Altered Cortical Causality after Remifentanil Administration in Healthy Volunteers: A Novel Approach for Pharmaco-EEG

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***Abstract*—Alterations in cortical causality information flow induced by remifentanil infusion in healthy volunteers was investigated in a placebo-controlled double-blind cross-over study. For each of the 21 enrolled male subjects, 2.5 minutes of resting electroencephalography (EEG) data were collected before and after infusion of remifentanil and placebo. Additionally, to assess cognitive function and analgesic effect, continuous reaction time (CRT) and bone pressure and heat pain were assessed, respectively. The causality information was extracted from the EEG by phase slope index (PSI). Among the features being reproducible between the two baseline recordings, several PSI features were altered by remifentanil administration in comparison to placebo. Furthermore, several of the PSI features altered by remifentanil were correlated to changes in both CRT and pain scores. The results indicate that remifentanil administration influence the information flow between several brain areas. Hence, the EEG causality approach offers the potential to assist in deciphering the cortical effects of remifentanil administration.**

I. Introduction

Use of electroencephalography (EEG) to assess drug effects on cortical oscillations is growing. Characterization of such drug-induced EEG effects is useful to gain knowledge on mechanisms of action underlying central acting drugs and for stimulating development of new drugs as for example analgesics [1]. A review of applications of EEG data in the study of analgesics has recently been published [2].

Remifentanil is an opioid analgesic, which is a potent mu-receptor agonist with a short onset and fast offset, and it is mainly used to relieve pain during surgery [3]. There are several studies, such as one by Egan and colleagues [4], who investigated the effect of remifentanil on spectral power of EEG signals in individual electrodes. However, to this point no studies exist which investigate the complex information flow between multiple electrodes.

To extract properties of multi-dimensional EEG signals, several types of pair-wise EEG connectivity and causality information flow methods exist. Such methods in very basic ways have been used previously to study analgesic effects of remifentanil [5]. In a previous study, Noh and colleagues [6] used a frontoparietal montage of EEG including F3, F4, Cz, P3 and P4 electrodes. They found that approximate entropy [7] was significantly correlated to remifentanil concentration.

In this study, we investigated complex causality information flow in 62 pairs of resting EEG signals by phase slope index (PSI) to study the cortical effects of remifentanil. PSI [8] reflects the average slope of the phase as a function of frequency (in the specified bandwidth). It measures causal interaction based on the temporal delays between two EEG signals and indicates which one leads the other. In other words, PSI reflects the flow of information between two EEG signals. Compared to several other causality measures, PSI is invariant to rescaling of data, and more importantly is found to be robust and less prone to spurious and misleading weak causality estimates between two signals (due to different signal-to-noise ratio, volume conduction, different overall spectral power or different spectral details) in comparison to actual causality methods due to temporal ordering [8], [9].

The aim of the current study was first to identify the reproducible PSI features at two baseline recordings (before remifentanil and placebo administration). Second, to identify the robust features being altered by remifentanil administration in comparison to placebo treatment. Third, to test if the altered PSI features were correlated to changes in sustained attention as assessed by a continuous reaction time test (CRT) and analgesic effect assessed by experimental bone and heat pain.

II. Methods

A. Participants

Twenty-one healthy male adult subjects (mean age 23.5 years, range 20 to 28 years; mean weight 79 kg, range 60 to 102 kg) volunteered to participate in this randomized double-blind placebo-controlled cross-over study. The study was approved by the Ethics Committee for the Region of Northern Jutland (N-20110014) and the Danish Health and Medicines Authority (EudraCT no. 2009-013465-26). Subjects were not allowed to use any analgesics for 14 days prior to enrollment and were asked to fast for six hours prior to study start. Immediately prior to the test session

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participants were required to pass a drug screen test as well as an alcohol test.

B. Experimental Details

Each subject was randomly assigned to first receive either remifentanil or placebo treatment, each with 30 minutes duration. Then after a two hour drug washout period, the other treatment (placebo or remifentanil) was administered on the same day. Resting awake EEG as well as CRT and quantitative sensory testing (QST) to standardized painful stimulation of bone pressure and heat pain were recorded at four stages. The stages included recordings during baseline (before infusion) and during infusion. The recordings during infusion were conducted at steady-state condition (25 minutes after infusion onset) of either remifentanil (dosage of 0.1 $\mu g\ kg^{-1}\ min^{-1}$) or placebo using an infusion syringe pump. Two identical 50 ml infusion syringes were prepared by a pharmacist with no other involvement in the study. One syring containing remifentanil 2 mg (Ultiva®, GlaxoSmithKline Pharma A/S, Denmark) dissolved in isotonic saline to a concentration of 60 $\mu g\ ml^{-1}$, and the other containing an equal amount of isotonic saline.

As a measure of sustained attention or vigilance, CRT was collected [10]. It is a computer test (Ekho program, Bitmatic, Denmark) in which 100 auditory beep signals (500 Hz, 90 dB) are delivered through headphones to the subject at random intervals of 2 to 6 seconds. The subject was instructed to press a button as soon as he heard the beep signal. The reaction time, i.e., the time from emission of the sound signal to activation of the button was measured in milliseconds. The CRT index was calculated as 50th percentile/(90th percentile-10th percentile), [10], [11].

For the assessment of painful sensations by QST, a standard numerical rating scale (NRS) was used with the range 0 to 10, where major points were: 0 = no pain, 5 = moderate pain and 10 = worst pain imaginable [12].

For bone pressure pain, pressure stimulation was performed on the dominant leg 15 cm below patella with a handheld algometer (Type 2, Somedic Production AB, Sweden) with probe diameter of 2mm. The force increase rate was 30 kPa/s adjusted to a probe size of 1 cm^2 [13]. Subjects were instructed to press a button when they experienced moderate pain corresponding to a NRS score of 5, which terminated the stimulation.

Heat stimulation was applied midway between the wrist and the cubital fossa on the right volar forearm using a 9 cm^2 thermode controlled by a computerized 'Thermo Tester' (TSA II NeuroSensory Analyzer, Medoc Ltd., Ramat Yishai, Israel). The temperature increased from a baseline of 32°C to a maximum of 52°C with a rate of 1°C/sec. Subjects were told to press a button when they experience moderate pain corresponding to a NRS score of 5. Three successive stimulations were performed, and the average of the temperatures to inflict moderate pain was used to calculate the analgesic effect to heat pain. Between the three stimulations, the temperature returned to baseline.

The EEG data was collected by 62 EEG electrodes (standard 10-20 EEG system) using Neuroscan SynAmp2 EEG and a standard EEG cap (Quick-Cap International, Neuroscan, El Paso, TX, USA). The data was recorded using a reference located between Cz and FCz and a 1000Hz sampling frequency. Impedance of the electrodes was kept below 5 kΩ. Subjects were instructed to rest with open eyes and gaze fixed on a remote point. During recordings the data was initially filtered by the Neuroscan software system using a bandpass filter with a passband of 0.5-200Hz. For each subject, a total of 2.5 minutes of resting awake EEG data was recorded for each of the four stages.

C. EEG Pre-processing

First, a notch filter (50Hz) was applied to remove power line signals and the EEG data was re-referenced to linked ear. Data was visually inspected by an EEG analysis expert to remove major artifacts and interpolate electrodes with low signal-to-noise ratio by the immediate neighbors. A low-pass filter with cut-off frequency of 37Hz was applied to improve manual inspection of the data, followed by an automated artifact removal process to remove parts with high amplitudes. This was realized by analyzing both a linked-ear reference version as well as a Cz re-referenced version of the EEG signal to detect parts that had amplitudes higher than 5.5 times the corresponding standard deviation (SD). Assuming Gaussian distribution for EEG signals, the choice of 5.5 SD as amplitude threshold provides the guarantee that no useful EEG information is discarded and at the same time, outlier signals (that are assumed to happen rarely and are associated with major artifacts) are discarded. Then these parts plus and minus 0.35 seconds of neighboring EEG data were discarded in all electrodes. On the de-artifacted data, a bandpass filter in the range 0.7-37Hz was applied to reduce the effect on phase estimates. Since de-artifacted data lengths were different among subjects, to make a fair comparison, we finally used the initial 88 seconds of pre-processed EEG data for further processing.

The EEG analysis depends on the reference system used, and as we were interested in discussing topological interpretation of our findings, we re-referenced the EEG data into the average reference [14].

D. Extracting Causality Features

To calculate the EEG causality, we used the PSI code by Nolte *et al.* [8] with a non-overlapping epoch length of 8 seconds and window length of 2 seconds with 50% overlap, corresponding to a frequency resolution of 0.5Hz. Normalized PSI values calculated as the mean over all epochs were used in the analysis, which was calculated by dividing each PSI estimate by the corresponding SD.

The PSI was calculated in the following frequency bands: delta (2-3.5 Hz), theta (4-7.5 Hz), alpha (8-12 Hz), and low beta denoted by beta1 (12.5-18Hz). It should be noted, that discarding information below 2Hz and above 18Hz, could help in partly reducing eye-movement/eye-blink and muscle artifacts.

E. Statistical Analysis

PSI is a signed measure and the null hypothesis is that the PSI estimates have a zero mean distribution [9]. To find PSI features that are more likely to reflect robust and actual causal relationships, we tested for non-zero median using a sign-test applied over the PSI estimates obtained from each

88 seconds of data. Similar to [15], a value of $p<0.01$ was considered as an indication of statistical significance. Descriptive statistics are reported as average (±SD).

To insure baseline reproducibility of reported PSI features with non-zero median (based on sign-test), we considered the PSI features to be reproducible if comparison of the two baseline recordings resulted in $p\geq0.3$ when applying a two-tailed paired-samples t-test. As the reproducibility was important for our analysis in order to select robust features altered by remifentanil infusion, the value of 0.3 (instead of 0.01) was chosen to obtain a slightly more reliable rejection of the hypothesis that baseline data were different.

The overall aim of the study was to search for PSI features that significantly changed due to remifentanil treatment, meaning a change from zero median to non-zero median or vice-versa. To validate that the identified alterations were not due to placebo effects, we compared changes due to remifentanil with the changes due to placebo treatment using a two-tailed paired samples t-test.

Baseline corrected alterations after remifentanil administration were calculated by subtraction of the corresponding baseline feature, and correlated to changes in CRT and QST using Pearson's correlation coefficient. For each clinical score, the relative change was calculated as 100*(POST-PRE)/PRE, where PRE and POST denote pre- and post-treatment values of the clinical score, respectively.

III. Results

Table 1 report the CRT indices, bone pressure pain and heat pain scores for the four conditions. All scores were changed significantly by remifentanil (all $p<0.001$), but not by placebo treatment ($p=0.18$ for CRT, $p=0.15$ for bone pressure pain, and $p=0.07$ for heat pain). Between the two baseline conditions, clinical scores were similar (all $p\geq0.2$).

A set of 44 PSI features were found to be reproducible between baselines, and furthermore differentiated the effect of remifentanil versus placebo. Several of these features were correlated as they correspond to closely-spaced electrodes. Table 2 lists the most significant features. PSI(band, e_1, e_2) denotes PSI at the specified band from electrode e_1 to e_2. Features are sorted based on frequency band and then based on EEG electrodes. A causality relationship from electrode e_1 to electrode e_2 means that e_1 is the driver and e_2 is the recipient. Fig. 1 shows the position of the EEG system used in the study as well as a drawing of altered information flow during remifentanil infusion.

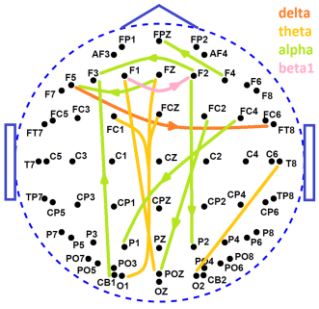

Figure 1. Simplified drawing of alteredinformation flow during remifentanil infusion compared to the baseline recording.

TABLE I. Statistics of continuous reaction time (CRT) index, bone and heat pain to induce moderate pain at baseline and during remifentanil (REM) and placebo (PLA) infusion. Mean (±SD) is reported.

Clinical score	REM baseline	REM infusion	PLA baseline	PLA infusion
CRT index	2.4 (±0.64)	1.8 * (±0.79)	2.4 (±0.53)	2.3 (±0.56)
Bone pressure pain (kPa/cm^2)	5417 (±1059)	7616 * (±1873)	5721 (±1050)	5466 (±1377)
Heat pain (^{o}C)	45.5 (±2.4)	47.9 * (±3.0)	45.7 (±2.9)	46.2 (±2.2)

*Significant changes, (baseline vs. during REM infusion), $p<0.001$.

TABLE II. Causality relationships changed by remifentanil treatment. The p values are shown for each feature based on sign-test as an indication of robustness of causality relationship in baselines as well as during remifentanil infusion.

feature	Baseline PLA, REM	REM
PSI(delta, F5, FT8)	$p\approx1, p=0.38$	$p=0.002$
PSI(theta, POZ, F1) *	$p=0.001, p=0.001$	$p=0.38$
PSI(theta, O1, FZ) *	$p=0.0002, p<0.0001$	$p\approx1$
PSI(theta, O1, FC1) *	$p=0.001, p<0.0001$	$p=0.66$
PSI(theta, O1, FCZ) *	$p=0.0002, p=0.0002$	$p=0.38$
PSI(theta, O2, T8) *	$p=0.001, p=0.001$	$p\approx1$
PSI(alpha, F4, FPZ)	$p=0.66, p=0.66$	$p=0.001$
PSI(alpha, FZ, F5)	$p=0.38, p=0.66$	$p=0.0002$
PSI(alpha, F2, F3)	$p\approx1, p=0.66$	$p=0.001$
PSI(alpha, CB1, F3)	$p=0.66, p=0.38$	$p=0.001$
PSI(alpha, F2, P2)	$p=0.38, p=0.38$	$p=0.0002$
PSI(alpha, FC4,P1)	$p\approx1, p\approx1$	$p=0.007$
PSI(alpha, FC2, POZ)	$p=0.66, p\approx1$	$p=0.001$
PSI(beta1, F1, F2)	$p\approx1, p=0.38$	$p=0.007$

* indicates loss of causality due to remifentanil, which means that the PSI became insignificant (i.e., median close to zero).

The first row of Table II, means that there were no robust causality at the two baselines, but there was a causality relationship after remifentanil infusion. Remifentanil destroyed the causality relationship in the five PSI features at theta band (identified with orange color in Fig. 1), while in others, remifentanil introduced causality.

Changes in PSI features were correlated to clinical scores. As shown in Fig. 2, we found several weakly significant correlations ($p\leq0.02$) between relative changes in PSI measure and clinical score. This reflects causality relationship between the associated brain regions due to remifentanil treatment and relative changes in CRT index or QST score (bone pressure pain or heat pain). All other possible correlations were insignificant (all $p>0.05$). Interestingly, the PSI features in delta and beta bands were correlated to the CRT, while the alpha band (known to be associated with pain perception) was correlated to changes in bone pain.

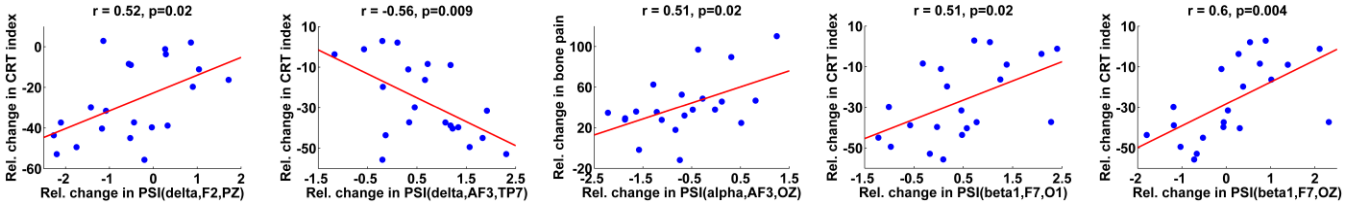

Figure 2. Scatter plots showing the correlations between relative changes due to remifentanil: CRT index or QST scores versus PSI causality measures.

IV. Discussion and Conclusion

Our analysis demonstrated that the causality characteristics of resting EEG data were altered by remifentanil infusion in healthy volunteers. We found several PSI features, which were reproducible at the two baseline conditions, but changed after remifentanil administration, while they remained unchanged after placebo treatment (or changed in an opposite direction of remifentanil effects).

As an area for future work, the effect of the following design parameters needs to be investigated: PSI normalization to unity standard deviation [8], data epoch and window lengths, range and bandwidth of frequency bands. Additionally, the final statistical significance value and robustness of PSI estimates needs to be explored, which may be utilized by applying graph theoretical measures to the pairwise connectivity measures to reduce dimensionality and explain the overall network properties of the data.

As our study included 21 healthy subjects, more data is needed to make clinically reliable conclusions and to validate the results. If validated in larger clinical studies, using EEG connectivity information flow to reflect spatio-temporal interactions between EEG signals, could provide evidence towards developing an objective method to understand the mechanism of action of analgesic drugs.

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