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# Wavelet analysis of single-sweep pharmaco-EEG: beta-band activity correlate to the analgesic effect of buprenorphine

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## Abstract

Pain is a widely present condition, with as many as 19 % of the European population suffering from chronic pain. In many cases pain is treated with opioids, but since little is known about the underlying mechanisms of opioid treatment, further studies are warranted.

This randomized, cross-over and double-blinded study included 15 healthy subjects in order to investigate the effects of buprenorphine administered through a transdermal patch. During treatment, measurements were made for blood plasma concentrations, occurrence of adverse effects and pain assessments. Evoked brain potentials (EPs) were recorded using electrical stimulation at the median nerve.

Features were extracted from the EPs using Continuous Wavelet Transform to detect the latency and amplitude of the most dominant waveforms within four frequency bands (delta, theta, alpha and beta). Features were log-transformed and baseline corrected before analysis using two-way repeated measures analysis of variance (ANOVA). Afterwards, features which exhibited significant differences compared to placebo treatment, were correlated with the clinical scores.

Significant differences between buprenorphine and placebo treatment were found for all amplitude features in all frequency bands, but not for latency (ANOVA). Correlation was found between the beta band feature and bone pain scores ( $P = 0.008$ ) as well as the plasma concentrations ( $P = 0.02$ ).

This study showed that features found in the EEG reflect the analgesic effect of buprenorphine. This discovery might be useful in clinical drug trials to monitor the analgesic effect.

**Keywords:** Pain, Buprenorphine, Electroencephalography, Continuous wavelet transform, Analgesic effect, Feature extraction, Pharmaco-EEG

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## 1. Introduction

Pain is widely present, with 19 % of the European and 25 - 30 % of the population in the USA population suffering from chronic pain [1, 2, 3]. Currently, pain treatment is based on the three-step ladder developed by the World Health Organization (WHO), which includes opioid treatment for moderate to severe pain [4]. Buprenorphine has been used for pain treatment for over 30 years utilizing various methods of administration. Interest in the drug has increased recently after delivery through a transdermal patch became possible, ensuring stable plasma concentrations, and increased patient compliance [5, 6].

This is partly due to the fact that the opioid-receptors affected by buprenorphine and its metabolite norbuprenorphine may be important in the treatment of bone-associated

pain which is difficult to treat in clinical practice. A previous study has found that buprenorphine provided a better analgesic effect with respect to bone-associated pain compared to another opioid, fentanyl [6]. Therefore, buprenorphine administered through a transdermal patch is a valid approach for the treatment of patients with persistent pain [6].

Pharmacological-Electroencephalography (EEG) using EPs has been proven as a viable tool for analyzing the analgesic effects of different drugs [7]. However it is important to make sure that differences found in the EEG are relevant for the study, and not caused by a general effect of the drug. Therefore any differences found in the EEG should correlate to the analgesic effect. Otherwise, the differences might describe another effect of the drug such as sedation, instead of the analgesic effect [7].

Previous EEG studies have analyzed the spectral energy of the signals using time-frequency methods. The

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most basic method is the short-time fourier transform, which analyses the signal through small windows. More recently the wavelet transform in which the signal is analyzed by compressing and extending a mother wavelet has become more popular. The wavelet transform provides a better time-frequency resolution than the short-time fourier transform, and studies have determined it to be superior for signal analysis of EEG [8, 9].

EP studies generally stimulate multiple times. This is done because the EP signal is relatively small compared to the background EEG activity [10]. During the averaging process, the background EEG cancels out, while the EPs synchronized to the stimulus become larger and the EP components become clear. However, this method has drawbacks as it only effectively preserves components of the EP that are phase-locked. Studies have shown that nociceptive input to the brain originating from the C-fibers are generally not phase-locked, and therefore removed in the averaging process [10, 11]. Therefore single-sweep analysis of the EP is preferable in order not to remove important data from the recording before analysis.

We hypothesized that buprenorphine induces changes in the EEG and that these changes can be correlated to the analgesic effect. The aims of this study were then **a)** to utilize the wavelet transform to find features in the Pharmacology-EEG of single-sweep EPs that exhibit differences in buprenorphine treatment compared to placebo and **b)** investigate if these differences correlate to the analgesic effects.

## 2. Materials and Methods

This randomized, cross-over and double-blind study was carried out at the research laboratories at Mech-Sense, Aalborg Hospital, Denmark. The protocol was approved by the local ethics committee (N-20070061) and the Danish Medicines Agency (EducaCT number: 2007-004524-21), and the study was carried out in accordance with the principles of Good Clinical Practice of the European Union.

### *Study design*

Twentytwo healthy male subjects (age:  $23.1 \pm 3.8$  years) without long-lasting pain complaints or lesions at the testing sites were included. In addition, routine medical examinations and blood samples were normal. Before inclusion, all subjects gave informed consent.

Each treatment was administered over 7 days with a 3 days follow-up. Subjects were hospitalized during the 7

days, in case of adverse effects. The treatment was administered through a transdermal patch, to achieve a stable release of the drug. The patches were administered by a nurse or pharmacist not otherwise involved in the project. Treatments were administered in random order, and both the subject and the investigator had no knowledge as to which treatment was being administered.

The treatment periods lasted 144 hours. During treatment 3 types of measurements were made at regular intervals. Blood plasma, EPs and pain measurements were performed, before administration of the transdermal patch as well as 24, 48, 72 and 144 hours after.

Adverse effects were reported by the subject as well throughout the treatment period.

### *Heat stimulation*

For the heat stimulation, an area of  $9 \text{ cm}^2$ , 10 cm proximal to the wrist of the right volar forearm, was heated using a "Thermo Tester" (TSA II NeuroSensory analyser, Medoc Ltd, Ramat Yishai, Israel).

The temperature was gradually increased from a baseline of  $32 \text{ }^\circ\text{C}$  at a rate of  $1 \frac{^\circ\text{C}}{\text{s}}$  to a maximum temperature of  $52 \text{ }^\circ\text{C}$ . The subjects were instructed to press a button when the heat tolerance threshold (HTT) was reached.

Three successive stimulations were performed, and the average was calculated.

### *Bone pressure stimulation*

Bone pressure stimulation was applied to a marked area on the right tibialis 15 cm below the patella. Since the site was marked, it was possible to stimulate the same area for all measurements.

Pressure stimulation was applied using a hand-held algometer (Type 2, Somedic Production AB, Sollentuna, Sweden) using a probe size of 2 mm in diameter. The pressure was gradually increased with a rate of  $30 \frac{\text{kPa}}{\text{s}}$ .

The subjects were instructed to press a button when the pressure tolerance threshold (PTT) was reached.

### *Adverse effects*

At each pain stimulation subjects were asked to report 4 of the most common adverse effects (nausea, drowsiness, dizziness and local irritation due to the patch). The Adverse effects were rated on a 4-point scale: nothing (1), light feeling (2), moderate feeling (3) or intolerable feeling (4).

### Evoked potentials

Electrical stimulation was performed using two bipolar electrodes (Neuroline 720, REF: 72001-K/12, Ambu a/s, Denmark). The electrodes were placed on the left volar forearm over the median nerve, 2 cm distal to the wrist with an inter-electrode distance of 1 cm.

The stimulation was controlled by a constant-current stimulator (Isolator Stimulator Noxi IES 230, JNI Biomedical, Klarup, Denmark).

The duration of each stimulation was 2 ms at the pain detection threshold (PDT).

EEG recordings were sampled at 1000 Hz at the Cz electrode (NuAmp, Neuroscan, El Paso, TX, USA).

Two identical recordings of 60 sweeps were performed at every recording time.

### Pre-processing

Data was filtered using a notch filter with cut-off frequencies at 49 and 51. Data was epoched from 50 ms before until 500 ms after each stimulus. Baseline correction and linear detrending were then applied to the epochs.

The data were cleaned manually to remove artifacts in the EEG by deleting the 5 worst sweeps from each recording, resulting in a total of 55 sweeps. Since 2 recordings were made at each time, 110 sweeps in total were accepted for each recording time.

To avoid the stimulation artifact, data were only analyzed from 25 ms after stimulation onset.

In order to make the data comparable between treatments, scaling was performed on the data by multiplication with a scaling factor. The two baseline measurements were used to determine the scaling factor by calculating the peak-to-peak amplitude of the average EP between 75 ms and 315 ms after stimulation onset, since it was determined by visual inspection that the main peaks in the signals occurred within this timeframe. The scaling factor was then calculated to give both baseline signals a peak-to-peak value of one. Afterwards all signals were scaled by the same amount as the corresponding baseline.

### Wavelet feature extraction

The Continuous Wavelet Transform (CWT) was implemented to find the most pronounced components in the EP within the different frequency bands; delta (0.5 - 4 Hz), theta (4 - 8 Hz), alpha (8 - 12 Hz), and beta (12 - 32 Hz). The objective was to find the amplitude and the latency of the largest component within each band. The basic principle of the method is shown in figure 1.

Based on the frequency bands, the scales used for analysis should cover 0.5 - 32 Hz. Using the center frequency of the wavelet scales were calculated to match these frequencies with a chosen interval of 0.5 Hz [12].

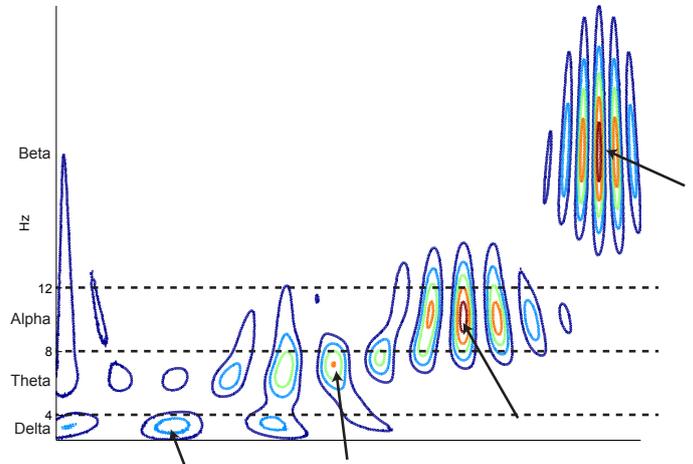


Figure 1: The principle in of the implemented wavelet features using a plot of generated data. The time-frequency contour plot is divided into the frequency bands by lines. Within each band the dominant component is identified with an arrow. The amplitude and latency of these components are extracted as features.

Each EP was decomposed using the CWT with a morlet wavelet for the calculated scales, resulting in a set of wavelet coefficients for each scale. The scales were then divided into the four different frequency bands and the maximum absolute wavelet coefficient was found within each band. This coefficient marks the most dominant waveform within that band and the absolute value was recorded as the amplitude (denoted as e.g.  $A_{Delta}$ ) with a corresponding latency (denoted as e.g.  $L_{Delta}$ ).

The features from each recording time were transformed using the natural logarithm and then averaged to give a single mean value for each recording time. Furthermore, features for each treatment were baseline corrected, by subtracting the baseline value from the following time points. This resulted in values that reflect the absolute difference between the baseline value and each subsequent recording.

### Statistical analysis and correlation with clinical scores

The baseline corrected wavelet features were analysed using two-way repeated measures analysis of variance (ANOVA), with time and treatment as factors. Features which exhibited statistically significant differences between treatment with placebo and buprenorphine were checked for correlation to the clinical scores. Correlation was performed between each clinical score and features

from buprenorphine treatment using the z-score and Pearson's linear correlation.  $P$ -value below 0.05 indicated statistical significance.

For correlation with the plasma concentration, the values obtained 24 hours after drug administration was excluded from the correlation. This is done to account for the delay before the analgesic effect of the drug will be present.

Since the features of interest were pre-hoc defined, adjustments for mass significance were not performed as to not discard important findings due to type II errors, which is a common problem using e.g. the Bonferroni correction [13].

### 3. Results

The study was completed for 15 out of 22 subjects. One left the study due to a job offer distant from the site, and another was hospitalized due to reasons unrelated to the study. Two subjects were excluded based on poor data quality of the EPs while 3 subjects were excluded due to several missing measurements caused by adverse effects preventing them to participate in the experiment.

Two subjects had few missing measurements (Subject 3: 24 hours and subject 6: 24 and 48 hours). The pain scores from these patients were interpolated from the other measurements, while the extracted features from these missing measurements were removed from the analysis.

An example of the recorded EPs are shown in figure 2 to illustrate difference between buprenorphine and placebo treatment.

Feature	Treatment	Time	Interaction
$A_{Delta}$	0.34	<b>0.03</b>	0.19
$A_{Theta}$	-	-	<b>0.03</b>
$A_{Alpha}$	-	-	<b>&lt;0.001</b>
$A_{Beta}$	-	-	<b>0.02</b>
$L_{Delta}$	0.33	0.06	0.33
$L_{Theta}$	0.82	0.63	0.99
$L_{Alpha}$	0.31	0.15	0.51
$L_{Beta}$	0.80	0.83	0.79

Table 1: Overview of the  $P$ -values obtained with two-way repeated measures ANOVA, for the different extracted features.  $P$ -values which are statistically significant are written in bold.

Each feature was analysed using two-way repeated measures ANOVA, and the results are summarized in table 1, which shows the  $P$ -values obtained from each

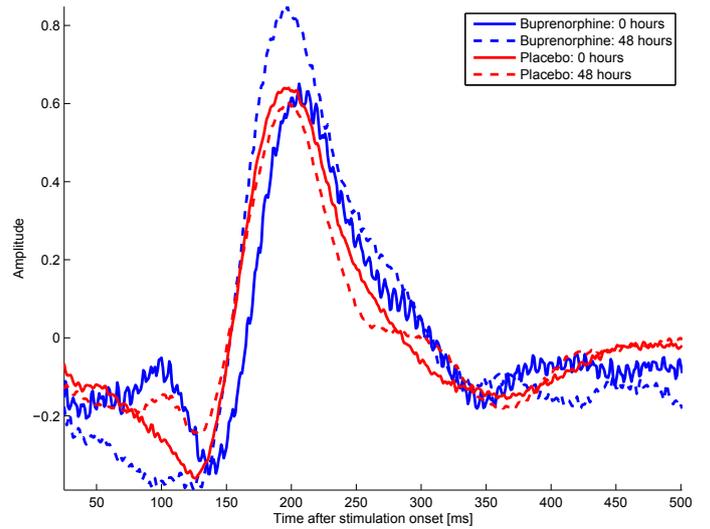


Figure 2: Plot showing evoked brain potentials from one representative subject. Evoked brain potentials are plotted for both placebo and buprenorphine treatment, before administration and 48 hours after, where the plasma concentrations are highest.

test, representing differences between treatments, recording times and if there is interaction between the two factors.

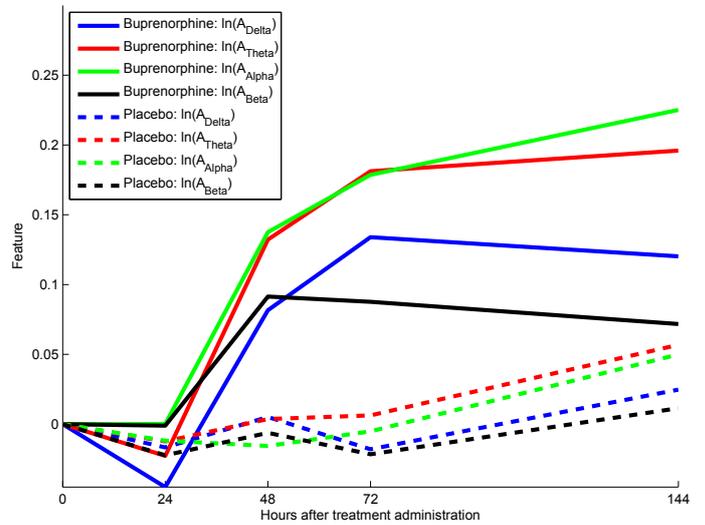


Figure 3: Plot of the development of amplitude features over time for all frequency bands.

Latency features showed no signs of significant differences (all  $P > 0.05$ , see table 1). Features for amplitude however, showed several significant changes. Significant differences were seen in the delta band between times, but not between treatments or interaction between treatment and time. Theta, alpha, and beta bands all showed a significant interaction between the recording time and treat-

ment. The development over time for the amplitude features is shown in figure 3. The amplitude features were all checked for correlations with the clinical scores and the results are shown in table 2.

Clinical scores	$A_{Delta}$	$A_{Theta}$	$A_{Alpha}$	$A_{Beta}$
Bone pain	0.79	0.81	0.77	<b>0.96</b>
Heat pain	0.59	0.66	0.67	0.84
Electrical pain	0.19	0.33	0.41	0.42
Adverse effects	0.02	0.06	0.04	0.33
Plasma concentration	0.83*	0.81*	0.74*	<b>0.98*</b>

Table 2: Correlations between the wavelet amplitude features for every frequency band and clinical scores. Statistically significant ( $P < 0.05$ ) correlations are marked in bold. \*: Note that a measurement (24 hours after treatment) has been removed before correlation. This is done to account for the delay before analgesic effect is present.

The correlations in table 2 show a significant relationship between the features for the beta band and the bone pain scores. Figure 4 shows the development over time for this feature, as well as the clinical scores.

#### 4. Discussion

This study investigated the pharmaco-EEG at the single sweep level before and during treatment with placebo or buprenorphine administered through a transdermal patch. It was found that treatment with buprenorphine caused an increase in beta-band activity (12 - 32 Hz) which correlated to the subjective scores for bone-associated pain ( $P = 0.008$ ) as well as the measured plasma concentrations of buprenorphine in the blood stream ( $P = 0.02$ ).

It is worth noting that single sweep analysis could be an important tool in the EEG analysis, since it prevents the loss of data due to the averaging process [11]. This seems important for this study, since the changes found in the beta band are high-frequency and might have been removed in the averaging process.

The stimulation was performed at the subjective PDT for each recording. This could be a problem for the results, since the observed differences in the EEG could be caused by increased stimulation current instead of the analgesic effect. However, had the stimulation been performed using a fixed current, the subject might not have found the stimuli to be painful during treatment with buprenorphine. It brings further credibility to the results that no features correlated with the electrical stimulation current. Had a significant correlation been present it would be likely that the differences in EEG were due

to the increased stimulation intensity, rather than the analgesic effect.

Before correlation with the plasma concentrations were performed, the values from 24 hours after treatment initiation was removed. This was done to account for the delay before the analgesic effect is present. This effect can be seen in figure 4B, where the plasma concentrations rise during the first 24 hours of treatment. However this is not the case for  $A_{Beta}$  or the bone-associated pain scores. The correction therefore seems reasonable since the plasma concentration 24 hours after treatment initiation does not reflect in the subjective scores for bone-associated pain.

In this study the plasma concentrations of buprenorphine in the blood was investigated. Norbuprenorphine, the metabolite of buprenorphine also has an analgesic effect and therefore might also affect the EEG [6]. It is possible that norbuprenorphine is affecting the EEG at lower frequencies, causing the low-frequency features to not correlate with the plasma concentrations. However since this is not reflected in subjective pain scores this effect is of less interest.

The study used only the Cz electrode for analysis. It is possible that the analgesic effect is reflected better at other electrodes. Other studies have used a more frontal electrode (Fz), since the depth-of-anesthesia is measured based on the EEG of frontal electrodes [14].

Analysis of EEG using CWT is common [7]. However features from CWT are usually the spectral indices, which reflect the overall energy within each frequency band. This study implemented a new feature for finding the latency and amplitude of the most dominant waveform in each frequency band. This approach can be vulnerable, since the feature is based on a single value from the entire wavelet decomposition, as opposed to all values for the spectral indices. It is possible that the spectral indices are better features for this application, since the latencies did not show differences between treatments. Therefore, the method should be tested against established methods such as the spectral indices.

The results from this study are reasonable since previous studies have determined buprenorphine to be especially effective against bone-associated pain [6]. Previous pharmaco-EEG studies have found the analgesic effect of pregabalin to cause a slowing of the EEG oscillations. This matches the results of this study where the activity in the low-frequency bands rise more than in the high-frequency bands (see figure 3) [7]. These findings might be used in clinical trials to monitor the analgesic effect of the analgetic [7, 15].

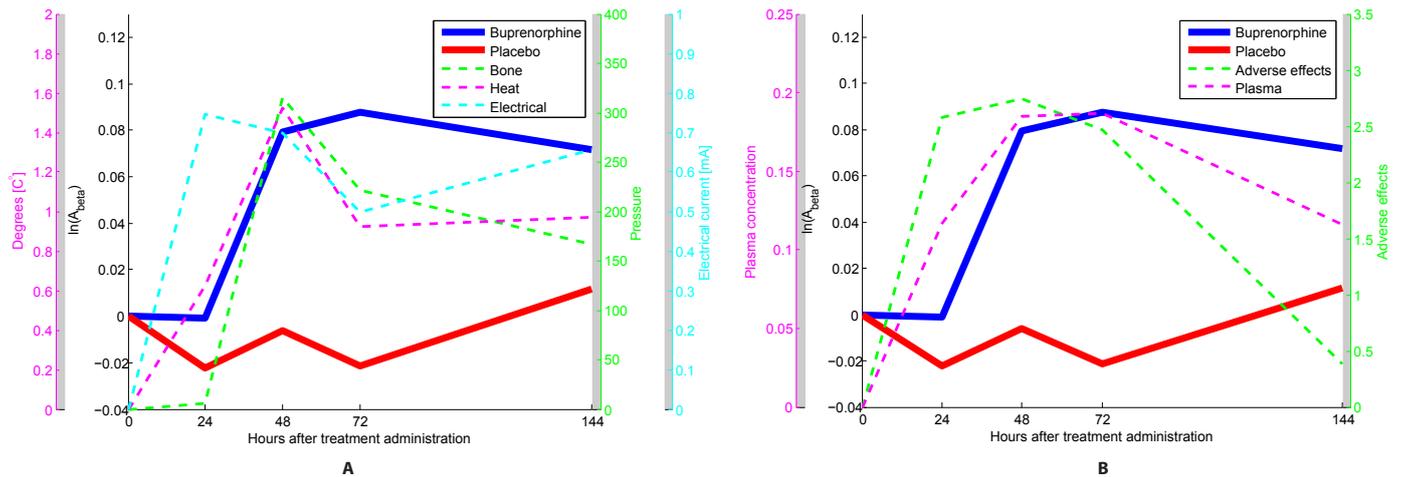


Figure 4: Figure showing the development of the  $A_{\beta}$  feature and clinical scores over time on separate scales to ease comparison. A: Graph of  $A_{\beta}$  and the subjective pain scores. B: Graph of  $A_{\beta}$  and the adverse effects and plasma concentrations.

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