A novel approach to pharmac-EEG for investigating analgesics: assessment of spectral indices in single-sweep evoked brain potentials

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Buprenorphine and fentanyl are both strong opioids with different pharmacological profiles.
- Electroencephalography can be used to assess the analgesic effect of drugs.
- Many different methods exist for analysis of electroencephalography, but little is known about the actual benefits of advanced methods.

WHAT THIS STUDY ADDS

- Single-sweep analysis of brain evoked potentials was sensitive to the effect of opioids.
- The analgesic effect of buprenorphine is reflected in the beta band.
- The results indicate that electroencephalographic 'fingerprinting' can be used to characterize the brain's response to analgesics.

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AIMS

To compare results from analysis of averaged and single-sweep evoked brain potentials (EPs) by visual inspection and spectral analysis in order to identify an objective measure for the analgesic effect of buprenorphine and fentanyl.

METHODS

Twenty-two healthy males were included in a randomized study to assess the changes in EPs after 110 sweeps of painful electrical stimulation to the median nerve following treatment with buprenorphine, fentanyl or placebo patches. Bone pressure, cutaneous heat and electrical pain ratings were assessed. EPs and pain assessments were obtained before drug administration, 24, 48, 72 and 144 h after beginning of treatment. Features from EPs were extracted by three different approaches: (i) visual inspection of amplitude and latency of the main peaks in the average EPs, (ii) spectral distribution of the average EPs and (iii) spectral distribution of the EPs from *single-sweeps*.

RESULTS

Visual inspection revealed no difference between active treatments and placebo (all $P > 0.05$). Spectral distribution of the averaged potentials showed a decrease in the beta (12–32 Hz) band for fentanyl ($P = 0.036$), which however did not correlate with pain ratings. Spectral distribution in the single-sweep EPs revealed significant increases in the theta, alpha and beta bands for buprenorphine (all $P < 0.05$) as well as theta band increase for fentanyl ($P = 0.05$). For buprenorphine, beta band activity correlated with bone pressure and cutaneous heat pain (both $P = 0.04$, $r = 0.90$).

CONCLUSION

In conclusion single-sweep spectral band analysis increases the information on the response of the brain to opioids and may be used to identify the response to analgesics.

Introduction

Opioids constitute the most potent analgesics currently known and are the prevailing drugs for treatment of moderate to severe chronic pain [1, 2]. However, the analgesic effect of opioids are influenced by genetic and environmental factors which makes the effect different between individuals [3–5]. This could explain why 40% of chronic pain patients are dissatisfied with their treatment [6]. Commonly the analgesic effect is assessed using subjective measures such as the visual analogue scale, but to date no robust objective measure has been presented for assessment of the analgesic effect of opioids [7].

Pharmaco-electroencephalography (EEG) recorded as evoked brain potentials (EPs) has been proven as a viable tool for analyzing changes in cortical activity following administration of different analgesics [2]. However, it is important to ensure that differences found in the EPs reflect the underlying analgesic mechanisms and are not caused by confounding factors such as sedation and other adverse effects. Therefore, alterations in the EPs should be correlated with the analgesic effect *per se* [8, 9].

During recordings of EPs, the typical approach is to apply multiple stimuli and calculate a signal average to improve the signal:noise ratio [10, 11]. During the averaging process, the background noise cancels out, while the potentials synchronized to the stimulus become larger and even smaller EP components become clear. Analysis of EPs has traditionally been carried out using either visual or automatic detection of amplitudes and latencies of the main peaks in the averaged EPs [9, 12, 13]. Another way of analyzing EPs is to compute the frequency content of the signals using time-frequency methods. Recently the wavelet transform has become popular for this purpose due to superior time-frequency resolution compared with, for example, the short time Fourier transform [14, 15].

Analysis of averaged EPs, however, has drawbacks as only components of the EP that are phase-locked (e.g. components that occur at the same time in relation to stimulus) are effectively preserved. Not all nociceptive inputs to the brain are phase-locked and therefore information is removed in the averaging process [11, 12, 16, 17]. Therefore, single-sweep analysis of the EP could be preferable despite the increased computational costs, in order to analyze detailed information. Single-sweep analysis has been used in analysis of diabetes patients and healthy volunteers where the use of single-sweep analysis allowed for classification between the groups [18]. However, the method has so far not been applied for analysis of pharmaco-EEG.

We hypothesized that advanced analysis of pharmaco-EEG recorded after opioid administration would enable extraction of characteristic properties (referred to as features) reflecting differences in the EPs that correlate with the analgesic effect. Hence, during treatment with two

strong opioids the aims of the present study were (i) to compare results from analysis of averaged and single-sweep EPs by visual inspection and spectral analysis and (ii) to identify an objective measure for the analgesic effect of opioids.

Methods

Study subjects

Twenty-two opioid-naïve healthy male subjects (age 23.1 ± 3.8 years) without long lasting pain complaints or lesions at the testing sites were included. In addition, objective examination, electrocardiography, blood pressure, pulse and respiration, routine blood and urine samples were all normal. Before inclusion, all subjects gave written informed consent.

Study design

This randomized, double-blind and placebo controlled three-armed cross-over study was carried out at the research laboratories at Mech-Sense, Aalborg University Hospital, Denmark. The protocol was approved by the local ethics committee (N-20070061) and the Danish Medicines Agency (EduraCT number: 2007–004524-21), and the study was carried out in accordance with the principles of Good Clinical Practice of the European Union. Data from the psychophysical assessment and the pharmacokinetics/pharmacodynamics have been reported previously [19, 20].

Each subject had a transdermal patch applied containing buprenorphine, fentanyl or placebo. Two patches were applied in each period in order to ensure blinding as the buprenorphine and fentanyl patches were not identical. Furthermore, the fentanyl patch was only effective for 3 days, whereas the buprenorphine patch was effective for 7 days [19]. A buprenorphine or an identical placebo patch was applied on the right shoulder and a fentanyl or an identical placebo patch was applied on the left shoulder. The patches were applied by a nurse or a pharmacist who was not otherwise involved in the study. The patch on the left shoulder was removed after 72 h and the patch on the right shoulder was removed after 144 h. Buprenorphine $20 \mu\text{g h}^{-1}$ (transdermal patch, Norspan® 144 h, Norpharma, Denmark) and fentanyl $25 \mu\text{g h}^{-1}$ (transdermal patch, Durogesic® 72 h, Hospital Pharmacy, North-Jutland, Denmark) were used in the study as they are considered equipotent [21].

Each treatment arm consisted of 7 days of treatment with a follow-up of 3 days and each treatment arm was separated by a washout period of at least 10 days. The subjects were hospitalized during the treatment period in case of adverse effects. Assessments of pain and EPs were performed prior to patch placement (0 h) and 24, 48, 72 and 144 h after patch placement. However, for fentanyl the recordings for 144 h were not used for analysis since the

patch was removed 72 h post-treatment. Only pain assessments that could be quantified (cutaneous heat, electrical pain and bone pain) and have been proven reliable over time were included. The pain assessments were performed in the same order every time with 3–5 min between tests.

Heat pain assessment

Heat pain assessments were performed on an area of 9 cm², 10 cm proximal to the wrist of the right volar forearm, using a 'Thermo Tester' (TSA II NeuroSensory analyzer, Medoc Ltd, Ramat Yishai, Israel). The temperature was gradually increased from a baseline of 32°C at a rate 1°C s⁻¹ to a maximum temperature of 52°C. The subjects were instructed to press a button when heat pain tolerance threshold (PTT) was reached. Three successive assessments were performed and the average was calculated. This pain assessment paradigm is based on the Peltier principle and has proven reliable and reproducible over time [22].

Bone pressure assessment

Bone pressure assessments were performed to a marked area on the right tibialis 15 cm below the patella. Since the site was marked, it was possible to apply pressure the same area for all assessments. Pressure was applied using a hand-held algometer (Type 2, Somedic Production AB, Solentuna, Sweden) using a probe size of 2 mm in diameter. The pressure was gradually increased with a rate of 30 kPa s⁻¹. The subjects were instructed to press a button when the pressure PTT was reached. This type of pressure mimics the bone pain evoked from the periosteum and has been shown to be reproducible [23].

Electrical stimulation

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. A computer-controlled constant current stimulator (Isolator Stimulator Noxi IES 230, JNI Biomedical, Klarup, Denmark) was used to deliver the stimulus with a duration of 2 ms at the electrical pain detection threshold (PDT). Stimulations were performed with an inter-stimulus interval of 5 s. Cutaneous electrical stimulation has been shown to be reproducible [24].

EP recordings

EP signals were recorded using vertex tin electrodes and amplified digitally (NuAmp, Neuroscan, Compumedics, Hamburg, Germany). One electrode was placed on the left ear lobe and served as reference, another was located at Cz (international 10–20 system) and a third electrode was placed 2 cm frontal to the Cz electrode, serving as ground. To reduce the impedance to below 5 kOhm the electrodes were mounted using electro gel. The Cz electrode was

chosen since it provides a reliable measure of the overall cortical neural processing and furthermore has been used in various previous drug studies, which makes comparisons more valid [25].

The recordings took place in a quiet room with dimmed light with all unnecessary equipment turned off to avoid artefacts and noise. Two identical recordings of 60 sweeps were performed at every recording time with an inter-stimulus interval of 5 s. Instructions and recordings were performed by a medical doctor and qualified research nurses, and patients were instructed to rest in a supine position with eyes open during recordings.

Pre-processing

The overall approach to the data analysis is presented in Figure 1.

The EPs were recorded in AC mode with sampling rate of 1000 Hz (Neuroscan software v 4.3, Compumedics, El Paso, Texas, USA) and processed in the following steps: (i) zero-phaseshift notch filtering (49–51 Hz) using a finite impulse-response filter with a slope of 24 dB/octave, (ii) zero-phaseshift band-pass filtering (1–100 Hz) using a finite impulse-response filter with a slope of 12 dB/octave, (iii) epoching in the time window 50 pre-stimulus to 500 ms post-stimulus, (iv) linear detrending, (v) baseline correction, (vi) rejecting artefact sweeps manually and (vii) calculating average of accepted sweeps. The data were cleaned manually to remove artefacts in the EPs by deleting the five worst sweeps from each recording, resulting in a total of 55 sweeps. Since two recordings were made at each time, 110 sweeps in total were accepted for each recording time. For the average analysis, all 110 sweeps were used to generate one single EP for each subject at each recording instance.

In order to make the data comparable between treatments, scaling was performed by multiplication with a scaling factor. This scaling was performed for each individual by calculating the peak-to-peak amplitude of the average baseline EP in the time frame containing the main peak (75 to 315 ms after stimulation onset). The scaling factor was calculated independently for each treatment arm as the factor resulting in a peak-to-peak value of 1 at baseline, and this factor was multiplied to all EPs for this specific subject and treatment. By this procedure, all subjects were scaled to have the same baseline amplitude to make results comparable over subjects and the changes in signal level during post-treatment recordings were still preserved [25].

Latencies and amplitudes

Peak amplitudes and latencies were determined by blinded visual inspection of averaged signals. The identified peaks were N1, P1, N2 and P2 (Figure 2). The peak-to-peak amplitudes were calculated by subtraction of the

Figure 1

Flowchart of the analysis method for the study. All evoked brain potentials were pre-processed in the same way and then subjected to three different methods for analysis. The results from each method were examined in a similar fashion in order to determine which of the methods provided the best performance

determined amplitudes, yielding three peak-to-peak amplitudes (PP_{N1P1} , PP_{P1N2} , PP_{N2P2}) and four latencies (L_{N1} , L_{P1} , L_{N2} , L_{P2}).

Spectral analysis of averaged EPs

The spectral indices were calculated in a similar fashion as previous studies to obtain a measure of the distribution of power within the individual frequency bands [8]. The continuous wavelet transform was applied to the EPs from 25 to 500 ms after stimulation onset in order to estimate the spectral indices in the standardized frequency bands for both the average and single-sweep potentials. This procedure was performed by squaring the time-frequency coefficients to obtain the power coefficients followed by integration over the entire time interval of the epoch and the following frequency limits: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz) and beta (12–32 Hz).

The complex Morlet wavelet was chosen in order to obtain optimal time-frequency resolution, and defined by a bandwidth of 2 Hz, a centre frequency of 1 Hz and a between-scale frequency resolution of 0.5 Hz [26].

Spectral analysis of single-sweep EPs

The spectral indices were also investigated at single-sweep level, using the same method as used for the averaged EP. This yielded individual spectral indices for each single sweep EP, which were then averaged to yield a single value for each recording time.

Baseline correction and normalization

In order to assess the altered response in the central nervous system, changes in spectral indices were calculated as the relative alteration in percentage from baseline. Hence, values for each time point were obtained by first

Figure 2

Grand means of evoked brain potentials at baseline and 24, 48, 72, and 144 h post-treatment for (A) buprenorphine, (B) placebo and (C) fentanyl treatment. Identified peak points N1, P1, N2 and P2 are depicted for each treatment. —, 0 h; —, 24 h; —, 48 h; —, 72 h; —, 144 h

subtracting the baseline absolute power from the absolute power at the actual time point, followed by division of the baseline absolute power.

Validation of results from single-sweep analysis

In the average procedure the spontaneous EEG activity which is not phase-locked to the stimuli, cancel out, and hence this type of EP represents only the response to the stimuli. On the contrary, single-sweep EPs is a sum of the spontaneous EEG activity and the neural response to the present stimuli. Hence, to validate that the observed changes in the single-sweeps are not a reflection of the altered spontaneous EEG, the spontaneous EEG has to be analyzed separately. This can be achieved by analyzing the signal segments between the EPs in the same fashion as the single-sweep analysis.

The between stimuli epochs were extracted from the EEG traces from 4000 ms to 50 ms prior to electrical stimulus. These epochs were decomposed by the same continuous wavelet transform and averaged. These spectral indices were analyzed for differences between drugs and placebo and any indices exhibiting significant differences were tested for correlation with pain assessments. These results will indicate whether the information found is specific to the EPs or is an effect in the spontaneous EEG which also resides in the EPs.

Statistical analysis

For each feature, both drugs were compared with placebo using two-way repeated measures analysis of variance (ANOVA), with the time of recording as within-subject factor (baseline, 24 h, 48 h, 72 h and 144 h) and treatment (placebo or drug) as between-subject factor. Fentanyl was only compared with placebo within the first 72 h of treatment as the fentanyl patch lasted only 72 h. Features which exhibited statistically significant differences between treatments were examined for correlations with pain scores. Correlation was performed between each type of stimuli and features from buprenorphine or fentanyl treatment using two-tailed Pearson's linear correlation. Features were examined for correlation with the electrical stimulation current in order to ensure that differences were not caused by an altered stimulation current. Fentanyl was not checked for correlation with bone pain, since a previous study using the same data has demonstrated that fentanyl did not have a significant analgesic effect on bone pain [19].

Since the features of interest were *pre-hoc* defined, adjustments for mass significance were not performed to avoid discarding important findings due to type II errors [27]. A *P* value below 0.05 indicated statistical significance.

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 discarded based on poor data quality of the EPs while three subjects were excluded due to several missing measurements caused by adverse effects preventing them participating in the experiment.

Two subjects had a few missing recordings, both at 24 h and one at 48 h post-buprenorphine treatment. The pain scores from these patients were interpolated from the adjacent measurements.

The routine medical examinations and blood samples were normal for all subjects.

A previous study established that buprenorphine attenuated bone and heat pain, whereas fentanyl only attenuated heat pain. Neither opioid had effect with regards to electrical pain [19].

Grand mean plots illustrating the average EP of all included subjects are shown in Figure 2.

Latencies and amplitudes

No significant differences between buprenorphine and placebo (all $F < 1.9$, all $P > 0.1$) or fentanyl and placebo (all $F < 2.5$, all $P > 0.05$) were found by visual assessment of latencies and peak-to-peak amplitudes of the averaged EPs.

Spectral difference plots

Spectral difference plots are shown in Figure 3 and 4 to visualize differences between average and single-sweep analysis as well as differences between treatments.

The averaged signals for each subject were decomposed into the time-frequency domain followed by a subtraction of the corresponding baseline recording, to obtain a visualization of the spectral differences as illustrated in Figure 3. The same procedure was repeated using single-sweep analysis and the results are shown on Figure 4. Since Figures 3 and 4 are shown using the same colour scale, it is possible to compare the results. The two figures show that the averaging procedure attenuates the spectral differences.

Spectral analysis of averaged EPs

The spectral analysis investigated differences in frequency distribution in order to evaluate more accurately the effect of the opioids on the EEG. The values from the spectral analysis of averaged EPs are shown in Table 1 along with 95% confidence intervals.

A significant difference was found between buprenorphine and placebo treatment in the theta band for the time factor. This therefore does not reflect a difference between treatments. No differences were significant in the delta, alpha and beta bands. Between fentanyl and placebo a significant decrease was found between treatments in the beta band. Otherwise, no significant differences were found for the delta, theta and alpha bands.

Significant differences found in the frequency bands were examined for correlations with heat and electrical pain. No correlations were found for any frequency bands (all $P > 0.05$).

Spectral analysis of single-sweep EPs

To investigate the EP frequency content more accurately, spectral analysis was performed using single-sweep EPs. Figure 5 illustrates the variability of the single-sweep EPs that is lost due to the averaging procedure.

Spectral indices extracted during single-sweep analysis illustrate the development over time as depicted in Figure 6. It is evident that power generally increased in the EPs due to treatment. Fentanyl rapidly increases EP power and then peaks at 72 h, where the patch is removed. For buprenorphine the increase happens more gradually and is more widespread in the frequency spectrum.

The increase in EPs was more distinct for low frequency bands, indicating that the distribution of power moved towards the low frequency spectrum. The values from the spectral analysis of single-sweep EPs are shown in Table 2 along with 95% confidence intervals.

Significant differences for time vs. treatment interaction between buprenorphine and placebo treatment was found for theta ($F = 5.1$, $P = 0.002$); alpha ($F = 5.6$, $P < 0.001$) and beta ($F = 3.0$, $P = 0.03$). There was no significant difference for the delta band (all $F < 2.0$, $P > 0.1$).

Significant differences between fentanyl and placebo were seen in the theta ($F = 2.9$, $P = 0.05$) and alpha ($F = 3.3$, $P = 0.03$) bands. However, for the alpha band the difference was found for the time factor only and, therefore, does not indicate differences between treatments.

The frequency bands that showed significant differences between the treatment arm and the placebo arm were examined at a group level for correlations with bone, heat and electrical pain. The results are summarized in Table 3.

For buprenorphine the beta band correlated with bone ($r = 0.9$; $P = 0.04$) and heat pain ($R = 0.9$; $P = 0.04$). Figure 7 illustrates the development over time for the correlated values. No correlations with pain ratings were found for other frequency bands. Neither buprenorphine nor fentanyl features correlated with the electrical stimulation current.

To validate the results from single-sweep EP analysis, the EEG segments between stimulations were also processed and analyzed in the same fashion for differences compared with placebo treatment. Significant increases were found in the theta ($F = 4.0$, $P = 0.006$) and alpha ($F = 4.4$, $P = 0.004$) bands for buprenorphine as well as theta ($F = 3.6$, $P = 0.022$) and alpha ($F = 4.5$, $P = 0.008$) bands for fentanyl. All other bands exhibited insignificant differences (all $F < 2.8$ and $P > 0.1$).

The theta and alpha bands were tested for correlation with pain assessments for both drug treatments but none correlated at a significant level (all $P > 0.1$).

Figure 3

Spectral difference plots of averaged evoked brain potentials (EPs). Each column represents the treatment and each row represents the recording time. The difference in frequency spectrums between the recording time and the baseline recording are shown. Red colours indicate increased activity, blue decreased activity and green no change in activity. The plots reflect the nature of the treatments; no major changes occur during placebo treatment. Fentanyl induces a decrease in low frequency activity in the early treatment period and then increases subsequently. Buprenorphine induces increased low frequency activity throughout the treatment. Please note that the colour scale in this figure and Figure 4 is similar, to allow for better assessment of the differences in the two methods

Discussion

In this study, we compared three different approaches to pharmaco-EEG analysis ranging from visual detection of peaks, spectral analysis of averaged EPs to spectral analysis of single-sweep EPs. By extracting detailed information from the single-sweep EPs, it was possible to demonstrate significant increases in the theta, alpha, and beta band

during buprenorphine treatment, and correlate the finding in the beta band with bone ($P = 0.04$) and heat pain ($P = 0.04$). For treatment with fentanyl an increase was seen in the theta band, which did not correlate with pain scores.

Methodological considerations

The traditional method for visual detection of amplitudes and latencies showed no difference between

Figure 4

Spectral difference plot for analysis of single-sweep evoked brain potentials (EPs). The plots reflect the nature of the treatments; no major changes occur during placebo treatment. Fentanyl induces a decrease in activity after just 24 h treatment and then increases subsequently, peaking at 72 h where the treatment ends. Buprenorphine induces increased activity from 48 h after treatment, peaking at 144 h

buprenorphine, fentanyl and placebo treatment. Furthermore, this method has some potential drawbacks as the visual detection of peaks is based on a subjective identification and studies using this method has been known to produce conflicting results [9, 11, 12, 28].

A more advanced method is spectral analysis of averaged EPs which has been used traditionally due to the poor signal:noise ratio of single-sweep EPs [17]. Using continuous wavelet transform on the averaged EPs we were able to demonstrate significant differences in the beta band for fentanyl, but no correlations with the

analgesic effect were found. However, this method 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 [11, 12, 29]. Furthermore, increased or decreased phase-locking of EP components will affect the amplitude of the averaged EP [16]. This might explain why the averaged EPs during fentanyl treatment showed significantly decreased activity which was not the case for single-sweep EPs. This suggests that the finding represents decreased phase-locking of beta

Table 1

Baseline corrected values from the evoked potential *average* analysis. Values are displayed as the average percentage of change from baseline with 95% confidence interval in square brackets

Feature	24 h	48 h	72 h	144 h
Buprenorphine				
Delta	-18.9 [-104.0, 66.1]	-1.9 [-123.9, 120.1]	20.5 [-30.3, 71.4]	15.2 [-75.3, 105.8]
Theta	-1.2 [-86.3, 83.8]	38.2 [-83.8, 160.2]	52.0 [1.1, 102.8]	54.5 [-36.0, 145.1]
Alpha	12.2 [-72.9, 97.2]	36.1 [-85.9, 158.1]	33.3 [-17.6, 84.1]	52.8 [-37.8, 143.3]
Beta	-4.4 [-89.4, 80.6]	12.0 [-110.0, 134.0]	-6.2 [-57.1, 44.6]	10.7 [-79.9, 101.2]
Fentanyl				
Delta	2.5 [-82.5, 87.5]	9.1 [-112.9, 132.1]	12.7 [-38.1, 63.5]	49.1 [-41.5, 139.6]
Theta	-22.9 [-107.9, 62.1]	-7.2 [-129.2, 114.8]	6.3 [-44.5, 57.2]	23.4 [-67.1, 114.0]
Alpha	-24.0 [-109.0, 61.0]	7.6 [-114.4, 129.6]	11.9 [-39.0, 62.7]	22.7 [-67.9, 113.2]
Beta*	-21.8 [-106.8, 63.3]	-14.2 [-136.2, 107.8]	-17.5 [-68.4, 33.3]	-1.9 [-92.4, 88.7]
Placebo				
Delta	13.3 [-86.3, 113.0]	18.8 [-73.3, 110.9]	10.2 [-60.9, 81.3]	11.7 [-81.1, 104.6]
Theta	12.6 [-87.0, 112.2]	17.5 [-74.6, 109.6]	11.4 [-59.7, 82.5]	27.0 [-65.8, 119.8]
Alpha	2.9 [-96.7, 102.5]	5.2 [-86.8, 97.3]	-0.1 [-71.2, 71.0]	20.9 [-71.9, 113.7]
Beta	7.8 [-91.9, 107.4]	18.4 [-73.7, 110.5]	-2.4 [-73.5, 68.7]	23.2 [-69.6, 116.0]

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

Figure 5

An example of an average potential and 30 of the single-sweeps from the same recording to illustrate the variability in amplitude and latency of the single-sweeps. —, average; —, single-sweeps

waves induced by fentanyl as opposed to differences in amplitude *per se*.

The averaging procedure was developed in order to detect only the EP itself by suppressing background EEG activity. However, there is much evidence to suggest that the averaging procedure removes important information from the signals [16, 17]. These findings are supported by this study, where we demonstrated significant power increases in more frequency bands using single-sweep analysis than average analysis. More importantly, the beta band correlated with bone and heat pain, and hence the analgesic effect. A possible pitfall when using single-

sweep analysis is the lack of background EEG suppression, making it hard to distinguish between the EP and background EEG. Validation analysis was carried out on the EEG segments between stimulations in order to investigate whether the altered spectral content was caused by the spontaneous EEG or as a response to the painful stimuli. In the analysis of the between stimuli segments differences were found in some bands, which also exhibited differences in single-sweep EP analysis, thus supporting the notion that both segments contain complimentary information. However, the beta band did not demonstrate differences for the between stimuli segments and neither did the other frequency bands correlate with any pain scores. Therefore the information extracted by single-sweep analysis representing the analgesic effect seems to reside only in the EP and is not the result of unsuppressed background EEG activity.

The study was carried out using experimental pain models and quantitative sensory tests on healthy volunteers. Clinically, determination of the appropriate drug is guided by the symptoms in the clinical settings. However, often other factors of the disease such as depression and co-medication act as confounders in these studies [30]. To minimize these confounders, experimental pain models can be beneficial since they allow the investigator to control the painful stimulus accurately [30, 31]. This study identified a possible biomarker for the analgesic effect of buprenorphine, which could be implemented in an algorithm for detection of analgesic efficacy. However, further studies are needed to investigate the behaviour of the biomarker in actual patients, where the disease might also influence the EEG.

The nociceptive input is carried by A δ - and C-fibres. However, the EPs represent nociceptive input mainly from

Figure 6

Percentual changes over time in frequency bands during single-sweep analysis relative to baseline values. (A) Features for buprenorphine and placebo treatment in frequency bands and (B) features for fentanyl and placebo treatment. —, drug – delta; —, drug – theta; —, drug – alpha; —, drug – beta; - - - , placebo – delta; - - - , placebo – theta; - - - , placebo – alpha; - - - , placebo – beta

Table 2

Baseline corrected values from the evoked potential average analysis. Values are displayed as the average percentage of change from baseline with 95% confidence interval in square brackets

Feature	24 h	48 h	72 h	144 h
Buprenorphine				
Delta	–5.8 [–44.6, 33.1]	14.6 [–33.9, 63.1]	28.8 [–22.8, 80.4]	25.6 [–28.9, 80.0]
Theta**	5.3 [–33.5, 44.2]	46.0 [–2.5, 94.5]	60.1 [8.4, 111.7]	73.3 [18.7, 127.7]
Alpha***	4.0 [–34.8, 42.8]	30.6 [–17.9, 79.1]	35.0 [–16.6, 86.6]	46.6 [–7.8, 101.0]
Beta*	7.1 [–31.7, 46.0]	16.0 [–32.5, 64.5]	16.5 [–35.1, 68.2]	10.1 [–44.3, 64.5]
Fentanyl				
Delta	–9.8 [–48.6, 29.0]	2.1 [–46.4, 50.6]	2.4 [–49.2, 54.0]	10.3 [–44.1, 64.8]
Theta*	–6.2 [–45.0, 32.6]	12.1 [–36.4, 60.6]	24.9 [–26.7, 76.5]	20.8 [–33.7, 75.2]
Alpha	1.1 [–37.8, 39.9]	15.9 [–32.6, 64.4]	25.9 [–25.7, 77.5]	21.6 [–32.8, 76.0]
Beta	–0.6 [–39.5, 38.2]	5.2 [–43.3, 53.7]	3.2 [–48.5, 54.8]	5.8 [–48.6, 60.3]
Placebo				
Delta	5.6 [–29.4, 40.6]	6.2 [–26.1, 38.6]	–1.4 [–34.3, 31.5]	4.6 [–33.1, 42.3]
Theta	3.6 [–31.4, 38.6]	4.8 [–27.5, 37.2]	5.3 [–27.6, 38.2]	16.0 [–21.7, 53.7]
Alpha	1.8 [–33.2, 36.8]	0.7 [–31.7, 33.1]	1.4 [–31.5, 34.4]	8.8 [–28.9, 46.5]
Beta	–2.3 [–37.3, 32.7]	–2.4 [–34.8, 30.0]	–3.9 [–36.8, 29.0]	–1.5 [–39.2, 36.2]

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

A δ -fibres, due to the slow conduction velocity of C-fibres. Tonic pain stimuli allow for more accurate analysis of C-fibre input, but lack the advantage of being precisely time-locked to a stimulus. In opioid studies EP analysis has often proved to be more sensitive to the drug effect despite the missing C-fibre input [2].

It is notable that significant results were obtained using a single Cz electrode for recordings. The vertex electrode only reflects the underlying brain activity in a restricted area and brain activity in other parts of the pain network is not recorded [25, 32]. However, for a clinical application mounting of a multi-channel EEG-cap is not practical,

Table 3

r values for correlations between single-sweep frequency bands and pain scores. Statistically significant correlations are marked in bold

Feature	Bone	Heat	Electrical
Buprenorphine			
Theta	0.77	0.69	0.45
Alpha	0.78	0.72	0.48
Beta	0.90	0.90	0.66
Fentanyl			
Theta	–	0.10	–0.33

Figure 7

Development of the spectral indices for the beta band from single-sweeps during buprenorphine treatment as well as pain scores for bone and heat. The development of the beta band features is correlated with both pain scores. —, buprenorphine – beta; —, bone pain; —, heat pain

which is why we limited the study to a single electrode. Since our results indicate that the analgesic effect of buprenorphine correlated with the beta-band, it seems that a simpler recording setup is viable for assessing the analgesic effect.

The electrical pain detection threshold was determined in each median nerve stimulation session. This was done to account for the fact that both buprenorphine and fentanyl are strong analgesics and should the same intensity as at baseline be given, the risk would be to stimulate at non-painful intensities and activate cortical networks different from the 'pain matrix'. Stimulation at the pain threshold ensured that all EPs reflect painful nociceptive input. This could lead to the assumption that the increased electrical current used for stimulation causes the increased energy in the EPs. However, since both fentanyl and buprenorphine proved to have no analgesic effect for electrical pain stimulations and the fact that no features correlated with the

electrical current used in stimulation, we believe that the observed changes reflect the analgesic effect [33].

Interpretation of findings in spectral indices

Other studies have investigated the analgesic effect of opioids using pharmaco-EEG [2, 31]. A previous opioid study of morphine using resting EEG found similar increases in several frequency bands as seen in the current study [34]. Another study of fentanyl found increases in delta, theta and beta bands following intravenous administration, whereas our study only found increases in the theta band [35]. However, this might be due to the different modes of administration used and the dosing. Another opioid study of meperidine using resting EEG also found an increase in the lower frequency bands, whereas the alpha band decreased [36].

Investigation of analgesics using pharmaco-EEG is not limited to opioids. In a resting EEG study following pregabalin treatment in patients with pain due to chronic pancreatitis, the power distribution was demonstrated to increase in the low frequency bands (mainly the theta band). By applying a 'support vector machine' to combine features it was shown that the EEG changes were correlated with the analgesic effect [8]. Another study found increased alpha and theta activity after treatment with ketamine [37]. Although these findings were obtained from the resting EEG, they correspond with the findings in this study in which increased activity was also demonstrated in both the theta and alpha band after treatment with buprenorphine. Though the activity also increased in the high frequency spectrum, the increase was more pronounced for the lower frequencies, meaning that the distribution of power moved towards the low frequency spectrum, in accordance with earlier findings [8]. Interestingly, this study suggests that although low-frequency components increase more dramatically during opioid treatment, they are not more sensitive to the analgesic effect of opioids.

Experimental studies without using drugs have found features in high frequency bands (beta and gamma) and correlated them with pain scores using laser evoked potentials [38, 39]. These studies have suggested that the saliency of the stimulus plays a large role in the EEG, indicating that it is not pain-specific but instead related to attention triggered by the sensory input. This means that features thought to reflect the painful response of the brain will not be present if the stimulus is delivered with a short inter-stimulus interval because the subject will be expecting the stimulus [39]. The stimulus for this study was delivered with a short and non-varying interval of 5 s. This is rather low compared with an inter-stimulus interval of 20 s used in other studies [38, 39]. This combined with the correlation with pain scores leads us to believe the differences reflect the analgesic effect of buprenorphine, rather than the saliency of the stimulus.

This study identified a possible biomarker for the analgesic effect of buprenorphine, but not of fentanyl and it could be argued that this weakens the results. However, since the two opioids mediate their analgesic effect through different opioid receptors and the formulation of the patches was not completely similar it is entirely reasonable to obtain different results [19]. This is further validated by the fact that differences have been proven between the pharmacokinetic/pharmacodynamic relationships of buprenorphine and fentanyl [20].

Clinical implications

The development and testing of new drugs is associated with large economic costs. Therefore, a method for assessing the therapeutic effects in early preclinical drug development is needed to account for confounding factors such as the placebo effect. The use of EPs as a biomarker for analgesic effect has previously been reviewed and represents promising results for establishing preclinical screens to determine efficacy for new drugs in the early stages of development [8, 32, 40]. The same method could also be established in the clinic to detect response to analgesics in patients with chronic pain.

In conclusion, in comparison with less advanced methods, single-sweep analysis is usable for investigation of EPs to identify biomarkers for the analgesic effect of strong opioids, thus proving the value of advanced processing methods in pharmaco-EEG. In future studies such analysis may be used to screen for the effect of analgesics.

Author contributions

Mikkel Gram: Neurophysiological analysis, discussion of results, preparation of manuscript

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Anders K. Nielsen: Neurophysiological analysis, discussion of results, preparation of manuscript

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All authors contributed equally to the final manuscript.

Competing Interest

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (avail-

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