**Submission ID #: 60333**

**Scriptwriter Name: Bridget Colvin**

**Project Page Link:** <http://www.jove.com/files_upload.php?src=18401613>

**Title:** **Computer-Based Multitaper Spectrogram Program for Electroencephalographic Data**

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**Author Questionnaire:**

**1.** Microscopy: Does your protocol involve video microscopy? No

**2.** Does your protocol demonstrate software usage? Yes

**3.** Which steps from the protocol section below are the most important for viewers to see?

n/a

**4.** What is the single most difficult aspect of this procedure and what do you do to ensure success?

n/a

**5.** Will the filming need to take place in multiple locations (greater than walking distance)? N

Section - Introduction

***Videographer: Interviewee Headshots are required. Take a headshot for each interviewee.***

1. **REQUIRED Interview Statements (Said by you on camera): All interview statements may be edited for length and clarity.**
   1. **Christopher O’Brien**: This protocol is significant because it enables users to develop customizable multi-taper spectrograms without prior signal processing knowledge **[1]**.
      1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.
   2. **Christopher O’Brien**: The main technical advantages of our program are the program's user-friendly design and the ability to create tapered spectrograms using computers without MATLAB licensing **[1]**.
      1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

**Ethics title card: (for human subjects or animal work, does not count toward word length total)**

* 1. Procedures involving animal subjects have been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Tennessee.

Section - Protocol

1. **Recording Electroencephalographic (EEG) Electrode Implantation and Initial Data Collection**
   1. Seven to ten days after the electrode implantation surgery, configure the data acquisition system to record all of the signals in millivolts **[1-TXT]** and obtain EEG recordings for the desired experimental duration **[2]**.
      1. WIDE: Talent at computer, configuring system, with monitor visible in frame**TEXT: See**[**https://www.jove.com/video/54908/high-density-electroencephalographic-acquisition-rodent-model-using**](https://www.jove.com/video/54908/high-density-electroencephalographic-acquisition-rodent-model-using) **for electrode implantation details**
      2. SCREEN: screenshot\_1: 01:21-01:41 *Video Editor: can speed up*
   2. Amplify and digitize the unfiltered EEG signals using the appropriate data acquisition instrumentation and software **[1]**.
      1. SCREEN: screenshot\_1: 01:51-02:01
   3. Then have two different individuals independently score each 10-second bin of the digital EEG (black trace) and EMG (blue trace) recordings as wakefulness, REM sleep, or non-REM sleep in an appropriate sleep scoring softwareprogram **[1-TXT]**.
      1. SCREEN: screenshot\_2: 00:12-00:22 **TEXT: REM: rapid eye movement**
2. **Spectrogram Computation**
   1. Download the compiled Multitaper Spectrogram Program. **[1]**.
      1. SCREEN: screenshot\_3: 00:24-00:33
   2. For spectrogram computation, obtain raw, unprocessed EEG data in an either EDF (E-D-F) or CSV (C-S-V) file format **[1-TXT]** and place the file into the same location as the compiled program file and launch the spectrogram program **[2]**.
      1. WIDE: Talent loading data, with monitor visible in frame **TEXT: For Windows, use compiled program, for Macintosh, run raw code file**
      2. SCREEN: screenshot\_3: 00:20-00:23
   3. Follow the pop-up prompts and select the appropriate file format, enter the entire EEG file name, and select the parameters for the spectrogram calculation **[1-TXT]**.
      1. SCREEN: screenshot\_3: 00:34-00:55 **TEXT: Calculation may take >3 min**
   4. Enter titles for both the spectrogram and EEG **[1]**.
      1. SCREEN: screenshot\_3: 03:20-03:38 *Video Editor: can speed up*
   5. Then click **File** and **Save** to save the resulting spectrogram and EEG trace in the desired file format **[1-TXT]**.
      1. SCREEN: screenshot\_3: 03:47-04:18 *Video Editor: speed up* **TEXT: See text for troubleshooting details**
   6. **Christopher O’Brien**: Include both the filename and the extension and place the file in the same location as the program. Do not include special characters or spaces in the file name **[1]**.
      1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Section – Results

1. **Results: Representative EEG Data** 
   1. This figure shows representative similarities and differences in the cortical EEG during wakefulness, non-REM sleep, and REM sleep **[1]**.
      1. LAB MEDIA: Figure 1A
   2. This hypnogram was used to plot the temporal organization of the states of sleep and wakefulness based on assessments of the EEG and EMG recordings **[1]**.
      1. LAB MEDIA: Figure 1B
   3. In contrast to the discretized hypnogram, a spectrogram can be used to illustrate highly dynamic changes in EEG frequency and power as a function of time **[1]** and to highlight the similarities between the cortical EEG signal **[2]** during wakefulness and REM sleep **[3]**.
      1. LAB MEDIA: Figure 1C
      2. LAB MEDIA: Figure 1C: JoVE Video Editor please add/emphasize WAKE arrow and text
      3. LAB MEDIA: Figure 1C: JoVE Video Editor please add/emphasize NREM and REM arrows and texts
      4. By comparing the WAKE, NREM, and REM segments one can visualize the changes in EEG power across the sleep/wake cycle.
   4. These multitaper spectrograms each summarize 4 hours of EEG recordings **[1]** after systemic administration of saline **[2]**, morphine **[3]**, buprenorphine **[4]**, or fentanyl **[5]**.
      1. LAB MEDIA: Figure 2
      2. LAB MEDIA: Figure 2: JoVE Video Editor please emphasize Saline graph
      3. LAB MEDIA: Figure 2: JoVE Video Editor please emphasize Morphine graph
      4. LAB MEDIA: Figure 2: JoVE Video Editor please emphasize Buprenorphine graph
      5. LAB MEDIA: Figure 2: JoVE Video Editor please emphasize Fentanyl graph
   5. This figure shows the use of spectrograms to visualize the effects of different opiates on cortical EEG power. The slow wave activity present in the saline condition**[1]**, is eliminated by morphine and buprenorphine **[2]** **[3]**.
      1. LAB MEDIA: Figure 2: JoVE Video Editor please place vertical boxes around the NREM/REM higher orange and yellow spikes in Morphine and Fentanyl graphs (as in Figure 1C) and label with NREM/REM text
      2. LAB MEDIA: Figure 2: JoVE Video Editor please place vertical boxes in .5-4 hertz range in Saline graph (similar to Figure 1C)
      3. LAB MEDIA: Figure 2: JoVE Video Editor please place vertical boxes around the WAKE shorter orange and yellow spikes in Morphine and Fentanyl graphs (as in Figure 1C) and add WAKE text
   6. After fentanyl administration, slow wave delta power can be observed **[1]**.
      1. LAB MEDIA: Figure 2: JoVE Video Editor please emphasize deep red bad at end of Morphine and Fentanyl graphs
   7. EEG changes illustrated by the spectrograms can be further quantified and expressed as the average dominate spectral power of each half frequency **[1]**.
      1. LAB MEDIA: Figure 3A
   8. For example, as illustrated in this graph, averaging the spectral power within specific EEG frequency bands revealed that **[1]**, in the 0.5 to 4-hertz frequency range, the EEG power was much higher with saline treatment **[2]** than with buprenorphine **[3]**.
      1. LAB MEDIA: Figure 3B
      2. LAB MEDIA: Figure 3B: JoVE Video Editor please emphasize blue S data bar in Delta data bar cluster
      3. LAB MEDIA: Figure 3B: JoVE Video Editor please emphasize red B data bar in Delta data bar cluster

Section - Conclusion

1. **Conclusion Interview Statements: (Said by you on camera) - All interview statements may be edited for length and clarity.**
   1. **Christopher O’Brien**: Mice with chronically implanted EEG and EMG electrodes remain healthy for many months, enabling novel studies of drug-drug interactions and chronic drug administration **[1]**.
      1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera
   2. **Christopher O’Brien**: Additionally, these techniques can provide new insights into efforts to develop chemical countermeasures to mitigate opiate-induced respiratory depression **[1]**.
      1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera