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## Computer-Based Multitaper Spectrogram Program for Electroencephalographic Data

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**TITLE:**

Computer-Based Multitaper Spectrogram Program for Electroencephalographic Data

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**KEYWORDS:**

neuroscience, fentanyl, morphine, buprenorphine, sleep, chemical threats

**SUMMARY:**

This protocol provides an open source, compiled MATLAB program that generates multitaper spectrograms for electroencephalographic data.

**ABSTRACT:**

Current web resources provide limited, user friendly tools to compute spectrograms for visualizing and quantifying electroencephalographic (EEG) data. This paper describes a Windows-based, open source code for creating EEG multitaper spectrograms. The compiled program is accessible to Windows users without software licensing. For Macintosh users, the program is limited to those with a MATLAB software license. The program is illustrated via EEG spectrograms that vary as a function of states of sleep and wakefulness, and opiate-induced alterations in those states. The EEGs of C57BL/6J mice were wirelessly recorded for 4 h after intraperitoneal injection of saline (vehicle control) and antinociceptive doses of morphine, buprenorphine, and fentanyl. Spectrograms showed that buprenorphine and morphine caused similar changes in EEG power at 1–3 Hz and 8–9 Hz. Spectrograms after administration of fentanyl revealed maximal average power bands at 3 Hz and 7 Hz. The spectrograms unmasked differential opiate effects on EEG frequency and power. These computer-based methods are generalizable across drug classes and can be readily modified to quantify and display a wide range of rhythmic biological signals.

**INTRODUCTION:**

EEG data can be productively analyzed in the frequency domain to characterize levels of behavioral and neurophysiological arousal<sup>1</sup>. Multitaper spectrograms transform the EEG waveform into time and frequency domains, resulting in the visualization of the dynamic signal power at different frequencies across time. The multitaper spectrogram uses Fourier analysis to produce spectral density estimations. Spectral density estimation separates a waveform into the

pure sinusoidal waves comprising the signal and is analogous to the diffraction of white light through a prism to see the entire spectrum of colors<sup>2</sup>. The multitaper spectrogram of the EEG represents the combined activity of multiple networks of neurons with discharge patterns that oscillate at different frequencies<sup>2</sup>. Due to its time shift invariant, the Fourier transform is considered the best transformation between time and frequency domains<sup>3</sup>. Fourier analysis also has a number of limitations. EEG signals are nonstationary. Therefore, small changes may not be perceived under Fourier methods and the analysis may change depending on the size of the data set. However, windowing is used when applying a Fourier transform to a nonstationary signal. This assumes that the spectrum of the signal changes only marginally over short periods of time. An alternate method for spectral analysis is wavelet transform which may be more appropriate for detecting brain disease<sup>3</sup>.

From a functional perspective, the different oscillations comprising an EEG signal are lower-level, trait phenotypes characteristic of higher-level, state phenotypes such as sleep and wakefulness<sup>2</sup>, or the loss of wakefulness caused by general anesthetics<sup>4-6</sup>. Regarding states of sleep and wakefulness, the spectrogram clearly illustrates that endogenously generated rhythms of sleep are continuous and dynamic<sup>7</sup>. Quantitative descriptions of states of sleep and wakefulness have traditionally involved a binning process that assigns a sleep or wake classification to each specifically defined epoch (e.g., 10 s) of EEG recording. These state bins are then plotted as a function of time. Time course data plots, often referred to as hypnograms, are used to differentiate normal sleep from sleep that is disrupted by disease, drug administration, changes in circadian rhythms, shift work, etc. A limitation of hypnogram plots is that they misrepresent EEG signals by expressing arousal states as square waveforms. Hypnogram plotting involves a discretization of arousal states<sup>2</sup> and does not permit a finely grained display of intermediate or transition stages. Furthermore, 10 s scoring epochs produce a discretization of time by imposing a lower limit on the time scale. The result of the discretization of both state and time is the loss of neurophysiological information regarding the dynamic interplay between states of consciousness<sup>2</sup> and drug-induced disruption of these states<sup>4</sup>. For example, different anesthetic agents act on different molecular targets and neural networks. Pharmacological manipulation of these neural networks reliably produces spectrograms unique to the drug, dose, and route of administration<sup>4</sup>.

The present protocol was developed to facilitate research regarding the mechanisms by which opioids alter sleep<sup>8</sup>, breathing<sup>9</sup>, nociception<sup>10</sup>, and brain neurochemistry<sup>11</sup>. This protocol describes the steps required to create a multitapered spectrogram for EEG analyses that can be completed using proprietary software or a system that does not have MATLAB licensing. C57BL/6J (B6) mice were used to validate the ability of this computer-based method to create novel EEG spectrograms during normal, undisturbed states of sleep and wakefulness and after systemic administration of opiates. The reliability and validity of the analyses were confirmed by systematic comparisons of differences between EEG spectrograms after B6 mice received intraperitoneal injections of saline (vehicle control) and antinociceptive doses of morphine, buprenorphine, and fentanyl.

Quantitative studies of neonatal mouse EEG dynamics have translational relevance by providing

a model for studies aiming to achieve a better understanding of neonatal human EEG<sup>12</sup>. Quantifying EEG dynamics is not merely descriptive and can contribute to machine learning approaches that can predict arousal based in part on EEG data<sup>13</sup>. The goal of the present report is to promote translational science by providing a widely accessible, user friendly code for computing multitaper spectrograms that characterize drug-induced changes in mouse EEG.

## PROTOCOL:

All procedures involving mice adhered to the Guide for the Care and Use of Laboratory Animals (8<sup>th</sup> edition, National Academies Press, Washington DC, 2011) and were reviewed and approved by the University of Tennessee Institutional Animal Care and Use Committee.

### 1. Implantation of recording electrodes and initial data collection

1.1. Purchase mice and keep them in a humidity- and temperature-controlled room with ad libitum access to food and water. Allow mice to adapt to their new environment for one week before surgical implantation of recording electrodes. The implantation procedure has been described in detail<sup>1,14</sup>.

1.2. Sterilize all surgical equipment.

1.3. Anesthetize mice with isoflurane at 2.5%–3% delivered in 100% oxygen.

1.4. Following the loss of the righting reflex, remove the mouse from the anesthesia induction chamber and transfer it to a stereotaxic frame.

1.5. Apply an ophthalmic ointment to both eyes.

1.6. Reduce the isoflurane to 1.7%, continuously delivered via a mask.

1.7. Make a midline scalp incision to expose the skull.

1.8. Drill two craniotomies above the left and right cortex (each at stereotaxic coordinates anterior = 1.0 and lateral = 3.0 relative to the bregma<sup>15</sup>).

1.9. Insert the EEG electrodes into each craniotomy and secure with dental acrylic.

1.10. Implant bipolar electrodes in the dorsal trapezius muscle for recording the electromyogram (EMG).

NOTE: The four electrodes are led to a wireless telemeter implanted subcutaneously above the lower right body quadrant. These surgical techniques can be viewed here (<https://www.datasci.com/services/dsi-surgical-services/surgical-videos>).



1.11. After the surgery, administer analgesic carprofen and place the mouse in a warm recovery cage. Observe the mouse until it is ambulatory. House implanted mice individually.

1.12. Upon full recovery from the surgery, handle mice daily and evaluate the quality of the EEG and EMG recordings.

1.13. Configure the data acquisition system to record all signals  $\pm 1,000$  mV.

1.14. Obtain EEG and EMG recordings for the duration needed.

1.15. Score each 10 s bin of the digital EEG and EMG recordings as wakefulness, rapid eye movement (REM) sleep, or non-REM (NREM) sleep using sleep scoring software.

NOTE: Among mouse strains there are genotype- and state-specific differences in EEG power expressed as a percentage of the total power<sup>16</sup>. In B6 mice, states of wakefulness are characterized by a  $\sim 75$ – $100$  mV, mixed frequency EEG, and by EMG signals showing prominent muscle tone with large increases in amplitude during movement. Criteria for scoring NREM sleep include a reduction in EMG amplitude relative to the EMG amplitude of wakefulness. The NREM sleep EEG has a slower frequency and increased amplitude ( $\sim 100$ – $150$  mV) compared to wakefulness. REM sleep is characterized by muscle atonia and an EEG signal that is similar to the EEG of wakefulness.

1.16. Instruct two individuals to independently score the same record. At least one individual should be blinded to the treatment condition. Concordance values between the two sleep scorers should be greater than 90%.

## 2. Facilities and equipment

2.1. Amplify and digitize unfiltered EEG and EMG signals using data acquisition instrumentation and software.

NOTE: The Chronux Spectral Analysis Toolbox developed in the Mitra Laboratory at Cold Spring Harbor Laboratory is used to express EEG signals as power in relation to time and frequency domains.

## 3. Spectrogram computation

3.1. If a Windows user, use the compiled program.

3.2. If a Macintosh user, run the raw code file.

3.3. Obtain raw, unprocessed EEG data in either EDF or CSV file format and place it in the same location as the compiled program file.

3.3.1. Name the data files using the following constraints: names must consist of **only** letters, numbers, underscores, or dashes.

3.3.2. Name the data files using the following constraints: filenames must **not** contain periods, commas, spaces, or any other symbols.

3.4. Download the compiled Multitaper Spectrogram Program.

3.5. Launch the spectrogram program and follow the pop-up prompts. Choose file type: \*.CSV or \*.EDF.

NOTE: Further program installation details are located in the readme.txt file.

3.6. Type the entire EEG file name (e.g., 419eeg.edf or 419.eeg.csv).

3.7. Select parameters for spectrogram calculation: **Default** or **New**. This step requires the longest processing time as the spectrogram is being computed. The mathematical windowing function (taper) provides statistically-independent estimates of the underlying spectrum. The longer the recording duration, the longer this step will take. On a PC platform running Windows 10 this required a maximum of about 3–4 min for a 4 h recording.

3.7.1. Use the following default spectrogram parameters:

Sampling frequency = 500 Hz. This represents the number of samples per second.

fpass = 0.3 Hz and 30 Hz. Fpass defines the frequencies of input and controls the range of frequencies supplied in the output.

Padding = 2. Padding works to finely interpolate the output without affecting the result calculation in any way. This may assist with visualization and the precise identification of spectral lines. The field is any integer from -1 and up.

Time-bandwidth product (NW) = 15. The product of the signal temporal duration and spectral width.

Number of tapers = 29. When choosing number of tapers, it is essential to use  $2NW-1$ . There is no limit on the number of tapers used. The more tapers used will result in the inclusion of tapers with poor concentration in the specified frequency bandwidth.

Trial average = 1. This parameter governs whether or not trial or channel averaging is performed. If this parameter is set to 0, there is no channel averaging and the function will output independent results for each trial or channel passed as input data. However, if the trial average is set to 1, the results output to the user are averaged over trials or channels.

Time to compute FFT ~30 s. Used to follow the evolution of the spectrum by calculating the spectrum over many small windows.

Step size of window for FFT computation = 5. The amount the sliding time window advances after each spectrum calculation is performed.

NOTE: The default spectrogram parameters stated in step 3.7.1 can be changed as needed.

3.8. Enter titles for both the spectrogram and EEG.

3.9. Save the resulting spectrogram and EEG.

3.9.1. Save figures by clicking **File | Save** in the figure window.

NOTE: The figures will provide program users with summaries that can be developed into publication-quality figures.

## 4. Troubleshooting

4.1. Download the sample mouse sleep EEG data for sample spectrogram computation.

4.2. Run program with the sample data to ensure the user is correctly using the program. Find the figures for these sample data in the appendix to ensure that the figures created from the sample data are accurate.

NOTE: All equipment and materials used have been provided in the **Table of Materials**.

## REPRESENTATIVE RESULTS:

The following figures illustrate the type of novel insights into EEG indices of brain excitability that are provided by spectrograms.

**Figure 1A** illustrates similarities and differences in the cortical EEG during wakefulness, NREM sleep, and REM sleep. Many investigators use these kinds of traces, along with EMG recordings (not shown), to quantify sleep and wakefulness. **Figure 1B** uses a hypnogram plot to convey the temporal organization of states of sleep and wakefulness based on assessments of EEG and EMG recordings. States were scored in 10 s epochs and these epochs were plotted as the hypnogram during the 14,400 s comprising the 4 h recording. Hypnogram plots do not illustrate the fact that transitions between states are continuous and nonlinear. In contrast to a hypnogram plot, the spectrogram (**Figure 1C**) illustrates highly dynamic changes in EEG frequency and power as a function of time. The spectrogram also highlights the similarities between the cortical EEG signal during wakefulness and during REM sleep. The three boxes superimposed on the spectrogram (**Figure 1C**) mark states identified as wakefulness (WAKE), NREM sleep, and REM sleep in the hypnogram above (**Figure 1B**) and are provided to assist with visualizing the detailed changes in EEG frequency and power. The spectrogram for the entire recording provides a nuanced

appreciation of the EEG as a continuous process.

**Figure 2** provides four multitaper spectrograms, each summarizing 4 h of EEG recordings after intraperitoneal administration of saline, morphine, buprenorphine, and fentanyl. All four recordings are from the same mouse and were begun 2 h after light onset. The opiates, but not saline, inhibited NREM and REM sleep, and increased the amount of wakefulness. A number of novel features are visualized by the spectrograms. The detection of novel EEG features suggests the potential application for opiate differentiation in a chemical threat environment. After the saline injection (**Figure 2A**) the greatest amount of power resided in the 2–4 Hz range, indicating NREM sleep. Note that the EEG spectrograms were fundamentally altered by opiate administration, and that each opiate caused unique spectral changes.

**Figure 3** demonstrates that EEG changes illustrated by the spectrograms can be quantified and expressed as average dominate spectral power of each half frequency (**Figure 3A**) and as the average spectral power within specific EEG frequency bands (**Figure 3B**). The greatest differences from saline were caused by buprenorphine and occurred in the delta and theta ranges.

#### FIGURE AND TABLE LEGENDS:

**Figure 1. Cortical EEG recordings used to create hypnograms and spectrograms.** (A) EEG waveforms recorded during wakefulness, NREM sleep, and REM sleep during a baseline (no injection) recording. Each trace shows 90 s of recording. (B) The hypnogram uses the height of the bars to convey state of consciousness (ordinate) versus 4 h of recording (abscissa). (C) Tapered spectrogram using a color bar to convey EEG power in decibels (dB, right ordinate) or spectral power density at different EEG frequencies in Hertz (Hz, left ordinate) as a function of 4 h recording time (abscissa). Black vertical lines have been added to the spectrogram to delineate one episode each of wakefulness, NREM sleep, and REM sleep. (Spectrogram parameters: sampling frequency = 500 Hz, fpass = 0.3 Hz and 30 Hz, padding = 2, time-bandwidth = 15, number of tapers 29, trial average = 1, duration of time to compute FFT ~30 s, step size of window for FFT computation = 5)

**Figure 2. Spectrograms illustrating changes in EEG power and frequency caused by opiate administration.** Each spectrogram plots EEG frequency in Hertz (Hz, left ordinate) and decibels (dB) of EEG power using a color bar (right ordinate) for 4 h (abscissa) after administration of (A) saline, (B) morphine, (C) buprenorphine, and (D) fentanyl. (Spectrogram parameters: sampling frequency = 500 Hz, fpass = 0.3 Hz and 30 Hz, padding = 2, time-bandwidth = 15, number of tapers 29, trial average = 1, time to compute FFT ~30 s, step size of window for FFT computation = 5)

**Figure 3. Opiates differentially altered the average EEG power within the delta and theta EEG frequency bands.** (A) Summarizes the average EEG power during each 4 h recording shown in Figure 2. Ordinate plots average EEG power at each half frequency (abscissa). Relative to the saline control, each of the other three functions show opiate-specific alterations in average EEG power. (B) Illustrates the average EEG power in four EEG frequency bands (delta, theta, alpha, and beta) after administration of saline (S), buprenorphine (B), morphine (M), and fentanyl (F). Color coding is the same for power functions in A and average power bands in B.

## DISCUSSION:

The program described here was developed to create a spectrogram using the nine steps outlined in protocol section 3, Spectrogram Computation. These steps involve acquiring the spectrogram program, ensuring the correct file format, and altering the computational parameters for the generation of unique user spectrograms. Users can create spectrograms that are tailored to a range of conceptual questions and experimental designs. In order to enhance the ease and efficiency of this development process, it is essential to provide the raw EEG data in the correct file format, named according to the constraints described above. Although example signals have been provided for mouse EEG data, the spectrogram program is readily applicable to human and non-human EEG data that are free of signal processing limitations.

The recommended approach for troubleshooting and method modification is to begin by analyzing a small data set. The major program outputs to consider include plots of the filtered EEG as well as the spectrogram. An appealing aspect of the tapered spectrogram is that it can be applied to a wide variety of periodic, biological signals. The variety ranges from long duration circadian (~24 h) rhythms<sup>17</sup> to very fast rhythms such as 1,000 Hz discharge rates of a Renshaw cell<sup>18</sup>.

Data formatting is a constraint of this spectrogram protocol. European data format (EDF) is widely used with EEG data. However, there are many other formatting options. For this reason, the raw code file has been included (see 3.2 above) in case the user would like to alter the file format. With regard to the raw program file, another limitation is the need for experience with the computer programming language in order to alter the file format. Not all investigators have access to the proprietary software and the full array of plug-ins. This protocol was developed to circumvent this issue by providing a compiled program that runs on a WINDOWS-based device without software licensing. This is achieved through the RUNTIME plugin which is included in the compiled program and does not require any software registration by the user.

This EEG spectrogram routine is a novel, open source, computer-based program that allows users to create personalized, multitaper spectrograms from a wide range of data. The user has complete control over all computational aspects of spectrogram generation. Without prior signal processing and computer programming knowledge, spectrograms can be difficult to generate. The protocol described here will facilitate spectrogram generation. Please see the supplemental material section for further signal processing readings and multitaper spectrogram guidance.

## SUPPLEMENTAL MATERIALS :

<http://chronux.org>

<http://www-users.med.cornell.edu/~jdvicto/pdfs/pubo08.pdf>

<http://www.fieldtriptoolbox.org/tutorial/timefrequencyanalysis/>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4502759/#SD3-data>

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#### DISCLOSURES:

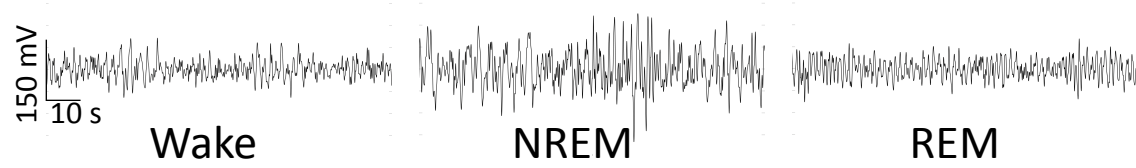
The authors have no conflicts of interest.

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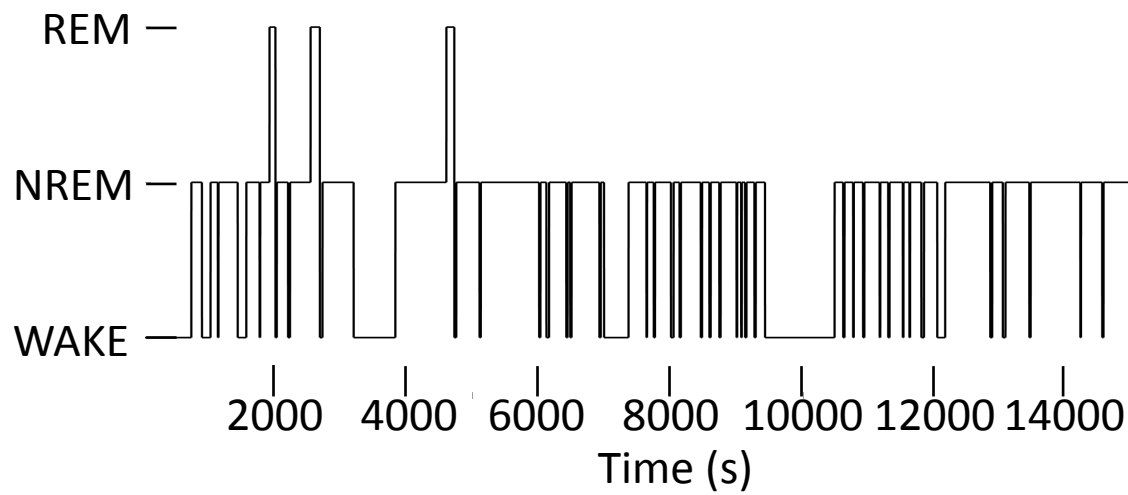
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401 thalamocortical cells discharging rhythmic (40 Hz) spike-bursts at 1000 Hz during waking  
402 and rapid eye movement sleep. *Neuroscience*. **56**, 1-9 (1993).
- 403

### A. EEG



### B. Hypnogram



### C. Spectrogram

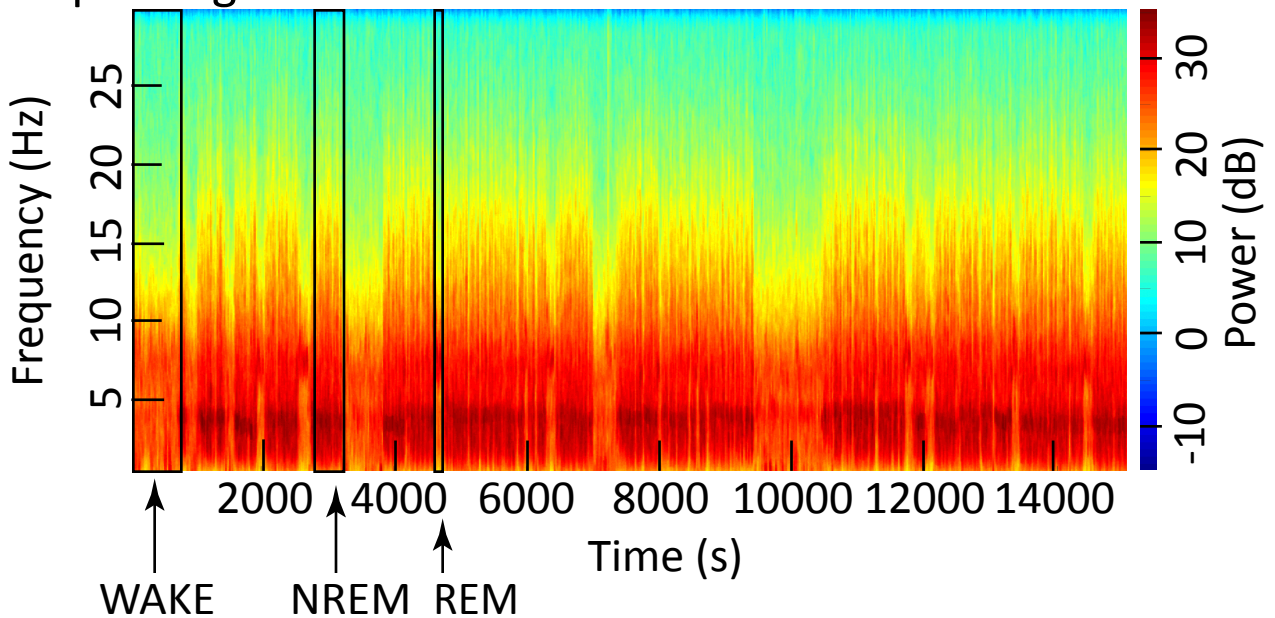


Fig. 1 O'Brien et al.



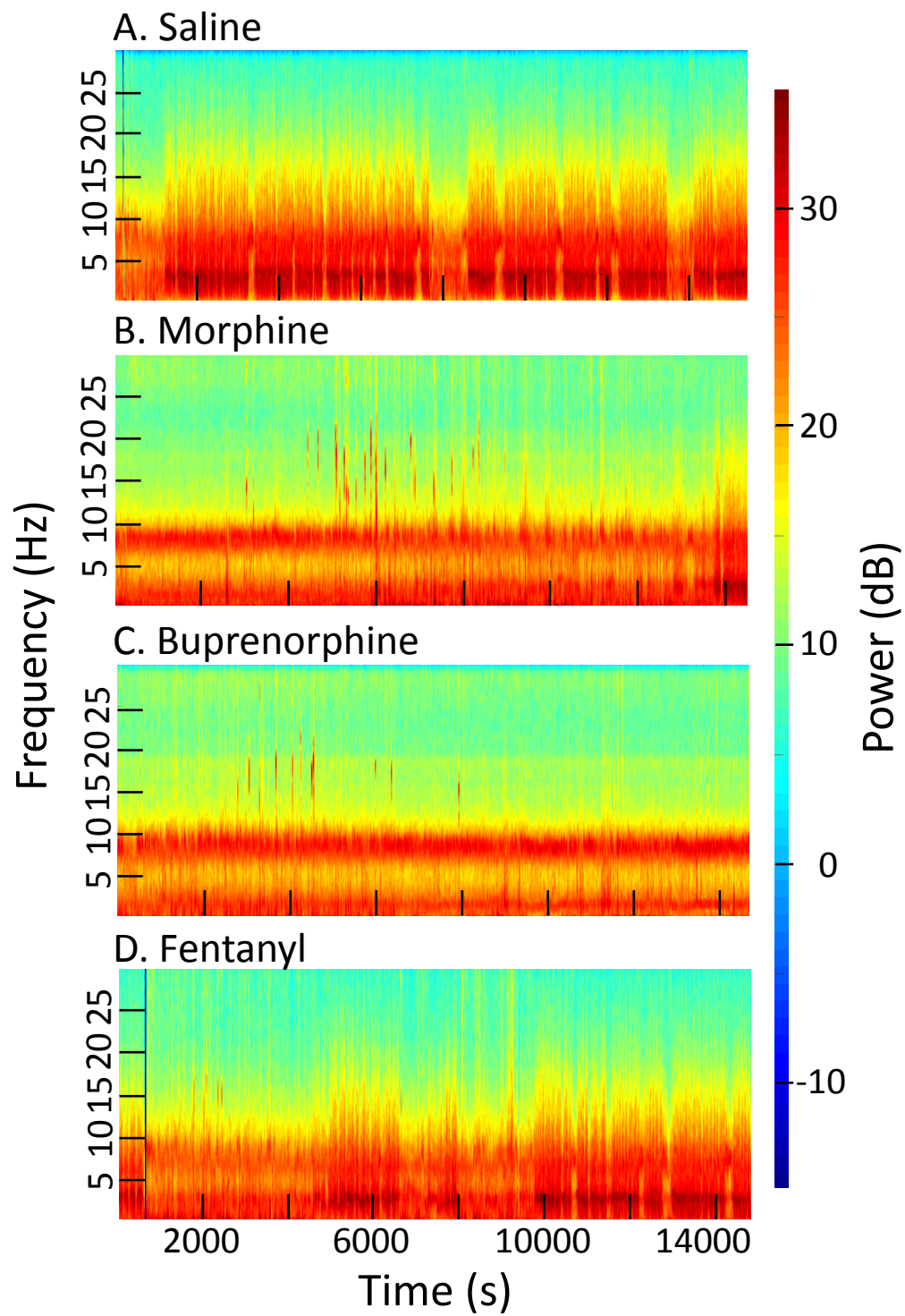


Fig. 2 O'Brien et al.

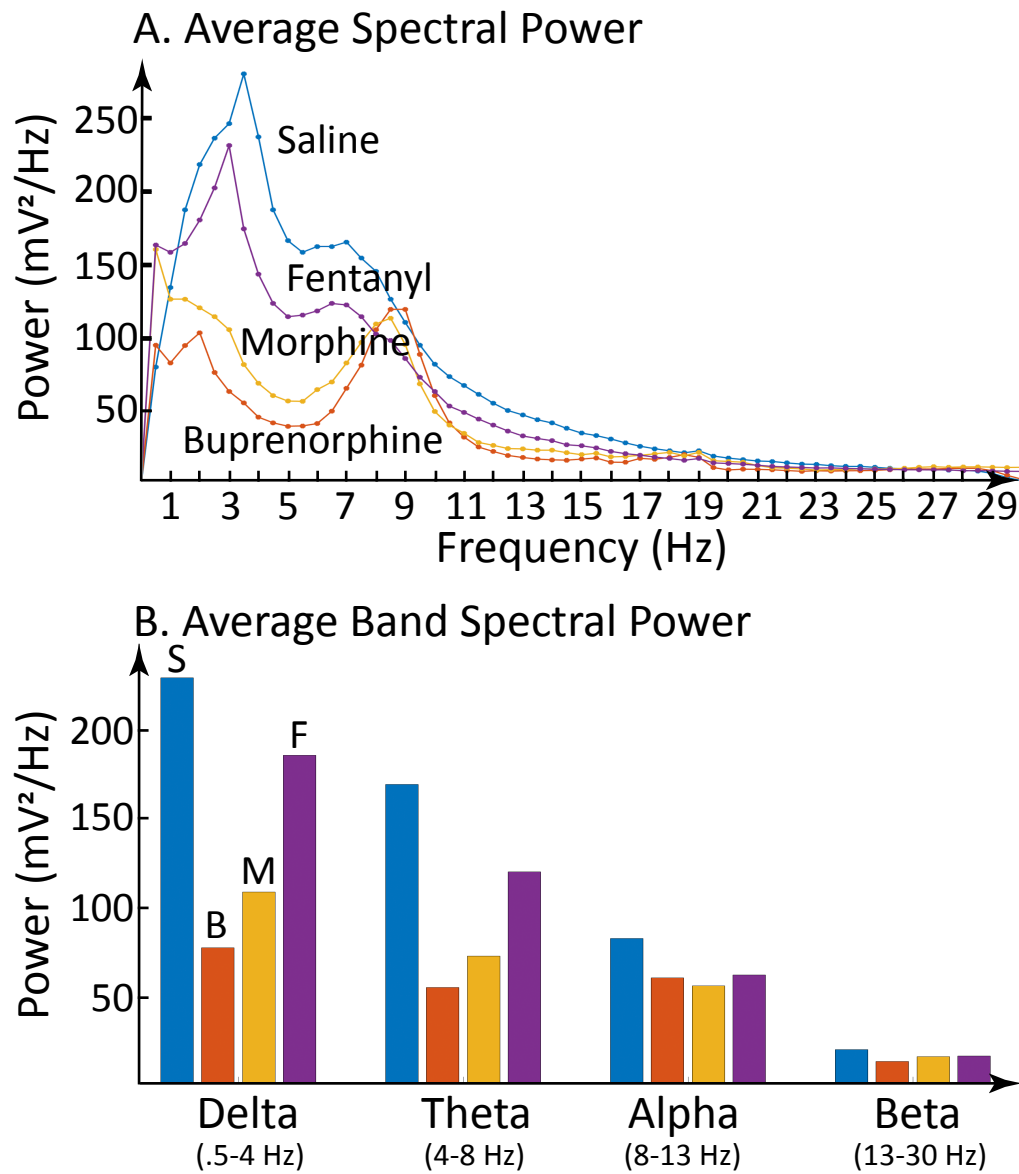


Fig. 3 O'Brien et al.

Name of Material/Equipment	Company	Catalog Number	Comments/Description
Dental acrylic	Lang Dental Manufacturing	C Jet powder and liquid	
EEG/EMG Amplifier	Data Science International	model MX2	
macOS Mojave	Apple	v10.14.4	
MATLAB	Mathworks	v9.4.0.813654	software for spectrogram comp.
Mouse anesthesia mask	David Kopf Instruments	model 907	
Neuroscore	Data Science International	v3.3.9317-1	software for scoring sleep and wakefulness
Ponemah	Data Science International	v5.32	software for EEG/EMG Data Acquisition
Stereotaxic frame	David Kopf Instruments	model 962	
Stereotaxic frame, mouse adapter	David Kopf Instruments	model 921	
Windows 10	Microsoft	v10.0.17763.503	
Wireless Telemeter	Data Science International	model HD-X02	

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We appreciate the prompt review and helpful suggestions for improving the manuscript (ms). Here we summarize concerns (in italics) and our response (regular font). All changes in the manuscript are identified by line number.

### Reviewer 1 Major concerns

1.1 *The authors should argue why multi-tapers are chosen as preferred method and not, e.g., windowed discrete Fourier Transform or wavelets.* As suggested, the ms has been revised to describe what we believe are major advantages of the multi-taper approach. We also acknowledge (with citations) some criticisms of multi-taper signal processing. Please see lines 51 to 59 of the revised manuscript (ms).

1.2 *The last table is not referred to in the text.* The revised ms corrects this omission which can now be seen on line 226 of the revised ms.

1.3 *In section 1.4, who are these "individuals": senior researchers or students ? Any prior knowledge on signal processing ?*

First author holds a B.S. degree in biomedical engineering

Second author <http://orcid.org/0000-0003-0627-2448>

Third author <http://orcid.org/0000-0002-2686-251X>

1.4 *In section 3.7.1, explain the keywords and give background information on fpass, padding, number of tapers, etc.* Thank you for stating that more clarification is needed. We have defined and provided background information of the parameters in section 3.7.1 of the revised ms, lines 188 to 212.

1.5 *In the figures, which multi-taper spectrogram parameters have been applied?* The parameters have now been stated in the figure captions of Figures 1 (lines 265 to 275) and 2 (lines 277 to 283).

### Reviewer 1 Minor concerns

1.6 *In figure 1 and 2, the right ordinate should be called 'colorbar'.* As suggested, "color bar" has been added for Figure 1 (line 269) and for Figure 2 (279)

### Reviewer 2 Major concerns

No major concerns noted.

### Reviewer 2 Minor concerns

2.1 *Please indicate experimental details such as company and cat# of EEG and EMG electrodes.* All equipment used for the data collection has been stated in the accompanying Table of Materials.

2.2 *Please indicate acquisition settings for EEG and EMG used for collection of example dataset.* Acquisition settings are defined in Section 1.13 on line 140.

2.3 *The author claim that this opensource code will be useful for other analysis - are they providing information on how to change the code for investigators that are not expert in coding*



*(i.e., people that would use this code)?* The purpose of the provided code is to create user generated multi-taper spectrograms for individuals that are not well-versed in computer programming. In the revised ms, we have provided supplemental materials for readers that are interested in tailoring the code to their own specific needs. Please see lines 332 to 335.

*2.4 Can this code be used to look at spectrograms from HPC LFP?* Yes, the code can be used to create spectrograms of hippocampal LFP recordings provided that the data format is .EDF or .CSV.

*2.5 It would be nice if the code could also help summarizing spectrogram results. Does the code allow for creating graphs and tables useful for publication from the results? It is not clear from the current manuscript.* Thank you for pointing out the need to address these points. The revised ms now explicitly states this on line 213-218.