Disruption of Cortical Connectivity during Remifentanil Administration Is Associated with Cognitive Impairment but Not with Analgesia


ABSTRACT

Background: The authors investigated the effect of remifentanil administration on resting electroencephalography functional connectivity and its relationship to cognitive function and analgesia in healthy volunteers.

Methods: Twenty-one healthy male adult subjects were enrolled in this placebo-controlled double-blind cross-over study. For each subject, 2.5 min of multichannel electroencephalography recording, a cognitive test of sustained attention (continuous reaction time), and experimental pain scores to bone-pressure and heat stimuli were collected before and after infusion of remifentanil or placebo. A coherence matrix was calculated from the electroencephalogram, and three graph-theoretical measures (characteristic path-length, mean clustering coefficient, and relative small-worldness) were extracted to characterize the overall cortical network properties.

Results: Compared to placebo, most graph-theoretical measures were significantly altered by remifentanil at the alpha and low beta range (8 to 18 Hz; all \( P < 0.001 \)). Taken together, these alterations were characterized by an increase in the characteristic path-length (alpha 17% and low beta range 24%) and corresponding decrements in mean clustering coefficient (low beta range −25%) and relative small-worldness (alpha −17% and low beta range −42%). Changes in characteristic path-lengths after remifentanil infusion were correlated to the continuous reaction time index (\( r = −0.57; P = 0.009 \)), while no significant correlations between graph-theoretical measures and experimental pain tests were seen.

Conclusions: Remifentanil disrupts the functional connectivity network properties of the electroencephalogram. The findings give new insight into how opioids interfere with the normal brain functions and have the potential to be biomarkers for the sedative effects of opioids in different clinical settings. (Anesthesiology 2015; 122:140-9)

Remifentanil is a potent central acting \( \mu \)-receptor opioid agonist used for balanced anesthesia and analgesia.\(^1,2\) Due to its remarkable titrability it has proven to be useful for scientific research and provides an ideal drug to study the mechanism of action underlying the effects of opioids.

During the last decade, electroencephalography has been increasingly used to study and monitor central acting drug effects. The advantage of electroencephalography is that it provides a reliable and direct measure of brain activity and has relatively low cost and ease of administration compared to other neuroimaging methods. Electroencephalography has previously been used to study central effects of remifentanil, and clear relationships between electroencephalographic alterations and remifentanil pharmacokinetics have been demonstrated.\(^3,4,5\) Most of these studies, however, focused on the classical power spectral density of the electroencephalogram. Thus, the complex information captured by electroencephalography was reduced to quantitative measures of brain rhythmicity. Consequently, these studies did not assess the brain connectivity and its relationship to cognitive function and analgesia.
networks that can be inferred from multichannel electroencephalography data in terms of functional connectivity. To characterize the complex networks in the brain, the characteristics obtained by functional connectivity may be subjected to graph-theoretical measures. Hence, graph-theory is built on a network that is reconstructed through functional connectivity analysis and decomposes the multiple connectivity features extracted from the multichannel electroencephalogram into a few composite measures of the overall brain network performance and functionality—for further details see figure 1. Analysis of functional cortical connectivity followed by graph-theory (and other advanced statistical methods) has provided valuable information on disease mechanisms underlying neurological and psychiatric disorders including autism, schizophrenia, bipolar disorder, and temporal lobe epilepsy. Also, in a recent study based on electroencephalographic alterations and analysis of cortical connectivity, disruption of fronto–parietal communication was shown to be a possible biomarker of anaesthesia.

In the current study, we investigated the effect of remifentanil and placebo infusion on graph-theoretical measures of functional cortical connectivity using multichannel electroencephalography. We hypothesized that in comparison to placebo; remifentanil infusion in healthy volunteers would induce significant changes in network properties of functional connectivity in the resting electroencephalogram. The aims of the study were as follows: (1) validate reproducibility of the graph-theoretical measures of cortical connectivity obtained from resting state electroencephalography, (2) investigate changes in graph-theoretical measures of cortical functional connectivity after remifentanil infusion compared to placebo, and (3) correlate putative changes in graph-theoretical measures to the sedative and analgesic effects of remifentanil as assessed by measurement of continuous reaction time (CRT) and experimental pain stimulation.

Materials and Methods

Study Subjects
Twenty-four healthy males were invited to participate in this study, which was approved by the Ethics Committee for the Region of Northern Jutland, Aalborg, Denmark (N-20110014) and the Danish Health and Medicines Authority (EudraCT No. 2009–013465). All subjects provided written informed consent. The study was conducted according to the Declaration of Helsinki and the rules of Good Clinical Practice. The Good Clinical Practice Unit at Aarhus University Hospital monitored the study, which was a locally approved extension of the main study registered with www.clinicaltrials.gov (NCT01375348). Data from the current study were collected as an explorative supplement to gain insight into mechanisms of action for remifentanil.

Before enrollment, all subjects underwent a clinical examination to ensure all were in good health with no history of chronic pain, psychiatric disorders, or drug abuse. Subjects were asked to fast for 6 h before experimental investigations and not use any analgesics for 14 days before study start. Immediately before the test session, participants were required to pass a drug screen and an alcohol test.

Study Protocol and Medication
The study was an investigator-initiated, double-blind, placebo-controlled, cross-over study of remifentanil in healthy volunteers. A diagram of the study protocol is illustrated in figure 2. Each session started with a pretreatment recording of resting state electroencephalography followed by an assessment of cognitive performance by CRT and subjective pain scores using quantitative sensory testing (QST) for bone and heat pain. After the pretreatment assessment, the subject was randomly assigned to either remifentanil or placebo infusions. Steady state was assumed after 25 min of infusion and independent recordings of electroencephalography, CRT, and QST were obtained again. After a 2-h drug washout period, a new pretreatment assessment of resting state electroencephalography, CRT, and QST was obtained and the second infusion was given (either placebo or remifentanil depending on the initial infusion). The second infusion was followed by reassessment of electroencephalography, CRT, and QST as described for the first session.

Blinding of treatment allocation was ensured by two identical 50 ml infusions, prepared by a pharmacist with no other involvement in the study according to a randomization plan generated at www.randomization.com. The remifentanil infusion contained 2 mg remifentanil (Ultiva®; GlaxoSmithKline Pharma A/S, Børby, Denmark) dissolved in 33.3 ml isotonic saline to a concentration of 60 μg/ml, while the placebo infusion contained an equal amount of isotonic saline. All infusions were administered by an infusion syringe pump (Codan Green Stream® SY-P; Codan Medizinische Geräte GmbH & Co KG, Lenzahn, Germany) ensuring a remifentanil dose of 0.1 μg kg⁻¹ min⁻¹.

Continuous Reaction Time
The CRT is a test of sustained attention and vigilance and has been well-validated in the Danish population. The CRT was tested in terms of the response to auditory stimuli using
a response unit and a push-button system controlled by commercially available software (EKHO; Bitmatic, Viby J, Denmark). One hundred auditory beep signals (500 Hz, 90 dB) were delivered through headphones to the subject at random intervals of 2 to 6 s. The subject was instructed to press a button as soon as he heard a beep signal. The reaction time, that is, the time from emission of the sound signal to activation of the button was measured in milliseconds. The CRT index

Fig. 1. Functional brain connectivity networks assessed in terms of graph-theoretical analysis. The multichannel electroencephalography recordings (A) are transformed into coherence (COH) measures (B). The symmetrical coherence matrix to the left contains all pair-wise couplings, which is represented on a circle to the right. A coherence value of 0 represents no coherence, while a value of 1 represents maximum coherence. The coherence values are mapped into appropriate weights of functional distance between electrodes (C), with the constraint that no coherence represents maximum distance, while maximum coherence is associated with zero distance. The coherence values and corresponding weights of functional distance are then subjected to graph-theoretical analysis (D). In this study, three graph measures were used for analysis. The mean clustering coefficient (i) is a measure of the level of clustered connectivity around electroencephalography electrodes. In the graph to the left, c is strongly connected to electrodes b and d, which are also connected. This forms a functional triangle, while no triangles are present in the figure to the right. Hence, in the graph to the left, groups of neurons forms a functional unit and have higher functional connectivity. The characteristic path-length (ii) is a measure of functional distance between any two electroencephalogram electrode pairs. In the graph to the left, the distance from b to e is longer than in the graph to the right, and hence information is transferred more rapidly in the latter graph due to the shorter path-length. Taken together, quantification of small-world networks (iii) provides a score of the balance between segregation and integration of the network. In the graph to the left, the network is perfectly ordered in contrary to the graph to the right, which is completely random. The small-network graph in the middle has a low path-length and high-clustering coefficient, which is known to be associated with normal brain function.
Khodayari-Rostamabad

and 10 = worst imaginable pain. Bone pain was assessed by pressure stimulation on the dominant leg 15 cm below the patella using a handheld algometer (Type 2; Somedic production AB, Hörby, Sweden) with a probe diameter of 2 mm. The pressure increase rate was 225 mmHg/sec of 2 mm. The pressure increase rate was 225 mmHg/sec adjusted to a probe diameter of 11 mm2 according to recommendations by Andresen et al. Subjects were instructed to press a button when they experienced moderate pain (numerical rating scale = 5), which terminated the stimulation. Heat pain was assessed by thermal stimulation (TSAII, NeuroSensory analyzer; Medoc, Ramat Yishai, Israel) on the right volar forearm midway between the wrist and cubital fossa. The stimulation intensity was increased by 1°C/s from a baseline temperature of 32°C to a maximum temperature of 52°C. Subjects were instructed to press a button when they experienced moderate pain (numerical rating scale = 5), which caused the stimulator immediately to return to the baseline temperature of 32°C. Three successive stimulations were performed, and the average temperature to inflict moderate pain was used for the analysis of analgesic effect to heat pain.

Electroencephalography Recordings and Data Preprocessing

Electroencephalography data was collected by 62 electrodes mounted according to the extended 10–20 system, using a Neuroscan SynAmp2 system (Neuroscan 4.3.1, El Paso, TX) and a standard electroencephalography cap (QuickCap, International, Neuroscan). The data were recorded using a reference electrode located between Cz and FCz, a ground electrode in the occipital area of the scalp and a sampling frequency of 1,000 Hz. Electrode impedance was kept below 5 kΩ. During recordings, data were bandpass filtered (0.5 to 200 Hz) using the Neuroscan software. Subjects were instructed to rest with open eyes and gaze fixed on a remote point. Although the open eyes design may introduce varying visual inputs, this design was chosen to avoid sleep patterns in the electroencephalogram after remifentanil infusion due to its sedative effect. Furthermore, all subjects were positioned likewise to minimize variation in visual input. For each subject, a total of 2.5 min of resting state electroencephalography data were recorded for each of the four conditions (i.e., before and after remifentanil and placebo infusions).

The preprocessing procedure was as follows. In the first phase, the 50 Hz power supply noise was suppressed by a notch filter (50±1 Hz), then a bandpass filter (1 to 70 Hz) was applied to attenuate high-frequency artifacts that may otherwise interfere with the visual inspection process in later steps. To validate data quality and to remove excessive muscle and movement artifacts, an electroencephalography expert visually inspected the linked ear referenced electroencephalography data. Furthermore, electrodes with low signal-to-noise ratio were interpolated by the immediate neighboring electrodes. All these steps were performed in Neuroscan EDIT 4.3 software (Neuroscan). Then, using Matlab R2011b software (Mathworks Inc., Natick, MA) the following analyses were performed: First, data were bandpass filtered (0.7 to 37 Hz). Second, rejection of artifact intervals with high amplitude was done (i.e., amplitudes 5.5 times the corresponding SD) in either the linked ear or the Cz referenced version of the data. Third, since the connectivity analysis depends on the electroencephalography reference system used, the electroencephalography data was rereferenced into the average reference to make results comparable to previous literature. Finally, to make a fair comparison between recordings, all recordings were reduced to equal length (initial 88 s of deartificated data) before performing connectivity analysis.

Electroencephalogram Functional Connectivity

The magnitude squared coherence (COH) between all electrode pairs was extracted from the multichannel electroencephalogram using the Welch’s modified periodogram averaging method with nonoverlapping windows. The window length was 2 s, resulting in 0.5 Hz frequency resolution. To partly reduce low-frequency eye-movement and eye-blink artifacts, we used the 2 to 30 Hz range for data analysis. The electroencephalogram coherence values were calculated separately for the following frequency bands: delta (2 to 3.5 Hz), theta (4 to 7.5 Hz), alpha (8 to 12 Hz), beta1 (12.5 to 18 Hz), and beta2 (18.5 to 30 Hz).

Graph-theoretical Analysis

Graph-theoretical analysis was applied to functional connectivity data to characterize the cortical network as illustrated in figure 1. In general, graph-theoretical analysis is used to identify overall network properties and can be...
constructed in several ways. First, the input to the graph should reflect the connection strength (functional distance) between electrodes and should therefore be considered carefully. As coherence measures are characterized by values on a continuous scale from 0 to 1 (0 = no coherence and 1 = maximum coherence), but with no information with respect to direction of information flow, a weighted undirected network design was applied. Second, the output measures should be selected appropriately to reflect relevant characteristics of the network. In this study, we extracted the characteristic path-length, mean clustering coefficient, and relative small-worldness to quantify complementary properties of the network, and taken together they can be used to characterize the overall network performance.

The characteristic path-length is a measure of the average functional distance between electrode pairs. It is a measure of how rapidly information from different specialized brain regions can be combined and thus provides a measure of functional cerebral integration. The mean clustering coefficient is the average level of clustered connectivity around electrodes. Hence, a high clustering coefficient indicates the presence of local groups of neurons making specialized functional units (e.g., sensory or motor areas). In contrast to the average path-length, the clustering coefficient thus provides a measure of functional cortical segregation. Networks that are both integrated and segregated are commonly called small-world networks, which reflect the balance of local segregation and global integration.

In this study, electroencephalogram electrodes were used in the sensor space as nodes and functional connectivity measures as edges, and the graph analysis was performed using the brain connectivity toolbox. The mean clustering coefficient was computed based on the definition in Onnela et al., while relative small-worldness was computed as mean clustering coefficient/characteristic path-length, which is a simplified version of the definition in Humphries and Gurney (2008). When calculating the characteristic path-length, the coherence values were mapped into appropriate measures of functional distance between electrodes by applying the inverse function f(COH) = −log(COH). By this mapping, a coherence value of zero (no connection) was associated with infinite distance, while a coherence value of 1 (maximum connection) was associated with zero distance.

### Table 1. Comparison of Continuous Reaction Time and Experimental Heat and Bone Pain between Remifentanil and Placebo Infusion

<table>
<thead>
<tr>
<th></th>
<th>Remifentanil</th>
<th></th>
<th>Placebo</th>
<th></th>
<th>Remifentanil vs. Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretreatment</td>
<td>Posttreatment</td>
<td>Pretreatment</td>
<td>Posttreatment</td>
<td></td>
</tr>
<tr>
<td>Continuous reaction time index</td>
<td>2.4 ± 0.64</td>
<td>1.8 ± 0.79</td>
<td>2.4 ± 0.53</td>
<td>2.3 ± 0.56</td>
<td>F = 14.1; P &lt; 0.001*</td>
</tr>
<tr>
<td>Heat pain (°C)</td>
<td>45.5 ± 2.4</td>
<td>47.9 ± 3</td>
<td>45.7 ± 2.9</td>
<td>46.2 ± 2.2</td>
<td>F = 23.1; P &lt; 0.001*</td>
</tr>
<tr>
<td>Bone pressure pain (mmHg/cm²)</td>
<td>40,630 ± 7,943</td>
<td>57,120 ± 14,050</td>
<td>42,910 ± 7,880</td>
<td>40,998 ± 10,330</td>
<td>F = 39.8; P &lt; 0.001*</td>
</tr>
</tbody>
</table>

* Significant result after adjusting for multiple comparisons.

**Statistical Analysis**

This was an exploratory study and therefore no formal power calculation was performed. The primary aim of our study was to investigate changes in graph-theoretical measures of cortical functional connectivity after remifentanil infusion. Previous studies using graph-theoretical measures on resting state electroencephalography after administration of analgesics and sedatives have typically enrolled between 10 and 30 subjects. Consequently, we considered a sample size of 20 subjects to provide sufficient statistical power. In order to allow for possible dropouts 24 subjects were enrolled.

Descriptive statistics are reported as mean ± SD unless otherwise indicated. Analyses of CRT, QST, and electroencephalogram coherence and graph-theoretical measures followed a two-step procedure: First, pretreatment conditions were compared using paired t tests. Second, differences between groups (i.e., remifentanil vs. placebo) were compared using analysis of covariance with the pretreatment value of the analyzed parameter as a co-variable. Significant changes in graph-theoretical measures were correlated to changes in CRT and QST using Pearson correlation coefficient. A P value of less than 0.05 was considered as an indication of statistical significance. In case of multiple comparisons, the Bonferroni correction was used. The software package STATA version 11.2 (StataCorp LP, College Station, TX) was used for the statistical analysis.

**Results**

Twenty-four healthy Caucasian male volunteers were screened for inclusion in the study. Two subjects had a positive urine drug screen and were excluded, while another subject was excluded due to a history of psychiatric disorder. Hence, 21 subjects were enrolled in the study. The average age was 23.5 ± 2.1 yr and the average body weight was 78.7 ± 10.4 kg.

**CRT and QST**

The CRT scores and QST parameters are reported in table 1. All assessments were comparable between the two pretreatment conditions (all P ≥ 0.2). After placebo infusion, no significant changes were seen in CRT scores (P = 0.18) or QST parameters (P = 0.07 for heat pain, and P = 0.15 for bone pressure pain). In contrast, all CRT and QST parameters were changed significantly by remifentanil infusion (all P < 0.001).
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Correlation Analysis
Putative correlations between the CRT index scores, QST pain parameters (bone and heat pain scores), and graph-theoretical measures influenced by remifentanil infusion (i.e., electroencephalogram frequency range alpha and beta1) were explored. A negative correlation between the characteristic path-length and CRT index was seen in the alpha band ($r = -0.57; P = 0.009$; fig. 5). No other significant correlations between the graph-theoretical measures and the CRT or QST parameters were evident after adjustment for multiple comparisons (all $P \geq 0.04$).

Discussion
The current study investigated how remifentanil and placebo affects graph-theoretical measures obtained from functional connectivity network measures in resting-state electroencephalography data from healthy volunteers. After remifentanil infusion a reduction in overall efficiency of cortical networks was seen in the alpha and beta1 frequency ranges (8 to 18 Hz) of the electroencephalogram, whereas no significant changes were seen after placebo treatment. Hence, remifentanil disrupted the complex cortical network subserving normal brain function. These alterations were associated with loss of stability of sustained attention, while no associations were found regarding the analgesic effect.

Remifentanil Effects on Cortical Network Properties
To our knowledge, the effect of remifentanil analgesia (i.e., subanesthetic concentrations) on electroencephalogram graph-theoretical measures has not been investigated previously. We found an increase in the characteristic path-length of the cortical networks with corresponding decrements in mean clustering coefficient and relative small-worldness. In experimental studies, neuronal networks optimized for complexity show small-world characteristics, and graph measures of these networks are comparable to real cortical networks.9 The retrieved values do not directly reflect any specific underlying neurobiological processes, but taken together, the findings translate to a reduction in overall efficiency of the networks between different neuronal centers, and an alteration of the balance between the integration and segregation of the networks.23 This is in contrast to most previous studies where small-worldness and scale-free properties were maintained during general anesthesia.24-28 However, previous studies have not used opioids for sedation, but typically used propofol or other anesthetics with an entirely different

Electroencephalogram Functional Connectivity
The average coherence across all pairs of electrodes for the four different conditions (i.e., the pre- and post-treatments to remifentanil and placebo) is illustrated in figure 3. Mean coherences at pretreatment conditions were comparable at all frequency bands (all $P > 0.1$). After remifentanil infusion, mean coherence was reduced in the alpha ($P = 0.007$) and beta1 bands ($P < 0.001$), while no differences were seen after placebo infusion (all $P > 0.1$).

The grand mean functional connectivity measures from all subjects for the four conditions are illustrated for the beta1 band in figure 4. Based on visual inspection, the overall functional connectivity was decreased after remifentanil infusion, with less long-range connections and less synchronized cortical activity primarily in the frontal and parietal brain areas. In contrast, no major differences were seen after placebo infusion.

Reproducibility of Pretreatment Graph-theoretical Measurements
Pretreatment graph measures are provided in table 2. All measures were comparable between pretreatment conditions for all frequency bands (all $P \geq 0.09$).

Remifentanil versus Placebo Effects on Graph-theoretical Measurements
When comparing the changes in graph-theoretical measurements of cortical network properties between remifentanil and placebo conditions in the beta1 band, a significant increase in characteristic path-length was seen, whereas the mean clustering coefficient and relative small-worldness decreased (all $P < 0.001$; table 2). For the alpha band a significant increase in the characteristic path-length was also seen ($P < 0.001$) as well as a decrease in the relative small-worldness ($P < 0.001$). Furthermore, a decrease in the mean clustering coefficient was evident ($P = 0.02$), although this was not significant after adjustment for multiple comparisons (table 2). Taken together, the alterations were characteristic for disruption of cortical networks during remifentanil treatment.

Fig. 3. Grand mean electroencephalogram coherence across all 62 electrodes for the four different conditions (i.e., the two pretreatment conditions, postplacebo and postremifentanil infusions). Data are provided for each frequency band. Error bars indicate SD. The mean coherence were different at alpha (8 to 12 Hz; $P = 0.007$) and beta1 (12.5 to 18 Hz; $P < 0.001$) bands when comparing pretreatment-adjusted remifentanil and placebo effects.
remifentanil receptor profile. Furthermore, most previous studies looking at graph-theoretical measures (including small-worldness) were based on functional resonance imaging. Hence, they did not measure the direct electrical activity in the brain, but rather indirect measures of activity based on changes in blood flow. Also, many anesthetics have effects on the cardiovascular system, which confounds the use of imaging methods. For these reasons the findings in the current paper cannot be directly compared to previous studies and more research is needed to study the unique effects of opioid induced sedation on cortical connectivity.

While multichannel electroencephalography and graph-theoretical analysis has not previously been used after remifentanil administration, simpler methods to address cerebral connectivity during remifentanil anesthesia based on pair-wise connectivity or associations have been reported. Hayashi et al. investigated resting state electroencephalography before and after induction of anesthesia using a combination of sevoflurane and remifentanil. They found that with the induction of anesthesia, frontal alpha activity measured by bicoherence was increased while occipital alpha activity was decreased. Decreased alpha coherence reflecting less synchronized cortical activity in frontal electrodes was also seen in our study and although the methods are different, they stress the importance of frontal changes during remifentanil administration.

The changes in functional connectivity after remifentanil were confined to the alpha and beta1 frequency ranges (8 to 18 Hz). This is in line with a previous study, where Kortelainen et al. studied the effect of adding remifentanil to propofol anaesthesia. A decrease in beta frequency (>14 Hz) activity was seen in both light and deep anesthesia, while increased alpha band (7 to 14 Hz) activity was seen during deep anesthesia only. Although we did not investigate deep anesthesia in the current study, the importance of alpha and beta1 bands modifications after remifentanil infusion was...
supported by this study. Finally, in a recent study Lee et al. used network analysis to explore the effect of propofol. They found disruption of "hub structure" in the same frequency bands as in our study. Hubs facilitate functional integration of the network and in their study they are interpreted to reflect the coordination of information flow in the brain. Although propofol is an anesthetic without major analgesic properties, the findings support those in the current study.

From a functional perspective, the retrieved changes in connectivity graph measures were associated with impaired cognitive function as indicated by the correlation to changes in CRT index. CRT is primarily a simple neuropsychological test of vigilance and sustained attention. Other studies involving mentally demanding tests of sustained attention and vigilance have demonstrated that functional network organization can change over relatively short time scales. Thus, increased path-length and an asymmetrical pattern of connectivity (right > left) in fronto–parietal regions were associated with decreased attention. In particular, our study showed that the increase in characteristic path-length was associated with changes in CRT. This further validates the findings since the characteristic path-length is a measure of functional cortical integration and cross-talk between distinct brain areas. Along this line, abnormal cortical connectivity seems to be a common trait for various neurological and psychiatric disorders including Alzheimer's disease, schizophrenia, and autism. These are characterized by varying degree of cognitive dysfunction related to abnormal integration of large-scale network (i.e., disconnection syndromes).

In contrast, no association to analgesia was seen. We have recently found changes in brain networks between insular and cingulate sources during morphine treatment in a study where pain specific evoked brain potentials to phasic stimuli were used. However, the current study explored the general properties of the brain in contrast to studies on evoked brain potentials. To our knowledge, there are no graph-theoretical studies on brain networks in patients with chronic pain, but our findings are supported by a previous study where withdrawal of opioids increased functional connectivity in the alpha and beta bands. Finally, it is well known that remifentanil may cause hyperalgesia rather than analgesia in many subjects. Although speculative the balance between analgesia and hyperalgesia (together with sedative effects on pain assessment) may therefore confound the results. In the experimental settings, phasic pain stimuli are also less sensitive to opioid treatment. Hence, it could be recommended in future studies to study the effects of remifentanil on long-lasting pain stimuli such as ischemia or other types of tonic pain.

### Methodological Considerations

Remifentanil is a fast acting opioid with a half-life of less than 20 min. Consequently a 2-hr washout period (equal

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### Table 2. Comparison of Functional Connectivity Measures between Remifentanil and Placebo Infusion

<table>
<thead>
<tr>
<th>Frequency Band</th>
<th>Network Measure</th>
<th>Remifentanil Pretreatment</th>
<th>Remifentanil Posttreatment</th>
<th>Placebo Pretreatment</th>
<th>Placebo Posttreatment</th>
<th>Remifentanil vs. Placebo</th>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Delta (2–3.5 Hz)</td>
<td>L</td>
<td>1.16 ± 0.26</td>
<td>1.05 ± 0.34</td>
<td>1.21 ± 0.28</td>
<td>1.15 ± 0.31</td>
<td>F = 0.48; P = 0.49</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.19 ± 0.09</td>
<td>0.26 ± 0.12</td>
<td>0.18 ± 0.04</td>
<td>0.20 ± 0.07</td>
<td>F = 2.46; P = 0.12</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.22 ± 0.30</td>
<td>0.33 ± 0.30</td>
<td>0.16 ± 0.07</td>
<td>0.21 ± 0.17</td>
<td>F = 1.67; P = 0.20</td>
</tr>
<tr>
<td>Theta (4–7.5 Hz)</td>
<td>L</td>
<td>1.13 ± 0.17</td>
<td>1.17 ± 0.20</td>
<td>1.16 ± 0.20</td>
<td>1.14 ± 0.19</td>
<td>F = 3.32; P = 0.08</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.19 ± 0.04</td>
<td>0.20 ± 0.05</td>
<td>0.17 ± 0.02</td>
<td>0.18 ± 0.03</td>
<td>F = 0.43; P = 0.51</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.18 ± 0.08</td>
<td>0.18 ± 0.08</td>
<td>0.16 ± 0.04</td>
<td>0.17 ± 0.05</td>
<td>F &lt; 0.01; P = 0.98</td>
</tr>
<tr>
<td>Alpha (8–12 Hz)</td>
<td>L</td>
<td>1.02 ± 0.16</td>
<td>1.19 ± 0.22</td>
<td>1.08 ± 0.19</td>
<td>1.04 ± 0.16</td>
<td>F = 28.6; P &lt; 0.001*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.22 ± 0.04</td>
<td>0.20 ± 0.04</td>
<td>0.21 ± 0.04</td>
<td>0.21 ± 0.04</td>
<td>F = 5.76; P = 0.02</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.22 ± 0.08</td>
<td>0.18 ± 0.06</td>
<td>0.20 ± 0.07</td>
<td>0.22 ± 0.07</td>
<td>F = 17.9; P &lt; 0.001*</td>
</tr>
<tr>
<td>Beta1 (12.5–18 Hz)</td>
<td>L</td>
<td>1.41 ± 0.26</td>
<td>1.75 ± 0.26</td>
<td>1.44 ± 0.24</td>
<td>1.40 ± 0.20</td>
<td>F = 46.1; P &lt; 0.001*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.16 ± 0.04</td>
<td>0.12 ± 0.03</td>
<td>0.15 ± 0.03</td>
<td>0.15 ± 0.02</td>
<td>F = 42.8; P &lt; 0.001*</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.12 ± 0.06</td>
<td>0.07 ± 0.03</td>
<td>0.11 ± 0.04</td>
<td>0.11 ± 0.03</td>
<td>F = 39.9; P &lt; 0.001*</td>
</tr>
<tr>
<td>Beta2 (18.5–30 Hz)</td>
<td>L</td>
<td>1.68 ± 0.27</td>
<td>1.84 ± 0.25</td>
<td>1.66 ± 0.23</td>
<td>1.66 ± 0.19</td>
<td>F = 7.83; P = 0.008</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.13 ± 0.03</td>
<td>0.12 ± 0.04</td>
<td>0.13 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>F = 2.03; P = 0.16</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.08 ± 0.04</td>
<td>0.07 ± 0.04</td>
<td>0.08 ± 0.03</td>
<td>0.08 ± 0.03</td>
<td>F = 1.77; P = 0.19</td>
</tr>
</tbody>
</table>

* Significant result after adjustment for multiple comparisons.

C = mean clustering coefficient; L = characteristic path-length; S = relative small-worldness.
to more than six half-lives) was considered sufficient time ensuring the brain to return to baseline condition before the second infusion was initiated. To validate the stability of the functional connectivity graph-theoretical measures of the electroencephalogram, the test re-test reproducibility of the two pretreatment recordings was evaluated. All measures were reproducible and thus confirm the stability of cortical connectivity during pretreatment conditions. Along this path, no change in functional connectivity and the associated graph-theoretical measures were observed after placebo infusion (analysis not shown), which further strengthen the validity of the changes seen in functional connectivity after remifentanil.

Some methodological limitations are present in this study. In order to control for confounding factors, enrolled subjects comprised a very homogenous group (i.e., opioid naive healthy males). Therefore, an extension of this study is needed to assess the effects of remifentanil on functional cortical connectivity in women and in the clinical conditions such as in chronic pain patients taking opioids on a regular basis. Additionally, it should be noted that we have used the sensor space for functional connectivity analysis rather than applying source localization first to use neuroanatomical reference points. This design was chosen to enable comparison to previous studies on remifentanil also reporting findings obtained from surface electrodes. Finally, it should be noted that electroencephalography and electromyography have an overlap in some frequency bands. To minimize the effect of the electromyography component, we applied both manual and automatic deartifacting to the data, and reduced the analyzed frequency range to 2 to 30 Hz.

Conclusions

Remifentanil administration is associated with significant changes in functional cortical connectivity including a reduction in overall efficiency of the cortical network. These modifications seem to disrupt the complex cortical network subserving normal brain function and are associated with cognitive performance but not with analgesia.

Acknowledgments

This study was supported by The Danish Cancer Society (Kræftens Bekæmpelse), Copenhagen, Denmark, and The Danish Agency for Science, Technology, and Innovation (Det Strategiske Forskningsråd), Copenhagen, Denmark (grant no. 10-092786).

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Drewes: Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Mølleparkvej 4, 9000 Aalborg, Denmark. amd@rn.dk. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY’s articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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