with severe pain or neuropathic pain develop the typical TCD pattern.[33] Previous studies investigating the resting EEG in patients with CP reported similar alterations in EEG activity, observed as an increase in power amplitude in the theta and alpha frequency band or shift towards lower PAF.[10,11,28] Although not confirmed, it is likely that similar changes in electrical brain activity have been developed in our study population. Pharmaco-EEG studies can help us understand underlying mechanisms of centrally acting analgesics by studying their cortical effects.

Analgesics and resting state EEG

A review on analgesia and EEG reported that opioids generally induced slowing of the spontaneous EEG, whereas other analgesics such as anticonvulsants produced inconsistent results.[7] However, most of these pharmaco-EEG studies were performed in healthy volunteers and results were not linked to clinical pain outcomes. One study in CP patients with pain observed that the analgesic effect of 3 weeks of pregabalin treatment was reflected as a slowing of brain oscillations.[8] THC has no status as proven analgesic in general, and in the current study, THC failed to demonstrate efficacy based on subjective outcome measures of pain. Moreover, a large placebo effect was shown, which could not be related to alpha indices in the resting state EEG. Graversen et al. did also not found a relation between changes in EEG indices and placebo response, suggesting that the slowing of EEG in the verum group reflects the underlying analgesic mechanisms of pregabalin.[8] Considering this may be true, one can propose that the absence of a clinical analgesic effect of THC is reflected by the absence of changes in the EEG. Alternatively, several studies in healthy subjects have shown that acute administration of THC disrupts neural oscillations. For example, Notage et al. demonstrated that delta, theta and high alpha amplitudes were all significantly reduced after a single dose of intravenous THC,[34] and Morrison et al. demonstrated a significant reduction in theta power.[35] In this light, it should be mentioned that patients in the present study used different types of medication, including opioids and other analgesics, with THC as add-on. THC did not produce alterations within the alpha spectrum of the resting state EEG in CP patients. Although we could not detect differences between subgroups of analgesics, possibly due to small subgroups, the variety of analgesics may have affected the EEG and masked a potential effect of THC.

Evoked potentials in chronic pain

Besides spontaneous EEG, chronic pain is associated with significant changes of early and late EP components in response to somatosensory and visual pain-related stimulation as well as cognitive tasks,[36-41] suggesting an abnormal brain functioning linked to cognitive

processes such as attention.[14] In particular, chronic pain was associated with delayed and enhanced stimulus processing of late P3 components using pain related EPs elicited by very similar electrical transcutaneous nociceptive stimuli in patients after breast cancer treatment and patients with trigeminal neuralgia.[20,21] Early components observed in the EP after electrical stimulation reflect sensory discriminative processes of stimulus perception, whereas these late EP components have been related to cognitive evaluative processes, such as attention and distraction and target/non-target responses.[42,43]

Pharmaco-EEG recorded as EPs, and particularly amplitudes, can be a viable and useful tool for analyzing changes in cortical activity following administration of different analgesics.[44] For example, opioids generally induced a decrease of the late component amplitude in EPs evoked by painful stimuli, whereas non-painful somatosensory stimuli were unaffected.[7] Weak analgesics, such as non-steroidal anti-inflammatory drugs, produced a fairly consistent decrease in P3 amplitudes. Most important, these changes in EP amplitudes were quite consistent with the clinical effect, i.e. the pain relief provided, and a few studies demonstrated dose dependent changes in EP amplitudes.[7] Acute administration of THC has been associated with reduced P3 amplitudes utilizing memory and reaction time tasks,[45,46] and dose dependently reduced P3 amplitudes using an oddball paradigm as neural correlate of cognition.[47] In an experimental human pain model, THC reduced the reported unpleasantness, but not the intensity of ongoing pain and hyperalgesia, that was positively correlated with right amygdala activity.[3] THC also reduced functional connectivity between amygdala and primary sensorimotor areas during the ongoing pain state, indicating that THC may target, although not selectively, the affective and more cognitive aspects of pain.[1,3]

In this context, we hypothesized that THC induces a reduction of P3 amplitude in response to painful electrical stimulation, reflecting a cognitive or attention component of pain.[48] However, no changes in amplitudes or latencies of both early and late EP components were detected immediately following a single dose of THC. Individual changes in EPs could not be associated with individual treatment responses, hampering the prediction of treatment outcome in individual chronic pain patients. Hence, the underlying central mechanisms and therapeutic potential of THC remain unclear.

Methodological considerations

This study has a number of limitations. First, different types of pain medication may have exerted multifarious influences on our results, i.e. other drugs could have affected the spontaneous EEG or induced a dampening effect on EPs. The sample size was too small to conduct subgroup analysis, and differences might have been missed between opioid subgroups due to a type I error. Second, electrical stimulation with the concentric electrode recruits predominantly Aδ fibers, but also to some extent Aβ fibers, and thus,

stimulation was not nociceptive-specific. Mouraux et al. showed that only low intensity electrical intra-epidermal stimulation below 2.5 mA are able to evoke Aδ-associated evoked brain responses.[49] In the present study we used transcutaneous electrical stimulation with stimulation intensities ranging from 0.9 to 5.9 mA, which indicates that we did not selectively stimulated Aδ fibers in all subjects. On the other hand, even when the stimulus is entirely noxious, the EP may not be nociceptive-specific.[50] Finally, a wide variety of methodologies among pharmaco-EEG studies impede direct comparison of results. This diversity involves differences in study procedures, such as electrode placement, selection of reference electrode and recording conditions, but involves even more a wide range of analysis methods. The analysis techniques used in the present study are generally most common, but has disadvantages since only phase-locked components of EPs are effectively preserved. Non-phase-locked nociceptive inputs to the brain are lost due to the averaging process. Gram et al. proposed a new methodology to classify single-sweep EPs recorded in pharmaco-EEG studies, which may provide interesting perspectives for future studies, since it reveals additional information to what is typically reported.[44]

Conclusions and future directions

A single dose of THC did not affect alpha indices of the resting state EEG nor EPs to pain related electrical stimuli in CP patients with chronic abdominal pain. Changes in subjective experienced pancreatic pain were negatively correlated with changes in PAP, indicating that individual treatment response was associated with enlarged peak power amplitude in the resting state EEG. Individual treatment responses could not be associated with changes in pain related EP components. Further long-term treatment studies are required to evaluate the anti-nociceptive effects of THC on pain related cortical activity.

Conflict of interest

The authors are primarily supported by their university medical center. Additionally, they received a grant from the European Union, the European Fund for Regional Development (EFRO, 'Here is an investment in your future'), and cooperate with Echo pharmaceuticals in a consortium conducting investigator-initiated phase 2 drug studies with Namisol®. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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Chapter 8

Pain-related cortical activity during tetrahydrocannabinol treatment in patients with chronic abdominal pain

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Submitted

ABSTRACT

The analgesic effectiveness of delta-9-tetrahydrocannabinol (THC), the most abundant psychoactive substance in cannabis, is inconclusive. Chronic pain is associated with maladaptive changes in neuronal electrical activity. In this study, cortical correlates of THC in relation to its analgesic potency are investigated utilizing electroencephalography (EEG). EEG recordings of 49 patients with chronic abdominal pain were assessed in a randomized, placebo-controlled, double-blind study involving administration of an oral tablet containing purified THC or identical matching placebos. THC was administered 3 times daily during a 50-52 day add-on treatment. Spontaneous EEG at a resting state and pain related evoked potentials (EPs) to noxious electrical stimuli were recorded prior drug administration on day 1 and last study day. EEG indices were associated with subjective measures of pain and analgesia. At day 50–52, THC did not affect the resting state EEG nor pain related EPs compared to placebo. A slightly significant delay in N1 latency at Cz was observed for THC in a subgroup of patients with postsurgical abdominal pain. Cortical correlates of THC could not be associated with its analgesic efficacy. However, clinical pain severity was associated with slowing of brain oscillations in a resting state, as well as with enhanced N1 and P3 amplitudes elicited by noxious electrical stimulation. In conclusion, pain related EEG indices were not affected by THC and did not reflect individual treatment responses in patients with chronic abdominal pain. More research is necessary to investigate central mechanisms and therapeutic potential of THC for individual patients with chronic pain.

INTRODUCTION

Chronic pain of moderate to severe intensity occurs in 19% of the adult Europeans and 30% of the US population. One-third of these individuals with chronic pain define their pain as severe and 40% reported inadequate management of their pain.[1,2] Pharmacological drugs are the key components of chronic pain management. However, only a minority of patients with chronic pain sufficiently benefits from the currently available treatments. This can be to some extent attributed to the variety of pain mechanisms underlying the chronic pain condition in each individual patient.

Chronic pain is associated with peripheral reorganization of afferent signalling and altered sensitivity for nociceptive afferents. At the level of the spinal cord, central sensitization contributes to an abnormal state of responsiveness or increased gain of the nociceptive system.[3] Furthermore, accumulating evidence shows that chronic pain underlies neocortical anatomical reorganization, functional connectivity changes, and abnormalities in resting state activity.[4] Analgesics targeting the underlying pain pathways may affect these abnormalities. Therefore, the relation between clinical drug efficacy and alterations in pain pathways should be studied for each drug. Accordingly, assessment of abnormalities in pain pathways prior to initiation of pharmacological treatment can predict drug effects and help in selecting the appropriate therapeutic strategy for individual patients. Thus knowledge of drug effects on neuronal electrical activity should be able to help us better understand the pathology underlying chronic pain.

So far, the analgesic effectiveness of delta-9-tetrahydrocannabinol (THC), the primary psychoactive substance in cannabis, is not evident. Multiple clinical studies have demonstrated that THC induces pain relief in patients with chronic pain, but this pain reducing effect remained absent in other randomized controlled trials.[5] In the current study, THC treatment did not reduce chronic abdominal pain compared to placebo using subjective outcomes of pain, which has been reported previously.[6] Several reasons might explain these opposing results, including variability among individual chronic pain patients and lack of a mechanism based treatment model.
THC induces a range of perceptual and cognitive alterations through the activation of cannabinoid CB1 and CB2 receptors.[7,8] CB1 receptors are predominantly found in the central nervous system in areas associated with the activation of an extended pain network in the brain,[9,10] while CB2 receptors are primarily expressed in immune tissues.[11] It is suggested that pain experience is determined by the integration of neuronal activity including but not exclusively involving these pain associated brain areas. In addition,

pain is not only associated with a spatially extended network of dynamically recruited brain areas, but also with complex temporal patterns of brain activity.[12]

Electroencephalography (EEG) has been demonstrated to be a useful method to detect abnormalities at different levels of the pain pathway in the temporal domain. Changes in EEG activity observed in the presence of chronic pain include a general trend towards increased alpha and theta power in the spontaneous EEG and low amplitudes of evoked potentials (EP) elicited by various stimuli.[13] EEG studies have demonstrated that chronic cannabinoid use is associated with disrupted oscillations in the theta and gamma band.[14] Additionally, controlled acute administration of THC resulted in decreased theta power, indicating that THC modifies neural brain oscillations.[15]

In this study, neural correlates of THC in relation to its analgesic potency are elucidated utilizing EEG. We investigated the effects of THC on EEG indices during resting state and pain related EPs elicited by noxious electrical stimulation in patients with chronic abdominal pain.

METHODS

Study population

Adult patients with chronic abdominal pain arising after a surgical procedure or resulting from chronic pancreatitis (CP) were included. Chronic pain was defined as persistent or intermittent abdominal pain on a daily basis for at least 3 months and severe enough for medical treatment (average numeric rating scale ≥ 3).[16] Key exclusion criteria were: daily cannabis use in past three years; history of hypersensitivity to THC; serious painful conditions other than postsurgical pain (PSP) or CP; significant medical disorder or concomitant medication that may interfere with the study or may pose a risk for the patient; major psychiatric illness in history; epileptic seizure in history; affected sensory input such as diabetic neuropathy; positive urine drug screen or alcohol test at screening or on study days; or participation in another investigational drug study within 90 days before study entry.

Eligible patients were recruited from October 2012 until July 2014, and stopped prematurely due to poor recruitment. All patients provided written informed consent. The study was performed in accordance with the Declaration of Helsinki, the International Conference on Harmonization Guideline on Good Clinical Practice. The study was approved by the medical ethical review committee region Arnhem-Nijmegen and the institutional review board. The pharmacokinetics, efficacy and safety of THC have been described elsewhere.[6] Clinicaltrials.gov identification numbers NCT01562483 and NCT01551511.

Study design and study treatment

This was an equally randomized (1:1), double-blind, placebo-controlled, parallel study. Patients were stratified into opioid and non-opioid users and equally randomized to receive THC or placebo treatment. Oral tablets with standardized THC content (Namisol®, Echo Pharmaceuticals, Weesp, The Netherlands) or identical matching placebos were administrated as add-on treatment. Study treatment lasted for 50-52 days, consisting of a step-up phase (day 1-5: 3 mg TID; day 6-10: 5 mg TID) and a stable dose phase (day 11-52: 8 mg TID). It was permitted to taper the dosage to 5 mg TID, when 8 mg was not tolerated. Patients and study personnel were strictly blinded until end of study. Patients were asked to continue their regular co-medication, including analgesics, according to prescription.

Study procedures

Patients were screened for eligibility based on demographics, medical history, concomitant medication, smoking habits, physical examination, 12-lead electrocardiogram (ECG), standard laboratory blood tests and urine screening tests (drug screening and pregnancy test). Several questionnaires were conducted prior study treatment, including Hospital Anxiety and Depression Scale (HADS), Pain Anxiety Symptoms Scale (PASS), Pain Catastrophizing Scale (PCS). Furthermore, all patients received a diary to daily report pain scores, add-on analgesics and adverse events. EEG measurements were conducted predose on day 1 and approximately 4 hours after administration of study medication on the last treatment day. Study days were carried out at the clinical research center of the Radboudumc.

EEG recording

Two types of EEG were recorded: spontaneous brain activity in a resting state and pain related EPs to noxious electrical stimuli. The resting state EEG was recorded for 1 minute with eyes closed. EEG was recorded using a multi-channel ActiCAP system (BrainVision, Brain Products GmbH, Germany) of 32 active electrodes according to the international 10-20 system. The ground electrode was placed at the forehead and the reference electrode in FCz position. EEG was recorded with a sampling rate of 2000 Hz and electrode impedances were maintained under 20 kΩ to ensure an optimal signal-to-noise ratio. Horizontal electrooculogram (EOG) was measured at the outer canthus of both eyes and vertical EOG above and below the left eye for artefact detection. Patients stayed in a separate quiet room sitting upright in a comfortable chair.

Stimulation protocol

Pain related EPs were extracted from the EEG by averaging repetitive stimulus responses within a stimulus block. A concentric surface electrode was attached to the non-dominant lower arm 10 cm distal from the cubital fossa. This concentric electrode activates mainly

nociceptive A-delta fibers in the superficial layer of the dermis, producing a pinprick-like pain sensation that is typical for A-delta fibers.[17] The individual pain threshold was determined by increasing the stimulus amplitude (0.1 mA/sec), starting at zero, until the pain threshold was achieved. This procedure was repeated for a second time. The stimulus amplitude was adjusted to 150% of the mean individual pain threshold. Patients received 20 painful electric doubled stimuli (pulse width of 2 ms; fixed inter-stimulus interval of 5 ms) delivered with a random inter-pair interval of 7-10 sec. Triggers were applied with an electric constant-current stimulator (Digitimer, model DS7A) using Presentation software (version 14.9). Experienced stimulus intensities were measured using a visual analogue scale (VAS_{stim}) from 0 cm (no pain) to 10cm (unbearable pain). VAS_{stim} were obtained at a random moment within a train of five doubled pulses, resulting in a total of 4 VAS scores within each stimulation block.

Signal analysis

EEG data were offline processed using Brain Vision Analyzer 2.0 software (band-pass filters: 1.0 and 80 Hz; notch filter: 50 Hz; sampling rate: 500 Hz; average reference montage; ocular correction[18]). The spontaneous EEG recordings were segmented into 12 epochs of 5 sec. Epochs were inspected for artifacts and semi-automatic rejected from further analysis if data exceeded an amplitude of 200 μV or exceeded the maximal allowed voltage step of 50 μV. Less than 2% of all epochs were rejected for further analysis. The power amplitudes of the EEG frequencies were computed using a Fast Fourier Transformation (FFT). To this end, epochs were multiplied by a Hanning window, Fourier transformed and spectral distributions were averaged across all epochs for each participant and electrode separately. Grand average power spectra were computed by averaging all scalp electrodes per treatment for each participant. Additionally, four regions of interest (ROI) were created by grouping the following electrodes: frontal (Fp1, Fp2, F3, Fz, F4), central (C3, Cz, C4), parietal (P3, Pz, P4, P8), and occipital (O1, Oz, O2).
Both power spectra and peak frequency analyses were performed on the spontaneous EEG. Spectral EEG power was calculated for selected frequency bands (theta: 4.0–7.5 Hz; alpha: 7.5–13.0 Hz; and beta: 13.0–30 Hz) by extracting the mean activity per ROI and per measurement. Peak alpha power (PAP) amplitudes were determined as the maximum value between 7.5-13 Hz and the peak alpha frequency (PAF) as the corresponding frequency. The gravity alpha frequency (GAF) was calculated by the weighted sum of spectral estimates divided by alpha power within the 7.5-13 Hz range.[19] The GAF is considered as more constant alternative compared to the more fluctuating peak method. EP recordings were segmented into -100 to 1000ms epochs time locked to stimulus onset. Less than 1% of all segments were removed by semi-automatic artifact rejection. After baseline correction (-100 to 0 ms) all residual epochs within a stimulus block were

averaged for each subject individually. Subsequently, the grand average EP for each block and group was calculated. Based on morphology and latency of the grand average EP, two distinct peaks (N100 and P300) were defined for the midline electrodes Fz, Cz, Pz and Oz. The N100 was defined as the largest negative amplitude value between 80 and 180 ms, and the P300 as the largest positive value between 180 and 400 ms. The maximum amplitude and corresponding latency of these peaks were calculated for each individual grand average EP.

Statistical analysis

SPSS software for Windows v.20 was used for statistical analysis. Repeated measures Analysis of Variance (RM-ANOVA) analysis were performed to test whether there were statistically significant differences between THC and placebo (between factor; 2 levels: THC and placebo) with respect to period (within factor; 2 levels: baseline and day 50) and EEG parameters, such as power spectra density per ROI (within factor; 5 levels: frontal, central, parietal, occipital and overall) and frequency band (within factor; 3 levels: theta, alpha and beta). VAS_{pain} at baseline and subgroups (chronic pancreatitis (CP)/ postsurgical pain (PSP)) were incorporated as covariates. Degrees of freedom were adjusted using Greenhouse-Geisser correction for within-subject factors with more than two levels. All EEG outcomes were analysed in a similar manner. No correction for multiple comparison was applied for explorative post hoc testing. Additionally Pearson's correlations or non-parametric Spearman tests were used, based on the data distributions, to investigate the relation between differential effects on EEG parameters and subjective outcomes of pain intensity. All statistical tests were performed two-tailed, and the limit for statistical significance was set at $P<0.05$.

RESULTS

Demographics and clinical outcomes

Forty-nine patients (22 CP/ 27 PSP) were included in the EEG analysis. Clinical and demographic characteristics of these patients are described in Table 1. As reported, no differences were observed regarding subjective outcomes of VAS pain between THC and placebo treatment [6]. Mean VAS pain scores reported on average were 1.6 cm (40%) after THC treatment compared to 1.9 cm (37%) after placebo treatment. A significant difference in VAS pain scores at baseline was observed between the THC and placebo group, corresponding to a mean (SD) of 4.0 (1.9) and 5.2 (1.8) cm respectively.

Table 1: Demographic and clinical characteristics.

	THC (n=21)	Placebo (n=28)
Gender (male/female)	9 / 12	15 / 13
Age (years)	53.0 (9.8)	53.2 (9.3)
Etiology of pain (CP/ PSP)	8 / 13	14 / 14
Concomitant medication		
None	0	0
PCM	3	11
NSAID	3	2
Weak opioids	3	5
Strong opioids	7	10
Antiepileptics	3	4
VAS pain baseline	4.0 (1.9)	5.3 (1.7)
VAS pain last day	2.4 (2.3)	3.5 (2.4)
VAS pain change	-1.6 (1.8)	-1.9 (2.2)
HADS anxiety	6.5 (3.6)	6.7 (4.5)
HADS depression	6.5 (4.5)	6.8 (4.0)
HADS total	12.9 (7.0)	13.3 (7.5)
PCS total	20.4 (9.7)	23.0 (12.0)
PASS total	63 (29)	64 (30)

Continuous data are expressed as mean (SD) and categorical data as numbers (n). Weak opioids were defined as codeine and tramadol. Strong opioids were defined as opioid-based therapies such as oxycontin, fentanyl and morphine. Abbreviations: CP= chronic pancreatitis, PCM=paracetamol, NSAID= non-steroidal anti-inflammatory drugs, VAS= visual analogue scale, HADS= hospital anxiety and depression scale, PASS= pain anxiety symptoms scale, PCS= pain catastrophizing scale.

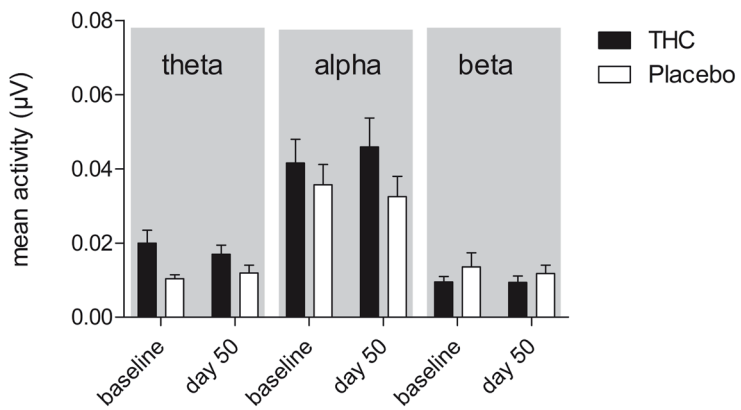

Figure 1: Mean (SD) absolute power spectra, expressed as mean activity (μV) averaged across all electrodes, within the theta (4.0–7.5 Hz), alpha (7.5–13.0 Hz) and beta (13.0–30 Hz) frequency bands at baseline and last day of treatment shown for THC (n=21) and placebo (n=28).

Table 2: Mean (SD) alpha indices per ROI measured baseline on study day 1 and at the last day of study treatment on day 50 for the THC and placebo group.

Alpha index	Day	ROI	THC (N=21)		Placebo (N=28)	
			mean	SD	mean	SD
PAP (μV/Hz)	1	Frontal	.15	.12	.16	.13
	1	Central	.21	.18	.18	.18
	1	Parietal	1.70	1.95	1.37	1.38
	1	Occipital	2.47	3.48	2.58	3.05
	50	Frontal	.17	.14	.17	.14
	50	Central	.25	.25	.18	.18
	50	Parietal	1.77	1.99	1.47	1.64
	50	Occipital	4.10	6.24	2.13	2.73
PAF (Hz)	1	Frontal	8.88	1.02	9.16	1.16
	1	Central	9.34	1.09	9.07	1.04
	1	Parietal	9.59	.81	9.23	1.11
	1	Occipital	9.57	1.03	9.27	1.03
	50	Frontal	8.96	1.14	8.91	.90
	50	Central	10.51	3.62	9.05	1.28
	50	Parietal	9.54	.95	9.30	.97
	50	Occipital	9.47	.94	9.08	1.05
GAF (Hz)	1	Frontal	9.60	.42	9.55	.43
	1	Central	9.72	.46	9.70	.42
	1	Parietal	9.66	.51	9.58	.60
	1	Occipital	9.64	.50	9.57	.64
	50	Frontal	9.62	.50	9.52	.45
	50	Central	9.77	.64	9.66	.40
	50	Parietal	9.73	.68	9.59	.59
	50	Occipital	9.68	.61	9.55	.63

Abbreviations: peak alpha power (PAP), peak alpha frequency (PAF), gravity alpha frequency (GAF), and region of interest (ROI)

Table 3: Mean (SD) electrical stimulation parameters for THC and placebo

	THC (N=21)		Placebo (N=28)	
	mean	SD	mean	SD
Mean electrical pain threshold (mA)	2.47	1.60	2.72	1.89
Stimulus intensity (mA)	3.70	2.41	4.06	2.86
VAS stimulus baseline (cm)	5.70	1.43	5.65	1.62
VAS stimulus day 50 (cm)	5.16	1.48	5.15	1.91

THC effect on resting state EEG

Grand average spectral density powers obtained from the resting state EEG and calculated per frequency band separately are shown in figure 1. No significant differences were observed between THC compared to placebo treatment ($F(1, 47) = 0.752$; $p = .390$) and between last day of treatment compared to baseline ($F(1, 47) = 0.215$; $p = .645$). Significant within subject effects were observed for ROI ($p < .001$) and frequency band ($p < .001$).

Regarding all alpha indices, no significant between subject effects were observed between THC and placebo treatment, and no significant within subject changes were observed between last study day and baseline measurements. PAP, PAF and GAF outcomes per ROI are presented for THC and placebo treatment in table 2.

Resting state EEG and clinical pain

No correlations were observed between power spectral densities and VAS pain scores at baseline in patients with chronic abdominal pain. However, PAF ($r = -.31$; $p = .03$) and GAF ($r = -.36$; $p = .01$) demonstrated significant negative correlations and PAP ($r = .27$; $p = .06$) a nearly significant positive correlation with VAS pain as shown in Figure 2, indicating that increased pain severity is associated with lower peak frequencies. Furthermore, no significant differences at baseline were observed in power spectral densities or alpha indices between the THC and placebo group.

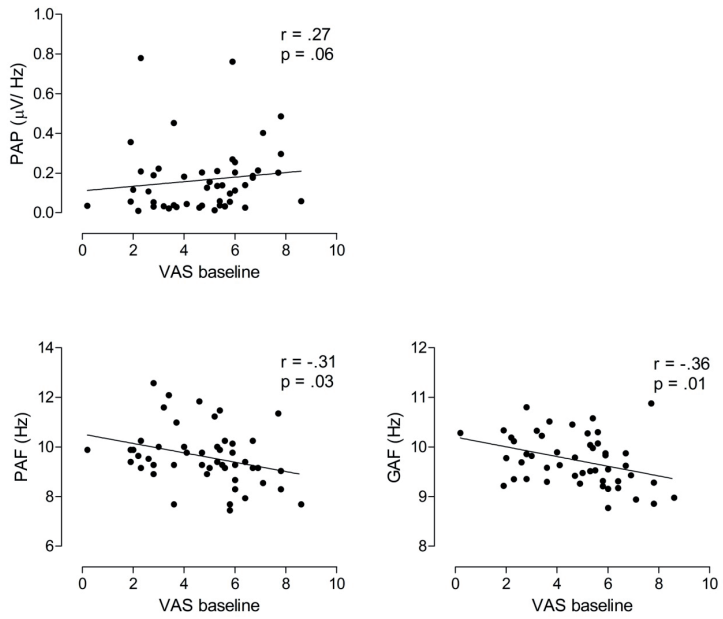

Figure 2: Correlations between clinical VAS pain scores and alpha EEG indices at baseline. Alpha indices include peak alpha power (PAP) shown in upper left panel, peak alpha frequency (PAF) shown in lower left panel, and gravity alpha frequency (GAF) shown in lower right panel.

Pain related electrical stimulation

Electrical pain thresholds were similar between treatment groups, overall resulting in mean stimulus intensities of 3.9 mA (range 0.9 – 12.8 mA). Both study groups showed a similar non-significant reduction in VAS_{stim} ($F(1, 46) = 0.103$; $p = .750$) at the end of study treatment compared to baseline (Table 3). Additionally, no treatment effect ($F(1, 46) = 0.009$; $p = .927$) was observed between THC and placebo concerning the VAS in response to pain related electrical stimuli.

THC effect on pain related Evoked Potentials

No changes were observed in pain related grand average EPs after 50-52 days of treatment compared to baseline (Figure 3 A-D). Corresponding scalp distributions of neural activity for N1 and P3 peaks demonstrate the largest activity at the vertex and stable topographic maps over time. No significant within subject effects over time were observed in peak amplitudes and latencies ($F(1, 46) = 0.583$; $p = .449$), and no significant effect was found between THC and placebo treatment ($F(1, 46) = 2.518$; $p = .119$) recorded over midline electrodes Fz, Cz, Pz and Oz. However, a significant subgroup effect was observed between CP and PSP patients ($F(1, 46) = 5.607$; $p = .022$). Further explorative post hoc analysis of subgroups revealed a significant treatment effect ($F(1, 25) = 6.729$; $p = .016$) in PSP patients, but no repeated measurement effect. Apparently, this treatment effect derived from N1 latency measured at Cz ($F(1, 25) = 6.037$; $p = .021$), representing a delay in the THC treatment group.

Baseline EPs and clinical pain

Baseline N1 amplitude at Cz were significantly correlated with clinical pain scores ($r = -.34$; $p = .017$), indicating that severe subjective pain is associated with enhanced N1 amplitudes. Additionally, baseline VAS pain scores demonstrated significant correlations with P3 amplitudes measured at Pz ($r = .300$; $p = .037$) and Oz ($r = .296$; $p = .039$), suggesting that clinical pain severity is also associated with enhanced P3 amplitudes.

Treatment response and EEG parameters

Changes in clinical pain scores at the last study day compared to baseline were not associated with alterations in EEG band activity nor with alpha EEG indices in both treatment groups. Additionally, this subjective measure of treatment response was also not correlated with changes in N1 or P3 components of the pain related EP. Hence, individual treatment responses, produced by THC and/ or placebo, were not reflected by alterations in the resting state EEG nor pain related EPs.

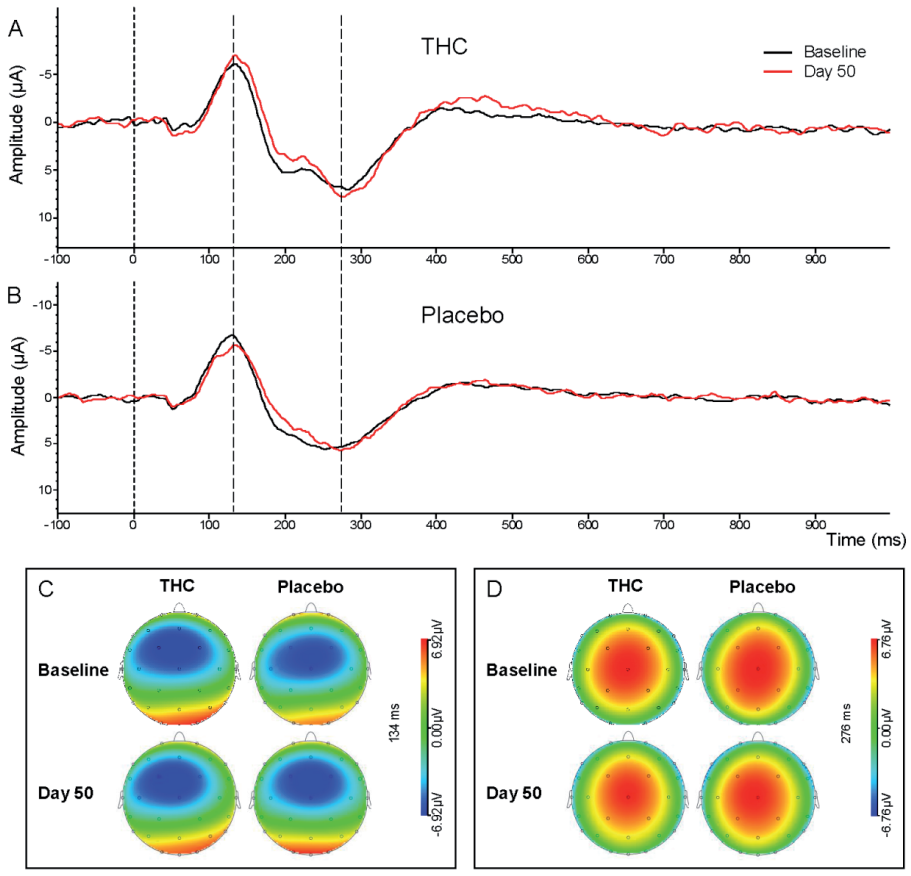

Figure 3 A-D: Pain related grand average evoked potentials at Cz recorded baseline and at day 50-52 in the THC group (panel A) and the placebo group (panel B). Corresponding group mean scalp distributions of neural activity for the N1 (panel C) and P3 (panel D) components, mapped at their peak latencies, recorded baseline and at day 50-52 in the THC and placebo condition.

DISCUSSION

This is the first study to our knowledge to evaluate the pharmacodynamic effects of a 50 days THC treatment on pain related EEG indices in patients with chronic abdominal pain. We demonstrated that THC did not affect spontaneous brain activity nor evoked pain processing compared to placebo after 50 days of treatment. Solely in PSP patients, a delay in N1 latency at Cz was observed for THC treatment compared to placebo. Moreover, cortical correlates of THC could not be associated with its analgesic efficacy, and thus, did not reflect individual treatment responses. However, clinical pain severity was associated with slowing of brain oscillations in a resting state, as well as with enhanced N1 and P3 amplitudes elicited by noxious electrical stimulation.

No therapeutic effects of THC

In the current study, THC did not show a beneficial effect on clinical pain experience after a 50-day treatment period compared with placebo in patients with chronic abdominal pain. Remarkably, subjective pain relief of approximately 40% was observed in both treatment arms.[6] A lack of observed analgesic efficacy on the clinical endpoint could be related to insufficient analgesic potency of the investigational drug, but can also be considered from a mechanistic point of view.

Chronic pain modulates neuronal activity

Pain results from the integration of nociceptive and contextual information (i.e. cognitive, emotional, and motivational) mediated by dynamic processes in the brain.[12] Two mechanisms are proposed to underlie chronic pain and its development: 1) increased responsiveness of nociceptive neurons in the central nervous system (central sensitization), and 2) alterations in central cognitive and autonomic processing.[3,20] In addition, a close relationship between chronic pain and psychological factors indicates that brain function plays a central role in chronic pain. This is supported by several neurophysiologic and functional imaging studies demonstrating changes in the frequency spectrum of the brain, that are believed to be causally involved in the development and maintenance of chronic pain [12]. In the current study, PAF and GAF were negatively correlated with VAS pain scores at baseline, indicating that increased pain severity was associated with slowing of brain oscillations in a default state. Several studies have shown this slowing of PAF in chronic pain compared to controls, including patients with chronic abdominal pain.[21-24] Moreover, multiple studies on EEG power spectra at rest reported that chronic pain patients displayed an increase of theta oscillations compared to controls.[23,25] Although these results are not fully consistent, chronic pain is generally associated with alterations in brain oscillations recorded at rest, which are attributed

to plasticity of the central nervous system. Similar signs of plasticity were observed in the current research population, enabling the study of potential antinociceptive effects of THC utilizing maladaptive EEG outcome parameters in this group of chronic pain patients.

THC and neuronal oscillations

In this study, we did not observe THC induced changes in resting state brain activity, whereas several other EEG studies have demonstrated that chronic cannabinoid use is associated with disrupted oscillations in the theta and gamma band.[14] Additionally, controlled acute administration of THC resulted in decreased theta power in healthy subjects,[15] demonstrating that THC has the ability to modify neural brain oscillations. We hypothesized that THC induces antinociceptive effects by reversing the increased theta activity or slowed alpha peak power in patients with maladaptive alterations resulting from chronic pain. Various explanations can be considered for the lack of EEG changes in our study. First, the analgesic efficacy of THC, evaluated by means of clinical pain experience, is still unclear and the optimal therapeutic dosage is not defined. Question is if in the absence of clinical efficacy, as also shown in this study, antinociceptive effects can be expected? Changes in the EEG could still occur, but these might be independent from clinical or antinociceptive drug efficacy. Second, placebo effects on pain perception are associated with decreased neural activities in pain modulatory brain regions and pain related EPs. A recent study using tonic muscle pain in healthy subjects revealed that placebo analgesia induced significant increases in alpha oscillations.[26] These changes in alpha amplitudes were strongly correlated with the placebo effect on pain reported experience. In the current study, a considerable placebo effect on clinical pain perception was observed, which may have affected brain oscillation of chronic pain patients in both treatment arms.

Pain related EPs

Chronic pain is frequently associated with changes of early and late EP amplitudes in response to somatosensory and visual pain related stimuli.[27] Delayed and enhanced P3 amplitude, using equivalent electrical noxious stimulation, was shown in patients with chronic pain after breast cancer treatment.[28] In the current study, we observed significant correlations between clinical pain experience and enhanced N1 and P3 amplitudes, suggesting that clinical pain intensity is associated with an increased responsiveness of pain related EPs. Early EP components are generally related to early preconciousness processes, reflecting somatosensory afferent input. By contrast, later components of EPs may reflect discomfort or emotional-motivational aspects, related to the manner in how the subject evaluates the stimulus.[29,30] These cognitive functions are also known to be

affected by cannabinoids. Furthermore, the frontal-limbic distribution of CB1 receptors in the brain suggests that cannabis may preferentially target the affective and more cognitive qualities of pain. This was supported in an experimental human pain model, where THC reduced the reported unpleasantness, but not the intensity of ongoing pain and hyperalgesia.[31] Contrary to our hypothesis, we could not detect changes in pain related EPs elicited by noxious electrical stimulation after THC treatment. A slightly significant delay in N1 peak was shown for THC in only a subgroup of PSP patients, though no correction for multiple testing was applied. Thus, a potential type I error needs to be taken into account. Additionally, other factors such as the observed clinical inefficacy on VAS pain, as well as selected study population and stimulation paradigm may have complicated this study. Acute administration of THC has been reported to reduce P300 amplitudes to auditory stimuli in healthy volunteers.[32] Therefore, it would be interesting to study pain related mechanisms of THC in an experimental human pain model utilizing nociceptive specific stimuli in order to increase the internal validity.

Methodological considerations

Several limitations to the current study have to be addressed. The sample size is susceptible to both type I and type II statistical errors, which cannot be ruled out completely. Besides, the study population was too small to conduct a reliable prediction analyses based on responders and non-responders in both treatment groups. Suppose that only a minor part of chronic pain patients benefits from THC treatment, then it is important to develop a method to predict its effect in an individual. Future studies are necessary in order to develop a clinical pharmaco-EEG model, based on underlying maladaptive mechanisms, to predict the efficacy of THC in individual chronic pain patients.

Furthermore, it should be mentioned that most patients continued regular prescribed pain medication during study treatment, as decided by the ethical committee. Hence, central acting drugs, such as frequently prescribed opioids and anticonvulsants, have an effect on the spontaneous as well as evoked EEG.[33] Overall, opioids induce increased activity in the delta band and a decrease of late component EP amplitudes evoked by painful stimuli, while the few studies on anticonvulsants such as pregabalin show inconsistent results.[33] Additionally, these drugs can induce sedative effects, which also influence EEG characteristics.

Finally, noxious transcutaneous electrical stimulation using the concentric electrode is not nociceptive-specific. Only low intensity electrical intra-epidermal stimulation below 2.5 mA are able to evoke Aδ-associated evoked brain responses,[34] while larger stimulation intensities were applied in this study. Therefore, it should be mentioned that beside Aδ fibers, also tactile Aβ fibers were stimulated, producing pain related EPs.

In conclusion, THC did not affect these alterations in spontaneous brain activity nor evoked pain processing. Correlations between clinical pain intensities and objective EEG outcomes suggest that chronic abdominal pain alters central pain processing recorded by EEG. Moreover, cortical correlates of THC could not be associated with its analgesic efficacy, and so, did not reflect individual treatment responses. Future studies are necessary to further explore underlying mechanisms and therapeutic potential of THC for individual patients with chronic pain.

ACKNOWLEDGMENTS

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Chapter 9

General discussion

Partly based on:

Systematic mechanism-orientated approach to chronic pancreatitis pain

Bouwense SA, de Vries M, Schreuder LT, Olesen SS, Frøkjær JB, Drewes AM, van Goor H, Wilder-Smith OH.
World Journal of Gastroenterology. 2015 Jan 7;21(1):47-59.

After tissue healing, pain may persist as chronic pain. Chronic pain has a major impact on quality of life.[1] Although the majority of publications on chronic pain address the treatment of this pain, an adequate approach to the prevention and treatment of chronic pain is still lacking. A key insight has been that nervous system processing of pain is not hard-wired: sensory processing in the nervous system typically changes as a result of noxious sensory inputs.[2] Acute nociception initially results in increased pain sensitivity (hyperalgesia) affecting the peripheral and central nervous system. When ongoing nociception (due to ongoing damage to tissues and nerves) is present, peripheral nervous system sensitization may occur. Furthermore, this nociceptive barrage will continuously excite the brainstem and brain leading to central sensitization. In the end the whole nervous system may become sensitized, leading even minor stimuli to become painful or to the presence of pain without nociceptive input.[2-4] This phenomenon of maladaptive plasticity of the nervous system manifests itself as altered pain processing in chronic pain patients.

A systematic approach to diagnosing altered pain processing in chronic pain

A suitable tool is required in order to facilitate a systemic approach to diagnose altered pain processing in patients with chronic pain. This tool should document:[4]

1. The function of the central nervous system
2. Changes in the function and structure of the central nervous system

Application of such a tool in the systematic approach to chronic pain permits a mechanism-orientated management enabling: (1) proper diagnosis and follow-up of chronic pain, (2) rationale for treatment choice and responder identification and (3) monitoring of pain treatment.[4]

Quantitative sensory testing (QST), electroencephalograpy (EEG) and magnetic resonance imaging (MRI) have increasingly been used in chronic pain disorders to describe maladaptive changes in (peripheral) nerves and the central nervous system. Increasing evidence from studies using these tools has provided us with important information on central pain processing and how it can be influenced by disease progression and treatments. Each tool has its own strengths and limitations, although the potential of EEG is largely unclear. We focused in this thesis on EEG to further investigate cortical sensory processing in the presence of chronic pain.

Rationale for EEG as diagnostic tool for chronic pain

As early as 1953, the EEG was being studied by Kirschbaum and colleagues in patients with pain due to peptic ulcers and functional gastric disorders.[5] Their study is an early example of the recognition of the brain-gut axis as a possible substrate for visceral pain

syndromes. In the more recent literature, studies using EEG in visceral pain syndromes mostly address chronic pancreatitis. Although the use of EEG can be demanding and complex, this technique is a potentially useful non-invasive tool for clinical practice. EEG has a poor spatial resolution, but superior millisecond-range temporal resolution compared to other neurodiagnostic instruments such as PET or (f)MRI, enabling direct measurements of neuronal processing.[6] EEG can be used in chronic pain conditions to study the brains' default state reflected by the resting state EEG (static element) and brain activity due to external stimuli reflected by event related or evoked brain potentials (dynamic element).[6]

Changes in resting state EEG activity associated with chronic pain

Alterations in the brains' default state as reflected by resting state EEG, particularly in the alpha band, have been observed in multiple studies in various chronic pain conditions. These changes typically consist of a shift of peak alpha or theta frequency to lower frequencies and/or a reduction in alpha or theta power.[7-11] Similar results were shown in our study investigating novel potential markers of chronic pain in the resting state EEG in patients with chronic pancreatitis. We observed that chronic pancreatitis pain is associated with alterations in the spontaneous brain activity, observed as a shift towards lower peak alpha frequencies (PAF). This shift correlates with the duration of pain, suggesting that PAF has the potential to be a clinically feasible biomarker for chronic pain (**chapter 3**).

Accordingly, Olesen et al. reported an increase in amplitude strength in the theta and alpha band in patients with chronic pancreatitis compared to healthy controls, and concluded that their findings reflected a slowed EEG rhythmicity in patients compared to controls.[12] In irritable bowel syndrome (IBS) patients, power spectrum analysis of the resting EEG also showed a decrease of alpha power percentage together with an increase of beta power percentage compared to healthy subjects.[13] These findings are similar to findings in other non-visceral (e.g. neurogenic) chronic pain syndromes, indicating that slowing of brain rhythmicity as a reflection of altered resting state CNS function may be a hallmark of the chronic pain state. Additionally, baseline results as obtained in our clinical parallel study (**chapter 8**), demonstrated that clinical pain severity was associated with slowing of brain oscillations in a resting state, as well as with enhanced N1 and P3 amplitudes elicited by noxious electrical stimulation. However, it is still unclear whether alpha activity is directly related to subjective pain experience, given that equivocal results are observed among clinical studies.

Alterations in evoked brain potentials associated with chronic pain

Evoked potentials (EPs) have been used to describe changes in the central nervous system, although the heterogeneity among previous studies impedes identification of a characteristic maladaptive pattern of chronic pain. We aimed to investigate brain processing in response to painful electrical stimuli in patients with chronic pain after breast cancer treatment (**chapter 2**). In this group of patients, we observed that persistent pain was associated with both delayed and enhanced stimulus processing as reflected in an increased latency and enhanced amplitude of the late EP component between 250–310 ms. From this study the key question raised: are these alterations in evoked brain potentials similar for all types of chronic pain including (postsurgical) abdominal pain?

Dimcevski et al. recorded EPs after painful stimuli given with a constant current electric stimulator at the three different sites of the upper gastrointestinal tract. Patients with chronic pancreatitis had a significantly decreased latency for the N1 and P1, while N2 latency was borderline significant compared to healthy subjects. No differences were found in amplitudes of the N1, P1, and N2 potentials.[14] Patients with chronic pancreatitis also showed hyperalgesia to electrical stimulation and prolonged latencies of early visceral EPs components in the frontal region of the cortex compared to healthy controls. Moreover, scalp distributions of EP amplitudes were more scattered and more posteriorly located in this patient group.[15] These changes are generally compatible with our findings in patients with non-visceral postsurgical pain (**chapter 2**). In contrast, stimulation of vagal nerve afferents in patients with gastroesophageal reflux disease did not reveal any difference in peak latencies or EP amplitude between gastroesophageal reflux disease (GERD) patients and controls.[16] IBS patients showed an altered generation or transmission of rectal EPs observed as a higher prevalence of cerebral evoked potential early peaks, and shorter latencies compared to healthy subjects.[17] Another study in IBS reported a shorter latency and a smaller amplitude of the early EP component after electrical painful stimulation in the sigmoid colon in IBS patients compared to healthy subjects.[18] These data suggest that central processing of painful stimuli is altered in different types of chronic (abdominal) pain. However, typical maladaptive characteristics could not be identified probably due to the heterogeneity in clinical (pain) characteristics, stimulation modalities and EEG analyzing methods.

Different stimulus modalities produce different cortical scalp potentials

In order to obtain EPs that are specific to nociceptive input, such input should be the result of physiological processing of nociceptive stimuli, i.e. involving selective activation of nociceptive Aδ/C-fibers in the periphery and recording resultant EPs generated in the cortex.[19] Brain mapping studies have established a positive relationship between the intensity of pain reported to nociceptive selective laser stimuli and EP amplitude.[20] In

the context of evoked EEG studies, it must be noted that in general the experimental visceral electrical stimulation of large and small peripheral afferents in different gut segments is painful, but not nociception specific.[21] Whether EPs resulting from stimuli entirely selective for nociceptive peripheral afferents represent the experience of pain or a more generalized response of heightened attention or arousal to afferent stimuli is current topic of debate.[20,22-25] Mouraux and Iannetti demonstrated that laser-evoked EEG responses reflect neural activities equally involved in processing nociceptive and non-nociceptive sensory inputs.[24] Thus, a stimulus entirely selective for nociceptive peripheral afferents does not imply that the elicited brain activity is nociception specific. However, even if EPs reflect neuronal activities that are unspecific for the nociceptive system, their generation still relies on the consequences of nociceptive activation and resultant changes in CNS state at both peripheral and central levels.[24]

EEG as tool to detect altered brain processing associated with chronic pain

To summarize, studies in chronic pain investigated both the resting state as well as the evoked EEG. The broad range of applied analysis techniques and stimulation methods hampers direct comparison of results. However, alpha activity in the resting state EEG has been shown to be affected in multiple chronic pain states, including chronic pancreatitis as demonstrated in our study, suggesting a change in the default state of the brain as a result of chronic pain. In addition, we observed that chronic pain was associated with delayed and enhanced stimulus processing in response to electrical noxious stimuli in patients after breast cancer treatment. Pain-evoked EEG studies in chronic pancreatitis patients also demonstrated alterations in dynamic pain processing reflected by prolonged latencies of visceral EPs and higher theta activity with prolonged persistence of the signal at a lower frequency during experimental visceral pain. Taken together, these EEG findings further support the concept that chronic (abdominal) pain conditions are associated with significant and ubiquitous alterations in resting state and evoked central pain processing, both nociceptive and non-nociceptive, interpreted as a sign of maladaptive plasticity.[26] The challenge now is to improve the sensitivity and specificity of EEG to allow the development as diagnostic tool for individual patients. Additionally, future EEG research should monitor fluctuations of perceived pain in longitudinal studies.

Treatments for chronic abdominal pain are lacking

Opioids are among the most prescribed and effective drugs for the treatment of moderate to severe pain in general. Although there is a consensus on their utility as a treatment for chronic cancer pain, their long-term use for chronic non-malignant pain remains controversial due to concerns about side effects, long-term efficacy, functional

outcomes, chronic toxicity and the potential for drug abuse. [27] Additionally, chronic administration of opioids can result in decreased pain thresholds and produce opioid-induced hyperalgesia. Opioid-induced hyperalgesia is a paradoxical effect, in that opioid therapy enhances or exacerbates pre-existing pain, while it is originally prescribed as analgesic.[28,29] This unintended and undesirable consequence of prolonged opioid exposure is likely the result of neural plasticity of the nervous system.[29] In addition, opioids may affect the absorption of other drugs by changes in gastrointestinal motility, sphincter function, intestinal fluid secretion and drug metabolism.[30-32] It is thus desirable to avoid prolonged opioid prescription, but the question arises: What are the alternatives for patients suffering from chronic abdominal pain?

As addressed in the first part of this thesis, chronic pancreatitis and chronic postsurgical pain display both visceral and neuropathic pain components, which are associated with maladaptive plasticity of the peripheral and central nervous system. Medical therapy is increasingly focused on a combination of medications by targeting different pain pathways. Over the past few years, several (psycho)pharmacological drugs, that are not normally considered analgesics, such as tricyclic antidepressants and anticonvulsants, have been investigated as adjuvant treatment option. Despite this, effective, safe and sustainable treatment options for chronic pain are still lacking.

The therapeutic potential of THC

Despite a long history of medicinal cannabis use in the treatment of pain, the analgesic properties of cannabis or THC are still ill-defined, particularly for chronic abdominal pain conditions. We did not observe any analgesic effect of a single dose of THC compared to diazepam, with no difference between opioid users and non-opioid users, in chronic pancreatitis patients (**Chapter 4**). Although pain was decreased after THC administration, a similar effect was observed after diazepam, which might be the result of placebo or sedative effects. Subsequent clinical trials by our group were designed to evaluate the efficacy of THC during a relatively long-lasting treatment period of 50-52 days (**Chapter 5**). In this parallel design study, THC also did not show a beneficial effect on chronic abdominal pain compared with placebo. Between the start and end of the study, VAS mean scores decreased by 1.6 points (40%) in the THC group compared to 1.9 points (37%) in the placebo group. Our findings are in contrast with other studies, since most previous studies in chronic non-malignant pain conditions demonstrated analgesic efficacy using a single dose or treatment periods of 2 to 15 weeks. [33-45] However, the majority of studies with cannabis-based medicines were conducted in patients suffering from central neuropathic pain in multiple sclerosis. The lack of observed analgesic efficacy in our clinical trials can be related to insufficient analgesic potency of the investigational drug, but it may also be related to 1) the selected study design and treatment dose, 2)

inter-individual variation in pharmacokinetic profiles, 3) a large placebo response, and 4) indirect anti-nociceptive effects. These aspects are further discussed in the following paragraphs.

Selecting the optimal study design and treatment dose

Randomized clinical trials are traditionally performed double-blinded, whereby both patient and investigator theoretically do not know or cannot distinguish the assigned treatment. However, the psychoactivity of cannabinoids may unmask their presence through the occurrence of recognizable side effects and consequently may result in poor concealment of group allocation.[46] This may bias participants toward their expectations and/or conditioning of pain relief, which can lead to incorrect assumptions regarding the efficacy of the drug. This is particularly so in studies utilizing self-reported subjective outcomes.[46] For this reason, low dose Diazepam was used as active placebo to prevent unblinding of patient and investigator in the single dose crossover study. However, interpretation of (secondary) outcomes appeared to be more complicated comparing two psychoactive substances. Therefore, we decided to use an inactive placebo in the parallel group study, which is less prone to unblinding for the reason that patients cannot compare both treatment arms in a parallel design. The observed pain relief in each treatment arm of both clinical trials were probably induced by placebo effects, which supports that we gained adequate blinding of patients. However, we should have evaluated the preservation of the blind-to-treatment allocation by asking participants whether or not they believed they received active study medication or placebo. It is important that future studies of psychoactive compounds use methodologies designed to counteract unmasking and incorporate blindness assessments in their study protocols.

In the single dose study, the THC dose was primarily based on the PK and PD results of an earlier phase I study with Namisol® in healthy young volunteers [47]. Previous studies with other cannabis-based compounds were inappropriate to select the optimal dose, because PK profiles depend among different formulations and administration routes. We used a novel tablet formulation of pure THC that was produced using an emulsifying drug delivery technology to improve the uptake of poorly soluble lipophilic compounds. This oral tablet demonstrated an improved bioavailability in healthy subjects. Previous studies of other cannabis-based compounds demonstrated linear dose-response curves for multiple pharmacodynamic parameters. Extrapolating these linear effects, we selected the maximal tolerable dose of THC as shown in the phase I study in order to induce the largest analgesic effect. However, it appears that a single dose might be insufficient to achieve adequate exposure duration. A lipophilic substance such as THC will diffuse to the fatty tissues immediately, and therefore, the THC concentration at the target site might be insufficient to modulate pain after single dose, explaining the

comparable effects between the experimental and control condition.
Subsequently, the single dose concentration-time curves were used to simulate multiple dosing strategies in order to determine the most optimal dosage regimen for a relatively long term treatment period. The treatment scheme, consisting of a step-up phase to habituate and a stable dose phase to induce the desirable effects, was semi-fixed only allowing dosage tapering in the stable dose phase with one step. Future studies should adjust the dosage for individual patients according to individual pharmacokinetic profiles and clinical effects [48,49].

Inter-individual variation in pharmacokinetic profiles

The underlying pathophysiology of chronic pancreatitis potentially alters the pharmacokinetics of orally administrated drugs, and consequently, the efficacy of a pharmacological treatment. Fibrotic destruction of the pancreas in chronic pancreatitis or after pancreatic surgical resections induces exocrine insufficiency and results in a decreased release of pancreatic enzymes and bicarbonate.[50] The pancreatic gland normally produces more than 2 L of secretions per day which is composed of water, bicarbonates and enzymes.[51] The impaired secretion of digestive enzymes into the duodenum leads primarily to fat malabsorption, which is clinically recognized as steatorrhea. Impaired pancreatic bicarbonate secretion in the intestines results in changes in the intraluminal pH, because of insufficient buffering of gastric acid by the bicarbonate. Reduction in duodenal pH results in inactivation of trypsine, amylase, and particularly lipase enzymes, and consequently leads to further impairment of fat digestion.[52,53] Fat malabsorption also results in a deficit of fat-soluble vitamins, [54] and may affect absorption of lipophilic drug formulations.[55] Additionally, opioid use, malnutrition and a history of alcohol abuse, common featured in chronic pancreatitis patient, may potentially influence the pharmacokinetics. We observed a delay in time to reach maximal plasma concentration and a spread in maximal plasma concentrations in chronic pancreatitis patients compared to healthy volunteers. These changes in pharmacokinetics of THC might be direct and indirect consequences of exocrine and endocrine pancreatic insufficiency. Several other factors, such as antidiabetics, cytochromes P450 enzyme polymorphism and pancreatic surgery, potentially enhanced inter-individual variations in the pharmacokinetics of THC, and accordingly, contributed to inter-individual variations in efficacy.

The problem of placebo responses

A large number of novel analgesics have failed to prove superiority over placebo in clinical trials, which has been ascribed to a large placebo response.[56] In our clinical trials, we observed 20% pain reduction after a single dose of diazepam, which was used as active placebo in the cross-over study, and 37% pain reduction in the placebo arm after

50-52 days of study treatment. Large placebo responses are quite common in chronic (abdominal) pain studies. A meta-analysis in patients with irritable bowel syndrome allocated to placebo observed an average placebo response of 38%.[57] Additionally, the placebo response rate in clinical trials evaluating treatment of pain in chronic pancreatitis was 20%. Factors that were associated with higher placebo responses in chronic pancreatitis patients were a multicenter design, a run-in period of less than two weeks, and absence of a washout in crossover trials.[58] Type of active medication, randomization ratio, and the number of planned face-to-face visits are expectancy mediating factors also influencing the degree of the placebo response.[56] The effect of baseline pain intensity on the placebo response is not clear, however, patients experiencing more fluctuations in pain demonstrate larger placebo responses compared to patients with less variability in pain over time.[59] Fluctuating pain patterns are typical for chronic pancreatitis, which might have enhanced the placebo effect.
Underlying mechanisms mediating the placebo effect can be derived from psychological and neurobiological perspectives. Two well supported psychological mechanisms are expectancy about the therapeutic benefit and conditioning from earlier experiences.[60] High expectations toward treatment efficacy of THC might have contributed to the substantial placebo response as observed in our studies. In clinical practice, placebo effects can be utilized by influencing patients' expectations in order to improve treatment effects. However, in clinical trials, placebo effects should be minimized to optimize differences between verum and placebo. Modifiable study characteristics that potentially affect placebo responses should be identified and optimized in order to increase the probability that a clinical study will show superiority of the study drug compared with placebo. Of these, increased sample size, longer trial duration and more frequent face-to-face visits were significantly associated with larger placebo response.[56,58] In retrospect, we should have reduced the number of study contacts between patient and study staff in our trials. In future clinical trials, patients' expectations should be assessed as an important factor affecting the magnitude of the placebo response. Baseline attitudes can be used as stratification factor in the randomisation procedure or patients' expectations can be used as co-variables.[61] Prior identification of high placebo responders and potential determinants of the placebo response can also improve study designs in order to separate specific treatment from non-specific contextual (i.e. placebo) effects. Improved study designs and outcome measurements are required for appropriate drug evaluation and personalized health care in chronic (visceral) pain research.

A mechanism-orientated approach to evaluate anti-nociceptive effects of THC

The number of chronic pain clinical trials reporting negative findings has increased.[62]

The explanation for these unsuccessful outcomes may involve selection, analysis, and interpretation of outcome measures as well as shortcomings in the design of these trials. For these reasons, several Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) recommendations have been developed which should be considered in future clinical trials.[62-64] Additionally, it is possible that poor understanding of patient heterogeneity in pathophysiologic mechanisms (i.e. maladaptive plasticity of the CNS) and treatment responses are a reasonable explanation for unsuccessful trials. In the first part of this thesis, we focused on the pathophysiology of chronic abdominal pain and identified several pain related changes in EEG outcomes. In part III, we evaluated these maladaptive changes in EEG outcomes during THC treatment. The ultimate goal was to provide a foundation for a mechanism-oriented treatment approach in which THC targets the specific mechanisms of a patient's pain as we proposed in **Chapter 6**. Therefore, resting state EEG and EPs to pain related electrical stimuli were recorded in both clinical drug studies, but we observed no THC effects on these EEG indices after a single dose of THC (**Chapter 7**) nor during a treatment period of 50-52 days (**Chapter 8**). It is nevertheless too early to draw conclusions regarding the potential of EEG based on these negative findings. According to the mechanism-oriented treatment approach, pharmacologic treatments can alternatively aim to suppress sensitization or other maladaptive neuroplastic changes with the secondary ultimate aim to reduce pain or to postpone more severe pain at the long run. If we were able to detect THC effects resulting in normalisation of EEG indices, it was interesting to perform long-term follow-up of these patients' subjective pain outcomes. For now, we can conclude that THC did not target underlying pathophysiologic mechanisms as identified in several EEG indices in this study population. Furthermore, patients' individual treatment responses could also not be linked to individual changes in EEG indices, which suggest that these EEG indices do not reflect treatment response utilizing subjective outcomes. This is reasonable, since these outcome measures are not similar reflecting different domains related to pain.

Clinical versus neurophysiological assessment of pain and drug efficacy

Because pain is a subjective experience and not only an objective bodily state, the choice of an adequate instrument to measure pain is critical to evaluate the efficacy of an analgesic. Pain is traditionally measured by means of subjective ratings of pain intensity. In contrast, nociception does not describe psychological pain, but refers to the physiological processing of information about the internal or external environment, as generated by the activation of nociceptors. Thus we have two types of pain assessments, reflecting different mechanistic processes and utilizing different instruments, but to a certain extent related to each other. Moreover, both assessments can provide essential insight in each other's underlying mechanisms.

In assessing the efficacy of analgesics in clinical trials, several confounders can bias subjective pain outcomes. Experimental pain outcomes, such as EEG, are without many confounders and therefore a valuable tool for evaluating analgesics in clinical trials.[65] Additionally, assessing analgesic effects by EEG in chronic pain patients may contribute to a mechanism-oriented classification of pain and thereby to a better understanding of the underlying symptoms. However, it should be mentioned that changes in pharmaco-EEG monitoring central analgesic mechanisms are not consistent, and experimental pain studies using EEG to identify patients who may benefit from treatment strategies targeting central pain mechanisms are limited. Moreover, there is a gap between scientific relevance of experimental pain models in clinical trials versus implementation of these neurophysiologic tools in clinical practice. Diagnostic instruments for both clinical and neurophysiological assessments of pain so far lack documented reliability for use in the individual patient.

Recommendations for future research

Although the negative results as observed in our clinical drug trials could also be explained by a lack of efficacy of THC or limitations in the design of the trials, it should be mentioned that multiple chronic pain trials report negative outcomes these days. Rather, a lack of knowledge regarding the cause underlying chronic pain and poor understanding of patient heterogeneity in pathophysiologic mechanisms and treatment response are important explanations for the negative outcomes in trials.

Ongoing research needs to focus on the mechanisms underlying different chronic pain conditions, devise methods for reliably identifying these mechanisms in individual patients, and develop treatments that target these mechanisms. A way to increase our knowledge in this respect is to measure the effect of pain and nociception on central pain processing in large-scale clinical studies using neurophysiological tools, such as QST, EEG or fMRI, before and after interventions and during disease progression.[77] Longitudinal data collections will help us understand pain related adaptations and evaluate therapies and guide us to the proper treatment for a specific patient at a specific disease stage. Developments in (f)MRI and EEG hold the most promise to add to our understanding of maladaptive plasticity of the central nervous system related to chronic pain. Moreover, combining these two techniques to obtain simultaneous high-spatial and high-temporal resolution scans offers exciting opportunities. Standardization of stimulation modalities, recording procedures and signal analysis is required. The challenge is then to improve the sensitivity and specificity of these techniques to allow their development as diagnostic tools for the individual patient.

Further research is required to investigate the analgesic potential of THC more comprehensively. However, it makes no sense to perform a new classical randomized

controlled trial. Future studies need more advanced methods, considering: 1) appropriate study designs to manage large placebo responses, 2) adequate patient (responder) selection, 3) individual treatment dosages based on inter-individual variation in pharmacokinetic profiles, 4) reliable neurophysiological outcomes to evaluate anti-nociceptive effects. Certainly, THC can also be an ineffective cannabinoid, but this cannot be confirmed from our studies. Therefore, advanced clinical studies are necessary in order to evaluate if THC might be effective for a certain pain condition and a selective group of patients using an individually determined treatment dosage.

Recommendations for clinical practice

To improve pain treatment in the long term, it is important to study the underlying (patho-) physiological mechanisms of pain as well as the underlying pharmacological mechanism of actions of new and existing analgesics. One aim of such research is that clinicians may be better equipped to choose the optimal analgesic and dose or to make informed decisions regarding analgesic rotation strategies in efforts to achieve the best individual patient outcome. Clinical practice can be very useful to obtain these data for scientific evaluation and to apply population-based evidence-based medicine subsequently. However, both subjective and experimental outcomes obtained in individual patients should be interpreted with caution, due to several limitations concerning validity and reliability of the diagnostic instruments. EEG has currently no proven added value for clinical practice. Until more objective measurements of (maladaptive) pain processing are perfected, clinical practice rely on the use of self-reported outcomes such as quality of life or pain scores.

Conclusions

We observed several changes in evoked EEG utilizing pain related EPs to noxious electrical stimuli in patients with chronic postsurgical pain and chronic pancreatitis. Alterations in resting state EEG were also observed in patients with painful chronic pancreatitis. These EEG findings further support the concept that chronic (abdominal) pain conditions are associated with significant and ubiquitous alterations in resting state and evoked central pain processing, suggesting that chronic pain involve changes in central pain processing mediated through mechanisms of neural plasticity. The ultimate goal of these efforts is to provide the foundation for a mechanism-based treatment approach in which therapeutic interventions target the specific mechanisms of a patient's pain.

In our studies, THC was not efficacious as add-on pain treatment in reducing chronic abdominal pain compared to placebo utilizing subjective pain outcomes. Future clinical studies should optimize study designs to adequately handle large placebo responses and choose advanced treatment outcomes to apply a systematic approach to chronic

pain. EEG can be a useful diagnostic instrument to analyze central pain processing and help us in understanding optimal mechanism orientated treatments for chronic abdominal pain. Future research should define the presence and pattern of altered pain processing for specific chronic pain disorders in individual patients, devise methods for reliably identifying these mechanisms in individual patients, and develop treatments that target these mechanisms.

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Chapter 10

SUMMARY

The objectives of this thesis were to investigate maladaptive mechanisms of neural plasticity underlying chronic pain, and to evaluate the analgesic and anti-nociceptive potency of oral tetrahydrocannabinol (THC).

The studies described in this thesis focused on three subjects:

1. To investigate potential neuroplastic changes in brain activity associated with chronic (postsurgical) pain using both spontaneous and evoked EEG recordings *(Part I)*.
2. To evaluate the therapeutic potential of a novel oral tablet containing purified THC for the treatment of chronic abdominal pain *(Part II)*.
3. To evaluate potential anti-nociceptive effects of THC utilizing pain related neuroplastic changes in spontaneous and evoked EEG *(Part III)*.

PART I: Cortical processing in chronic (postsurgical) pain

Women who undergo breast cancer surgery have a high risk of developing persistent pain. In **chapter 2**, we investigated brain processing of painful stimuli using evoked potentials (EPs) recorded in the electroencephalography (EEG) in patients with persistent pain after breast cancer treatment. Nineteen patients (8 women with pain, 11 without pain), treated more than one year ago for breast cancer via surgery (mastectomy or lumpectomy and axillary lymph node dissection) and/or chemo/radiotherapy were recruited and compared to eleven healthy female volunteers. Changes in cortical processing were recorded in the EEG utilizing pain related EPs to noxious electrical stimuli. The presence of chronic pain was associated with delayed and enhanced stimulus processing as reflected by an increased latency and enhanced amplitude of the EP positivity between 250-310 ms (P260). Compared to healthy volunteers, breast cancer patients had a speeding of (reduced P260 latency) and a tendency towards a less intense (smaller P260 amplitude) stimulus processing. These results suggest that the two conditions, i.e. treatment and pain persistence, have opposite effects regarding cortical responsiveness. The main conclusion of this study is that persistent pain after breast cancer treatment is associated with neuroplastic changes in cortical activity shown as delayed and enhanced stimulus processing.

Changes in spontaneous EEG activity have been observed in several chronic pain populations, suggesting that chronic pain involve changes in central pain processing mediated through mechanisms of neural plasticity. However, this was not yet investigated for chronic visceral pain conditions. We observed alterations in the resting state EEG of patients with painful chronic pancreatitis compared to matched healthy

controls, which is described in **chapter 3.** Chronic pancreatic pain was associated with slowing of the spontaneous brain activity, observed as a shift toward lower peak alpha frequencies (95% CI diff [−.68 to −.01 Hz]; $P<0.05$). No significant group differences were found in peak power amplitudes between chronic pancreatitis patients and healthy controls. The shift in peak alpha frequencies was correlated with the duration of pain, showing increased reductions with longer pain durations, and suggesting that peak alpha frequency is a potential biomarker for disease progression of chronic pain. These findings help us understanding the underlying pathology of chronic pain and assisting in diagnosis, establishing optimal treatment, and studying efficacy of new therapeutic drugs in chronic pain patients.

PART II: Efficacy and safety of tetrahydrocannabinol in chronic abdominal pain

The second part of this thesis focused on the therapeutic potential of a novel oral tablet containing purified tetrahydrocannabinol (THC) for the treatment of chronic abdominal pain. We performed two phase 2 clinical drug studies evaluating the clinical efficacy, pharmacokinetics, pharmacodynamics, pharmacogenetics, safety and pain related neuroplastic changes in spontaneous and evoked EEG after oral THC. In **chapter 4**, the results of a randomized, single dose, double-blinded, placebo controlled crossover study in patients suffering from abdominal pain related to chronic pancreatitis are presented. We found that a single dose of oral THC was not efficacious in reducing chronic pancreatic pain compared with the active placebo diazepam (mean diff THC - diazepam -.17; 95% CI diff [-.95 to .61]; p=.65). THC was absorbed with an average T_{max} of 123 minutes, which was similar for opioid and non-opioid users. The absorption of THC was delayed in several chronic pancreatitis patients, resulting in an increased variability compared to healthy volunteers, most probably due to underlying pathology and concomitant medication use. Overall, oral THC produced reliable pharmacokinetic profiles and was generally well tolerated with mild to moderate adverse events. Long term treatment effects of THC during a treatment period of 50-52 days were further evaluated in a second study. The results of this randomized, double-blind, placebo-controlled study evaluating the analgesic efficacy, pharmacokinetics, and tolerability of oral THC in patients with chronic abdominal pain, are described in **chapter 5**. Sixty-five patients with chronic abdominal pain after surgery or due to chronic pancreatitis were randomly assigned to groups given the THC tablet or matching placebos. Subjects in the THC group were given the tablet first in a step-up phase (3 mg, 3 times daily for 5 days and then 5 mg, 3 times daily for 5 days) followed by a stable dose phase (8 mg, 3 times daily until day 50–52). Preceding and during the entire study period, patients were asked to continue taking their own medications (including analgesics) according prescription. Contrary to our hypothesis, THC did not show a beneficial effect on chronic abdominal pain compared

with placebo (F(1, 46) =.016; *P*=.901). Between the start and end of the study, VAS mean scores decreased by 1.6 points (40%) in the THC group compared to 1.9 points (37%) in the placebo group. Additionally, no differences were observed in secondary efficacy outcomes such as quality of life, appetite level and pharmacodynamic parameters. Oral THC was generally well absorbed resulting in reliable pharmacokinetic curves. Seven patients administrating THC discontinued study treatment due to adverse events compared with two patients in the placebo group. All (possibly) related adverse events were mild or moderate.
We demonstrated that THC, administered 3 times daily during a 50-day treatment period, was safe and well-tolerated, however, did not relief pain. A large placebo response was present in this study, a finding that is in concordance with other chronic visceral pain studies. We advised designing future studies in a more optimal way, avoiding these large placebo effects.

Chapter 6 provides an overview of clinical trials that have been conducted to investigate the analgesic efficacy of various cannabis-based products with standardized THC content for chronic non-malignant pain. The majority of these trials reported improvement in pain scores in favour of products containing ∆9-THC (dronabinol). However, analgesic effects were generally weak and placebo effects were considerable in the control arms. Common limitations of these trials, that potentially bias treatment outcomes, are discussed in this review.
Underlying pain mechanisms, including plasticity of nociceptive and cognitive pain processing, may explain the varying analgesic effects of dronabinol in particular chronic pain states. To date, regulatory authorities assess the therapeutic potential of new analgesics based primarily on the patient's subjective pain experience. We discussed a mechanism-based approach beyond the measurement of subjective pain relief for future research, to evaluate the therapeutic potential of dronabinol in chronic pain management.

PART III: Neuronal mechanisms of tetrahydrocannabinol
In the first part of this thesis, we concluded that chronic abdominal pain can be to some extent attributed to maladaptive mechanisms of neural plasticity underlying chronic pain. In the second part, we could not observe clinical efficacy of oral THC and recommended a mechanism-based approach to evaluate the anti-nociceptive effects of THC in chronic pain management. In the studies reported in the last part of this thesis, we investigated the underlying pain processing mechanisms of THC by evaluating pain related neuroplastic changes in spontaneous and evoked EEG as described in **chapter 2** and **3**.

Chapter 7 addressed the potential anti-nociceptive effects of THC by investigating underlying pain related cortical activity in the crossover study reported in **chapter 4**. We investigated whether a single dose of orally administrated THC alters 1) the resting state EEG and 2) EPs to pain related electrical stimuli in patients with chronic pancreatic pain. A concentric electrode delivering electrical stimuli at the lower arm was used, which activates mainly nociceptive A-delta fibers and produces a pinprick-like pain sensation. Both EEG measurements were consecutively conducted predose and time-locked at 1:10, 2:10, 3:10, and 5:05 hours after administration of study medication. We primarily demonstrated a good test-retest reliability of all resting state alpha EEG indices in CP patients. The intra-subject variability of evoked EEG parameters showed fair to good agreement, supporting the use of these EEG parameters for further research. However, we could not detect THC-related effects on alpha indices of the resting state EEG nor on EPs to pain related electrical stimuli in chronic pancreatitis patients. VAS scores of electrical transcutaneous nociceptive stimulation maintained stable over time, indicating that acute evoked pain was also not affected by THC. These results are in line with the overall subjective experience of pancreatic pain patients, in whom no analgesic effect of THC was observed (**chapter 4**). Furthermore, individual changes in subjective pain scores were correlated with EEG indices to assess whether EEG changes were linked to underlying analgesic responses and not caused by confounding factors such as sedation and other adverse effects. A significant negative correlation was found between change in VAS pain scores and change in peak alpha power, indicating that individual treatment response is associated with enlarged peak power amplitude. Further analysis of subjective measures of individual clinical pain scores in relation to objective measures of EEG parameters at baseline did not reveal any correlation.
Cortical correlates of THC were further elucidated and reported in **chapter 8.** We explored the effects of THC after a 50-day treatment period on pain related EEG indices in patients with chronic abdominal pain. We demonstrated that clinical pain severity was associated with slowing of brain oscillations in a resting state, as well as with enhanced N1 and P3 amplitudes elicited by noxious electrical stimulation. Correlations between clinical pain intensities and objective EEG outcomes suggest that chronic pain alters central pain processing recorded by EEG. Utilizing these maladaptive EEG outcome parameters in chronic pain, THC did not affect spontaneous brain activity nor evoked pain processing compared to placebo after 50 days of treatment. Solely in postsurgical pain patients, a delay in N1 latency was observed for THC treatment compared to placebo. Moreover, cortical correlates of THC could not be associated with its analgesic efficacy, and thus, did not reflect individual treatment responses.

The main findings of this thesis are discussed with respect to recent literature in **Chapter 9**. Part of this general discussion is a mechanism-oriented approach to pain in chronic pancreatitis. Recommendations are addressed for future research and clinical practice. Future clinical studies should optimize study designs to adequately handle large placebo responses and choose advanced diagnostic tools to apply a systematic approach to chronic pain. The presence and pattern of altered central pain processing for specific chronic pain disorders in individual patients needs further investigation. Additionally, methods for reliably identifying these mechanisms in individual patients and treatments that target these mechanisms are required.

SAMENVATTING

Pijn is een onplezierige, sensorische en emotionele ervaring, die geassocieerd is met actuele of potentiële weefselschade. Acute pijn heeft een belangrijke beschermingsfunctie voor ons lichaam en verdwijnt normaal gesproken zodra de weefselschade hersteld is. Acute pijn kan door onverklaarde redenen blijven bestaan en overgaan in chronische pijn. Chronische pijn is een persisterend, multifactorieel gezondheidsprobleem en staat onder invloed van lichamelijke, psychische en sociale factoren. Ongeveer 19% van de Europese volwassen bevolking kent een periode van matige tot ernstige chronische pijn.

Hoewel de oorzaak van chronische pijn nog onduidelijk is, lijkt centrale sensitisatie een belangrijke rol te spelen in het ontstaan en aanhouden van persisterende pijnklachten. Centrale sensitisatie gaat gepaard met een toegenomen respons van nociceptieve neuronen in het centrale zenuwstelsel op normale afferente input of zelfs door afferente input onder het drempelniveau van de neuron. Hierdoor is pijn niet langer gekoppeld aan de aanwezigheid, intensiteit of duur van een specifieke perifere stimulus, maar is pijn het gevolg van neuronale veranderingen in het centrale zenuwstelsel. Pijn werkt dan niet langer als effectief alarmsignaal, maar is heviger dan te verwachten of ontstaat spontaan. Wanneer centrale sensitisatie is opgetreden wordt de behandeling en genezing van de pijnklachten lastiger. Huidige pijnbehandelingen schieten vaak te kort of zijn niet geschikt voor langdurige toepassing. Cannabinoiden, waaronder tetrahydrocannabinol (THC), bieden een potentiele nieuwe toegang voor de behandeling van chronische pijn.

De doelstelling van dit proefschrift was om de onderliggende maladaptieve neuronale veranderingen van chronische pijn te onderzoeken, alsmede de pijnstillende en anti-nociceptieve eigenschappen van orale THC te evalueren.

De studies beschreven in dit proefschrift concentreerden zich rondom drie onderwerpen:

Deel I neuroplastische veranderingen in hersenactiviteit geassocieerd met chronische (postoperatieve) pijn onderzocht door analyse van het spontaan elektro-encefalogram (rustEEG) en opgewekte potentialen (evoked potentials (EP's)).

Deel II therapeutisch potentieel van een nieuwe orale tablet bestaande uit pure THC voor de behandeling van chronische buikpijn.

Deel III anti-nociceptieve effecten van THC onderzocht door middel van rust EEG en pijn gerelateerde EP's.

DEEL I: Corticale verwerking van pijnlijke stimuli in patiënten met chronische (postoperatieve) pijn

Vrouwen die een borstkankeroperatie moeten ondergaan, hebben een hoog risico op het ontwikkelen van chronische pijn. In **hoofdstuk 2**, hebben we de verwerking van pijnlijke stimuli in de hersenen van patiënten met persisterende pijnklachten na een borstkanker behandeling onderzocht door gebruik te maken van EP's in het EEG. Negentien patiënten (8 vrouwen met pijn, 11 zonder pijn), die meer dan één jaar geleden zijn behandeld voor borstkanker middels een borstbesparende operatie (lumpectomie) of volledige borstamputatie (mastectomie), inclusief volledige okselklierdissectie en/ of chemoradiatie, werden vergeleken met elf gezonde vrouwen van ongeveer dezelfde leeftijd. Veranderingen in corticale verwerking op pijnlijke elektrische stimuli werden in het EEG geregistreerd door middel van pijn gerelateerde EP's. De aanwezigheid van chronische pijn was gerelateerd aan een vertraagde en versterkte stimulus verwerking, wat zich uitte als een verhoogde latentietijd en verhoogde amplitude van de positieve piek tussen 250-310 ms (P260) van de EP. In vergelijking met gezonde vrijwilligers liet de gehele groep borstkankerpatiënten echter een vervroegde P260 piek en tendens naar kleinere P260 amplitude zien.

Deze resultaten suggereren dat de twee condities, borstkankerbehandeling en chronische pijn, tegenovergestelde effecten laten zien in corticale respons. De belangrijkste conclusie van deze studie is dat chronische pijn na borstkankerbehandeling geassocieerd is met neuroplastische veranderingen in corticale activiteit, zichtbaar als een vertraagde en verstrekte stimulus respons.

Veranderingen in spontaan EEG zijn in een aantal chronische pijn populaties reeds geobserveerd, wat suggereert dat chronische pijn veranderingen in de centrale pijnverwerking met zich meebrengt door neuroplasticiteit van het centraal zenuwstelsel. Deze hypothese was echter nog nooit onderzocht voor chronische buikpijn. In **hoofdstuk 3** hebben wij het rust EEG van patiënten met chronische buikpijn als gevolg van chronische pancreatitis vergeleken met vergelijkbare gezonde proefpersonen. Hiervoor werd een Fast Fourier Transformatie (FFT) toegepast op het EEG signaal, wat verdere analyse in het frequentiespectrum mogelijk maakt. Wij observeerden in patiënten een verschuiving van het EEG naar lagere piek alfa frequenties. Chronische pancreatitis pijn bleek dus geassocieerd met een vertraging van de spontane hersenactiviteit. Er werden geen significante verschillen gevonden in piek alfa amplitude tussen chronische pancreatitis patiënten en gezonde proefpersonen. De verschuiving in piek alfa frequentie was gecorreleerd met de duur van de pijn, waarbij langdurigere pijn grotere verschuiving liet zien, wat suggereert dat piek alfa frequentie een potentiële biomarker is voor ziekteprogressie van chronische pijn.

Deze bevindingen helpen ons de onderliggende pathologie van chronische pijn te begrijpen, aanvullende diagnoses te stellen en de optimale behandeling te kiezen. Daarnaast biedt het EEG een alternatieve methode om de effectiviteit van nieuwe analgetica te onderzoeken in patiënten met chronische pijn.

DEEL II: Effectiviteit en veiligheid van THC in chronische buikpijn

Het tweede deel van dit proefschrift concentreert zich op de therapeutische toepassing van een nieuwe tablet, bestaande uit pure THC geïsoleerd uit de *cannabis sativa* plant, voor de behandeling van chronische buikpijn. We hebben twee fase 2 geneesmiddelenstudies uitgevoerd om de klinische effectiviteit, farmacokinetiek, farmacodynamiek, farmacogenetica, veiligheid en pijn gerelateerde neuroplastische veranderingen in spontaan en opgewekt EEG van orale THC te onderzoeken. De resultaten van een gerandomiseerde, dubbelblinde, placebo gecontroleerde studie met een eenmalige dosering THC in patiënten met buikpijn als gevolg van chronische pancreatitis zijn beschreven in **hoofdstuk 4**. We vonden dat een eenmalige dosering orale THC niet effectief is in het reduceren van chronische pancreatitis pijn in vergelijking tot de actieve controle diazepam. De THC werd geabsorbeerd met een gemiddelde tijd tot maximale plasmaconcentratie van 123 minuten, wat vergelijkbaar was tussen opiaat gebruikers en niet opiaat gebruikers. De absorptie van THC was in een aantal chronische pancreatitis patiënten vertraagd, wat resulteerde in een verhoogde variabiliteit tussen patiënten in vergelijking met gezonde proefpersonen. Dit is meest waarschijnlijk het gevolg van de onderliggende pathologie en het gebruik van comedicatie. Wij concludeerden dat orale THC betrouwbare farmacokinetische profielen liet zien en goed getolereerd werd met alleen milde tot matige bijwerkingen. De lange termijn effecten van THC tijdens een behandeling van 50-52 dagen werden verder onderzocht in een tweede studie. De resultaten van deze gerandomiseerde, dubbelblinde, placebo gecontroleerde studie, naar de effectiviteit, farmacokinetiek en tolerantie van orale THC in patiënten met chronische buikpijn, zijn beschreven in **hoofdstuk 5**. Vijfenzestig patiënten met chronische buikpijn als gevolg van chirurgie of chronische pancreatitis werden *at random* in twee groepen verdeeld om THC tabletten of overeenkomstige placebo tabletten te krijgen. Proefpersonen in de THC groep begonnen met een opstapfase (3 mg, 3 maal per dag voor 5 dagen en dan 5 mg, 3 maal per dag voor 5 dagen) gevolgd door een stabiele doseringsfase (8 mg, 3 maal per dag tot dag 50–52). Patiënten werden tijdens de gehele studieperiode gevraagd hun bestaande medicatie (inclusief pijnstillers) volgens voorschrift te blijven gebruiken. In tegenstelling tot onze hypothese liet THC geen voordeliger effect zien op chronische buikpijn in vergelijking met placebo. Tussen start en einde van de studie gingen pijnscores (visual analoge scale (VAS)) in de THC groep met 1.6 punten (40%) omlaag in vergelijking met 1.9 punten (37%) in de placebo

groep. Daarnaast werden er geen verschillen gevonden in secundaire uitkomstmaten zoals kwaliteit van leven, eetlust en farmacodynamische parameters. Orale THC werd over het algemeen goed geabsorbeerd resulterend in adequate farmacokinetische plasma concentratie profielen. Zeven patiënten in de THC groep hebben voortijdig de studiebehandeling beëindigd wegens bijwerkingen in vergelijking met twee patiënten in de placebo groep. Alle (mogelijk) gerelateerde bijwerkingen waren mild tot matig van aard.
Wij hebben in deze studie kunnen aantonen dat THC, bij 3 maal daags gebruik voor een behandelperiode van 50-52 dagen, veilig was en goed getolereerd werd, maar geen pijnstillende werking heeft. Een aanzienlijk placebo effect werd waargenomen, wat frequenter geobserveerd wordt in studies met chronische (buik)pijn patiënten. Wij adviseerden toekomstige studies alternatieve studiedesigns te kiezen om dit placebo effect te reduceren.

Hoofdstuk 6 geeft een overzicht van klinische studies die gedaan zijn naar de effectiviteit van diverse op cannabis gebaseerde producten met gestandaardiseerde THC inhoud voor chronische niet kanker gerelateerde pijn. Het merendeel van deze studies rapporteert een verbetering in pijnscores bij producten van THC (dronabinol). De analgetische werking was echter zwak en er werden substantiële placebo effecten gevonden. De zwakke kanten van deze studies, welke mogelijk de behandeluitkomsten hebben beïnvloed, worden in deze review besproken.
Onderliggende pijnmechanismen, waaronder plasticiteit van nociceptieve en cognitieve pijnverwerking, verklaren mogelijk de variatie in analgetisch effect van THC in specifieke chronische pijn condities. Tot op heden beoordelen regelgevende instanties het therapeutisch potentieel van nieuwe analgetica voornamelijk op basis van de subjectieve pijnervaring van de patiënt. Wij bediscussieerden een op mechanismen gebaseerde aanpak die verder gaat dan het meten van subjectieve pijnverlichting, om voor toekomstig onderzoek het therapeutisch potentieel van THC bij chronische pijn te evalueren.

DEEL III: Neuronale mechanismen van THC
In het eerste deel van dit proefschrift concludeerden we dat chronische buikpijn in zekere mate kan worden toegeschreven aan maladaptieve mechanismen van neurale plasticiteit, die ten grondslag liggen aan chronische pijn. In het tweede deel konden we de klinische werkzaamheid van orale THC niet observeren en werd een op mechanisme gebaseerde aanpak aanbevolen om de anti-nociceptieve effecten van THC bij chronische pijn te evalueren. In de studies die in het laatste deel van dit proefschrift zijn beschreven, hebben we de onderliggende pijnverwerkingsmechanismen van THC onderzocht door

pijn gerelateerde neuroplastische veranderingen in het spontane en opgewekte EEG te evalueren, zoals beschreven in **hoofdstuk 2** en **3**.

Hoofdstuk 7 ging in op de mogelijke anti-nociceptieve effecten van THC door de onderliggende pijn gerelateerde corticale activiteit te onderzoeken in de cross-over studie gerapporteerd in **hoofdstuk 4**. We onderzochten of een enkele dosis oraal toegediende THC effect heeft op de rusttoestand van het EEG en EP's op pijnlijke elektrische stimuli bij patiënten met chronische pancreatitis. Er werd een concentrische elektrode gebruikt, die voornamelijk nociceptieve A-delta vezels activeert middels elektrische stimuli op de onderarm en hierbij een naaldachtige pijnsensatie produceert. Beide EEG metingen werden achtereenvolgens uitgevoerd voor inname van studiemedicatie en op 1:10, 2:10, 3:10 en 5:05 uur na toediening van de studiemedicatie. Allereerst hebben we een goede test-hertest betrouwbaarheid aangetoond van alfa indices in het rust EEG in chronische pancreatitis patiënten. De intra-individuele variabiliteit van de EP componenten op baseline vertoonde een redelijk tot goede overeenkomst en ondersteunde het gebruik van deze EEG parameters voor verder onderzoek. We konden echter geen THC gerelateerde effecten op alfa indices van het rust EEG of EP's detecteren op pijn gerelateerde elektrische stimuli. Pijnscores van de elektrische transcutane nociceptieve stimulatie bleven stabiel in de tijd, wat aangeeft dat acute opgewekte pijn ook niet door THC werd beïnvloed. Deze resultaten komen overeen met de algehele subjectieve ervaring van patiënten met pancreaspijn, bij wie geen analgetisch effect van THC werd waargenomen (**hoofdstuk 4**). Bovendien werden individuele veranderingen in subjectieve pijnscores gecorreleerd aan EEG parameters om te beoordelen of individuele veranderingen in het EEG verband hielden met onderliggende pijnstillende effecten en niet werden veroorzaakt door verstorende factoren zoals sedatie of andere onbedoelde effecten. Een significante negatieve correlatie werd gevonden tussen de verandering in pijnscores en verandering in piek alfa amplitude, wat aangeeft dat individuele behandelingsrespons geassocieerd is met een verhoogde alfa activiteit. Verdere analyse van individuele subjectieve pijnscores op baseline in relatie tot objectieve metingen van EEG parameters leverde geen correlaties op.

Corticale correlaten van THC werden verder onderzocht en gerapporteerd in **hoofdstuk 8**. We onderzochten de effecten van THC na een behandelperiode van 50-52 dagen op pijn gerelateerde EEG indices bij patiënten met chronische buikpijn. We toonden aan dat de klinische pijnscore geassocieerd was met vertraagde hersenoscillaties in rusttoestand, evenals met versterkte N1- en P3-amplitudes opgewekt door nociceptieve elektrische stimulatie. Deze correlaties tussen klinische pijnintensiteit en objectieve EEG uitkomsten suggereren dat chronische pijn de centrale pijnverwerking beïnvloedt, wat

door middel van EEG kan worden geregistreerd. Gebruikmakend van deze maladaptieve EEG uitkomstparameters bij chronische pijn, had THC na 50-52 dagen behandeling geen invloed op de spontane hersenactiviteit noch op de pijnverwerking. Alleen bij patiënten met postoperatieve pijn werd een vertraging in N1-latentie waargenomen voor THC behandeling in vergelijking met de placebo. Bovendien konden corticale correlaten van THC niet geassocieerd worden met de analgetische werkzaamheid ervan en weerspiegelden ze dus niet het individuele behandelingseffect.

De belangrijkste bevindingen van dit proefschrift worden besproken met betrekking tot recente literatuur in **hoofdstuk 9**. Een deel van deze algemene discussie is een mechanisme-georiënteerde benadering van pijn bij chronische pancreatitis. Aanbevelingen zijn gericht op toekomstig onderzoek en de klinische praktijk. Toekomstige klinische studies zouden studiedesigns moeten optimaliseren om adequaat om te gaan met potentiële placebo effecten en geavanceerde diagnostische hulpmiddelen kiezen om een systematische aanpak op chronische pijn toe te passen. De aanwezigheid en het patroon van maladaptieve centrale pijnverwerking voor specifieke chronische pijnstoornissen moet verder worden onderzocht. Daarnaast zijn methoden voor het identificeren van deze maladaptieve mechanismen bij individuele patiënten en behandelingen die op deze mechanismen zijn gericht essentieel voor een succesvolle behandeling van chronische pijn.

Appendices

Dankwoord

Het is alweer 8 jaar geleden dat ik met dit onderzoeksproject begonnen ben en ik heb er altijd met veel plezier aan gewerkt. De veelzijdigheid van het project, zowel inhoudelijk als projectmatig, lag mij goed, maar ook de mensen om mij heen hebben hier een belangrijke rol in gespeeld. Ik wil graag iedereen bedanken die op eigen wijze een bijdrage heeft geleverd aan de totstandkoming van mijn proefschrift. Een aantal mensen wil ik graag in bijzonder bedanken.

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Prof. dr. Vissers, beste Kris, dank voor de prettige en constructieve overleggen. In het begin bespraken we hoofdzakelijk projectgerelateerde onderwerpen, maar je kende m'n interesse in de organisatie en nam me daar later geregeld in mee. Dank voor het positieve meedenken, de razendsnelle correcties en het bewaken van mijn doelen.

Dr. Wilder-Smith, beste Oliver, je was als pijnexpert en bedreven wetenschapper nauw betrokken bij dit project. Jouw visie op chronische pijn heeft een belangrijke stempel gedrukt op de studies binnen dit proefschrift. Dank dat je altijd de tijd voor me nam en me nooit zonder oplossing weer liet vertrekken. Ik waardeer je oprechte interesse voor de persoon en de wetenschap.

Leden van de manuscriptcommissie: prof. dr. Burger, prof. dr. Roozendaal en prof. dr. Huijgen, hartelijk dank voor het beoordelen van mijn proefschrift op zijn wetenschappelijke inhoud.

Graag wil ik alle gezonde proefpersonen en patiënten bedanken voor hun deelname en inzet. In totaal hebben 157 proefpersonen minimaal één, maar meestal diverse bezoeken gebracht aan het Radboudumc voor mijn onderzoek. Veel dank daarvoor!

Het zaadje van mijn wetenschappelijke interesse voor neurofysiologisch pijnonderzoek werd gepland tijdens mijn afstudeerstage op de afdeling anesthesiologie, pijn en

palliatieve geneeskunde. Hans en Emanuel, wat een voorrecht om 9 maanden met jullie een kamer te mogen delen. Ik heb ontzettend genoten van de inhoudelijke discussies, foute grappen en wederzijdse betrokkenheid. Helemaal tof dat we vrienden zijn geworden en nog altijd bij elkaar terecht kunnen voor een goed advies (oplossing: er moet altijd meer gedronken worden). Vanaf heden is het dr. Mooi voor jullie.

Beste collega's van het Donders Instituut, afdeling biologische psychologie, ik heb me als vreemd eendje altijd erg welkom gevoeld. De congressen naar Krakau waren iedere keer weer fantastisch. Dr. Tineke van Rijn, dank voor je tijd en moeite om je in de EEG analyse te verdiepen. Prof. dr. Gilles van Luijtelaar, dank dat je in mijn corona wilt zitten. De samenwerking wordt voortgezet met dr. Joukje Oosterman. Zeg Joukje, wanneer gaan wij naar Krakau?

Diverse afdelingen zijn betrokken geweest in de uitvoer van de geneesmiddelen studies. In bijzonder wil ik Simone Hins-de Bree, Jackie van Gemert en Hettie Maters van het CRCN bedanken voor hun ondersteuning en het GCP-proof maken van onze studies. Ook zonder onze consortiumpartners, Echo Pharmaceuticals en afdeling Geriatrie van het Radboudumc, hadden we dit project niet kunnen uitvoeren. Het ging niet altijd makkelijk, maar met vallen en opstaan hebben we toch een prachtig resultaat neergezet. Emanuel, Luuk en Dagmar, dank voor jullie directe inzet op dit project. Helaas bleek het voor beperkte periodes, maar je bent nooit te laat om je ambities bij te stellen.

Beste onderzoekers van de afdeling Heelkunde, ik heb ondertussen heel wat collegae onderzoekers voorbij zien komen, maar de goede sfeer in de groep blijft een constante factor. Het begon op kamer 3.47, waar meestal hard gewerkt werd, maar op z'n tijd ook een hoop onzin verkondigd werd. Tjarda, je hebt het maar zwaar met ons te stellen gehad (en nog steeds wil je een kamer met me delen). Chema, Dagmar, Willem en Sander, leuk dat we af en toe nog onderzoeksherinneringen oprakelen, want goede imitaties kunnen oneindig herhaald worden.

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About the Author

Marjan de Vries was born August 28th 1983 in Havelte, The Netherlands. After high school in 2002 (CSG Dingstede, Meppel), she decided to move to Nijmegen to study physical therapy at HAN University of Applied Sciences. She became particularly interested in neurophysiology of pain and did her first scientific work on collateral involvement of sensory nerves in patients with peripheral facial paralysis (dr. Carien Beurskens at Radboud University Medical Center). After graduation in 2006, she started working as physiotherapist, but she struggled with the limited evidence available in clinical practice. She went travelling to South Africa and did voluntary work as physiotherapist in HIV clinics in Durban. In 2007, she started Biomedical Sciences at Radboud University Nijmegen and focused on clinical human movement sciences. She obtained her master's degree in 2010 after a major internship on neurophysiology of pain under supervision of dr. Emanuel van den Broeke (Department of Anaesthesiology, Pain and Palliative Medicine at Radboud University Medical Center and Donders Institute for Brain, Cognition and Behaviour).

In the fall of 2010, prof. dr. Harry van Goor asked her for a research position on a European funded project aimed to perform phase II clinical drug studies with cannabis based tablets (Department of Surgery at Radboud University Medical Center). This research project offered ample opportunities to develop herself in a broad field of pharmacology, neuroscience and (experimental) pain. Additionally, she became familiar with the comprehensive laws and regulations applicable to studies with non-registered medicines, and assumed the role of project manager. In 2014, the Department of Surgery offered her a position as research manager, in which she can expand and share her knowledge and experience in all aspects of research up till now.

Marjan is living together with her husband Martijn Duinkerke and their daughter Benthe.

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Radboud Institute for Health Sciences
PhD Portfolio

Institute for Health Sciences
Radboudumc

Name PhD candidate:	M. de Vries	**PhD period:**	16-10-2010 – 21-11-2018
Department:	Surgery	**Promotor(s):**	Prof. dr. H. van Goor
Graduate School:	Radboud Institute for Health Sciences		Prof. dr. K.C.P. Vissers
		Co-promotor(s):	Dr. O.H.G. Wilder-Smith

	Year(s)	ECTS
TRAINING ACTIVITIES		
a) Courses & Workshops		
• Basiscursus Regelgeving en Organisatie van Klinische trials (BROK)	2011/2015	1.75
• Introduction course for PhD students	2013	1.75
• Academic Writing	2013	3.0
• Scientific Integrity	2014	0.4
b) Symposia & congresses		
• NEURONUS, IBRO & IRUN Neuroscience, Krakow, poster presentation	2011	1.5
• European Federation of IASP Chapters (EFIC), Hamburg, poster presentation	2011	1.5
• International Association for the Study of Pain (IASP), Milan, poster presentation	2012	1.5
• The 11th IASP Research Symposium Brain and Pain, Arnhem	2013	0.5
• NEURONUS, IBRO & IRUN Neuroscience, Krakow, oral presentation	2013	1.5
• International Association for the Study of Pain (IASP), Buenos Aires, poster presentation	2014	1.5
• NEURONUS, IBRO & IRUN Neuroscience, Krakow, oral presentation	2014	1.5
• Patient-Reported Outcomes in Surgery Conference, Washington	2015	0.75
• 5th International Congress of The Special Interest Group on Neuropathic Pain (NeuPSIG), Nice	2015	0.75
• International Association for the Study of Pain (IASP), Yokohama, poster presentation	2016	1.5
• International Association for the Study of Pain (IASP), Boston	2018	1.0
c) Other		
• Reviewer scientific papers, Journal of Pain Research	2014 -	0.5
• Reviewer scientific papers, Pain Practice	2018 -	0.1
TEACHING ACTIVITIES		
d) Lecturing/ education		
• Research internship (OMB2), Medicine and Biomedical Sciences	2011-2014	1.5
• Writing a research proposal (OMB4), Biomedical Sciences	2015-2016	0.5
• Meet the PhD, Biomedical Sciences	2016-2017	0.4
• Scientific project, Medicine and Biomedical Sciences	2016-2017	0.5
e) Supervision of internships		
• Supervisor research internships, Master Medicine, Radboud University. Students: Steef van den Broek, Marjolein Rol, Josianne Luijten, Jitse Harder, Daan Wolbrink, Ilse van Langeveld, Brigit Wrapstra, Charley Baars, Floris Heutink, Maikel Immens.	2012-2017	10.0
• Supervisor master thesis, Pharmacology, University of Utrecht. Thesis: The pharmacokinetic Profile of THC in Chronic Pancreatitis. student: Sana Hamad	2013-2014	2.0
• Supervisor Honours program, Radboud University. Thesis: Influence of Exercise on Visceral Pain. Student: Laura van Weerdenburg	2014-2015	1.0
• Supervisor Honours program, Radboud University. Thesis: An improved method for the recording of pinprick-evoked brain potentials. Student: Bart de Vries	2015-2016	1.0
• Supervisor master thesis, master arts-klinisch Onderzoeker (A-KO), Maastricht University. Thesis: Virtual Reality's effects on evoked potentials and pain scores in healthy volunteers: a randomized cross-over study. Student Roman Assmann.	2016-2017	2.0
TOTAL		**27.0**

Radboud University Nijmegen Medical Centre

Stellingen behorende bij het proefschrift:

Tetrahydrocannabinol in Chronic Pain
Cortical Mechanisms of Pain and Analgesia

Marjan de Vries

1. Chronische pijn na een borstkankerbehandeling leidt tot een versterkte maar vertraagde verwerking van pijnlijke stimuli in het centraal zenuwstelsel. *Dit proefschrift*

2. Chronische pancreatitis pijn leidt tot een vertraagde hersenactiviteit in rust. *Dit proefschrift*

3. Het pijnstillend effect van THC bij chronische pancreatitis patiënten berust op een placebo respons. *Dit proefschrift*

4. Er is onvoldoende goed bewijs van effectiviteit van cannabis 'medicatie' op chronische pijn. *Dit proefschrift*

5. THC in een orale tablet geeft een betrouwbaar farmacokinetische profiel en is veilig in gebruik voor patiënten met chronische pijn. *Dit proefschrift*

6. Het grote placebo effect van de 'wietpil' in een trial bij patiënten met chronische buikpijn wordt veroorzaakt door de hoge verwachtingen die deze patiënten hebben van deze pil. *Dit proefschrift*

7. EEG is een betrouwbare methode om de gevolgen van centrale sensitisatie in patiëntengroepen met chronische pijn te detecteren. *Dit proefschrift*

8. Wetenschappers zouden moeten worden beloond om negatieve resultaten open access te publiceren.

9. *'Dokter. Feit dat hij komt is halve genezing.' Herman Pieter de Boer, schrijvend kunstenaar*

10. Een investering in kennis betaalt zich terug met de hoogste rente. *Benjamin Franklin, 1750, Poor Richard's Almanac*