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# Electrophysiology for biomedical engineering students: a practical and theoretical course in animal electrocorticography

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**Albarracín AL, Farfán FD, Coletti MA, Teruya PY, Felice CJ.** Electrophysiology for biomedical engineering students: a practical and theoretical course in animal electrocorticography. *Adv Physiol Educ* 40: 402–409, 2016; doi:10.1152/advan.00073.2015.—The major challenge in laboratory teaching is the application of abstract concepts in simple and direct practical lessons. However, students rarely have the opportunity to participate in a laboratory that combines practical learning with a realistic research experience. In the Biomedical Engineering career, we offer short and optional courses to complement studies for students as they initiate their Graduation Project. The objective of these theoretical and practical courses is to introduce students to the topics of their projects. The present work describes an experience in electrophysiology to teach undergraduate students how to extract cortical information using electrocorticographic techniques. Students actively participate in some parts of the experience and then process and analyze the data obtained with different signal processing tools. In postlaboratory evaluations, students described the course as an exceptional opportunity for students interested in following a postgraduate science program and fully appreciated their contents.

electrocorticography; cortical oscillations; signal processing

BIOMEDICAL ENGINEERING PROGRAMS may be developed from a traditional engineering curriculum. This is the case at the Faculty of Exact Sciences and Technology of the University of Tucumán. The Biomedical Engineering Program focuses on electrical engineering related to biomedical applications. The curriculum includes fundamental biology courses such as Biology, Anatomy, and Human Physiology (four semester-based credits). However, the Biomedical Engineering career also offers optional courses that students can take during the last year of the program. These courses include theoretical and experimental classes designed to help students in the development of their Graduation Projects.

Studies in neurophysiology are not included in the core curricular program because of the complexity of the topics and the specialized equipment that students would have to master. However, it is possible to carry out experimental work with a reduced group of students interested in developing a final project in this scientific field.

The exercise described here is included in one of those optional courses, the Graduation Project. The course is taught jointly by instructors in neurophysiology and biomedical engineering, and students receive theoretical instruction on these topics before entering the laboratory.

It is important to note that these experiments, like others that we have previously described (1), are part of the instructor's research work; therefore, students participate in a real research work and the recordings obtained are used for further work.

Electrocorticography (ECoG), also called intracranial electroencephalography (EEG), is a signal recording technique that takes place under the skull but not in the brain itself. Compared with the acquired signals on the scalp (EEG), ECoG recordings have characteristics that make them particularly suitable for basic research in neuroscience. These features include a higher spatial resolution than EEG (i.e., 10ths of millimeters vs. centimeters), broader bandwidth (i.e., 0–200 vs. 0–40 Hz), higher amplitude (i.e., maximum of 50–100 vs. 10–20  $\mu$ V), and far less vulnerability to artifacts (6, 7). At the same time, because ECoG is recorded by subdural electrodes and thus does not require electrodes that penetrate into the cortex, it is likely to have greater long-term stability and might also be safer than single neuron recording (10, 14).

Intraoperative ECoG has been traditionally used in the surgical management of medically refractory partial epilepsies to identify the location and limits of the epileptogenic area, to guide the extent of resection, and to assess its completeness (3, 8). ECoG signals are mainly composed of the sum of postsynaptic potentials of the pyramidal neuron dendrites recorded directly from the exposed surface of the cortex. Population signals such as local field potentials reflect the coordinated activity of a small neural ensemble. ECoG recordings are a gap between the scalp (EEG) and multiunit recordings, an intermediate level of study that provides an estimate of the coordinated activity in larger neural populations than intracortical local field potentials (20).

The abundant connectivity between neurons in the brain is functionally reflected by the presence of oscillations that can be observed with ECoG recordings technique. In general, oscillations can be characterized by their frequency, amplitude, and phase. These signal properties can be extracted from neural recordings using time-frequency analysis. Amplitude changes are considered to result from changes in synchronization within a neural ensemble, also referred to as local synchronization. In general, rhythmic electroencephalographic activity is commonly subdivided into five major frequency bands:  $\delta$  (0.5–3.5 Hz),  $\theta$  (4–7 Hz),  $\alpha$  or  $\mu$  (8–12 Hz),  $\beta$  (13–30 Hz), and  $\gamma$  (30–200 Hz) (19, 5). For example, motor activity is associated with changes in  $\beta$ -frequency oscillations, which have a range of 15–30 Hz and peak at 20 Hz (4). Another prominent group of such brain oscillations has frequencies between 9–13 Hz in humans and 12–15 Hz in cats. These activities are known as

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“rolandic  $\mu$ -rhythms” in humans and sensorimotor rhythms in cats and rats (12, 21).

Movement or preparation for movement is typically accompanied by a decrease in  $\mu$ - and  $\beta$ -rhythms, particularly contralateral to the movement. This decrease has been labeled “event-related desynchronization.” Its opposite, rhythm increase or “event-related synchronization,” occurs after movement and with relaxation (13).

Although neural oscillations in human brain activity are mostly investigated using EEG recordings, they are also observed using more invasive recording techniques such as ECoG. The practice presented here consists in obtaining ECoG signals from the primary motor area, and the challenge for students is to identify and analyze these activities in the rat brain recordings by using suitable signal processing methods.

## MATERIALS AND METHODS

### Students

No more than five students could participate in this laboratory exercise. Students were given a handout on the experimental protocol and on the theoretical basis of the experiment. This included a background in ECoG and signal processing.

The experiment focused on ECoG recording of the motor cortex and the processing of this signal. Students assisted the instructor in the surgery for electrode implantation. After a postoperative period (required for animal recovery), students obtained cortical signals from an awake animal. A circular treadmill enclosed within a box of acrylic and wooden walls was designed and built in our laboratory to allow recording while the animal was moving. A stepper motor, activated by a controller system, allows treadmill movements to both sides. This apparatus is provided with a video camera for recording the animal gait during the ECoG signal acquisition.

The experiment was divided into the four activities.

### Procedure for Electrode Implantation

Although this procedure was performed by the instructor, students observed and took notes, especially about details concerning electrode placements. As the stereotaxic coordinates of the corresponding cortical area have been previously identified by researchers, it was not necessary to map the area.

The following materials were used for this procedure:

1. Heating pad
2. Stereotaxic device
3. Dissecting microscope
4. Surgical instruments
5. Physiological saline solution (0.9%)
6. Drill
7. Bipolar electrodes (PlasticsOne)
8. Dental cement

This procedure was performed in accordance with recommendations of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and institutional standards for the care and use of animals in research.

One Wistar adult rat weighting 250 g was anesthetized with ketamine-xylazine (150/10 mg/kg ip) and placed in a stereotaxic instrument (Narishige) with body temperature maintained at 37°C using a servo-controlled heating pad. The animal was regularly checked for hindpaw reflexes and injected with additional doses of anesthetic (20–30% of the initial dose) as needed.

Stereotaxic coordinates corresponding to hindlimb activation in the primary motor cortex were determined by the instructor in previous experiments (AP: -1, L: 2/-2) (12). These coordinates corresponded to the cortical motor area that activated hindlimb movements.

Two bipolar electrodes (MS303, PlasticsOne) were implanted subdurally. Two holes were made in the skull on either side of the midline following the previously specified coordinates. A third hole made in the occipital region served as a reference electrode. Special care was taken during the electrode placements in order not to damage cortical surface. Finally, the electrodes were fixed to the skull with dental acrylic, leaving free the connections for posterior recordings.

### Animal Habituation to the Treadmill

Habituation is an extremely simple form of learning, in which an animal, after a period of exposure to a stimulus, stops responding (17, 2).

Changes in the life of a rat can stress it. In this context, moving the animal to the treadmill and activating its movements can both produce animal stress. To reduce this problem, students habituated the animal to stay in the box during the treadmill movements.

Once the rat had recovered from the surgical procedure (~5 days), students placed the animal on the treadmill for a few minutes and for 5 consecutive days before the final experiment.

### Recordings in the Awake Animal

Before the beginning of the experiment, students were familiarized with the software of the acquisition system to be able to change the acquisition parameters for the different experiments. In addition, students learned how to retrieve the data obtained to analyze it after the laboratory work.

ECoG recordings were conditioned and acquired with a Biopac Student Lab PRO system. MP30 multichannel amplifier units (16 bits of analog-to-digital resolution) were set to acquire signals from two ECoG channels at 1,000 Hz (sample frequency), 120 dB (amplifier gain), and bandwidths of 0.05–100 Hz. The configuration of the acquisition parameters (sample rate, channel configurations, and recording time) were performed with BLS PRO software for Windows.

The treadmill box had a bright LED indicator that switched on at the time the treadmill was driven. At the same time, a pulse train was generated and recorded by the acquisition system to correlate ECoG recordings with the treadmill activation and video camera recordings (Fig. 1). Therefore, it was possible to synchronously record all the signals.

### Signal Processing

This part of the experiment included the following three activities:

*A. Analysis of video recordings.* Students identified animal positions (e.g., rest, walking, and running position) during the recording sessions (Fig. 2). Students used a marker to synchronize the video-graphic frames and ECoG acquisition system (Fig. 1).

*B. Time-frequency analysis of ECoG recordings.* The time-frequency analysis of ECoG recordings is shown in Fig. 3. The instructors recommended that students use the spectrogram via short-time Fourier transform because it is the time-frequency representation more commonly used (16, 15). The short-time Fourier transform (STFT) was defined as follows:

$$\text{STFT}\{x(n)\}(m, \omega) \equiv X(m, \omega) = \sum_{n=-\infty}^{\infty} x(n) \times \omega(n-m) \times e^{-j\omega \times n} \quad (1)$$

where  $x(n)$  is the discrete signal and  $\omega(n)$  is a window. The sequence  $x(n) \times \omega(n-m)$  is a short-time section of the signal at time  $n$ . The square modulus of the short-time Fourier transform is referred to as the spectrogram (SPEC) (22). The spectrogram was defined as follows:

$$\text{SPEC}\{x(t)\}(\tau, \omega) \equiv |X(\tau, \omega)|^2 \quad (2)$$

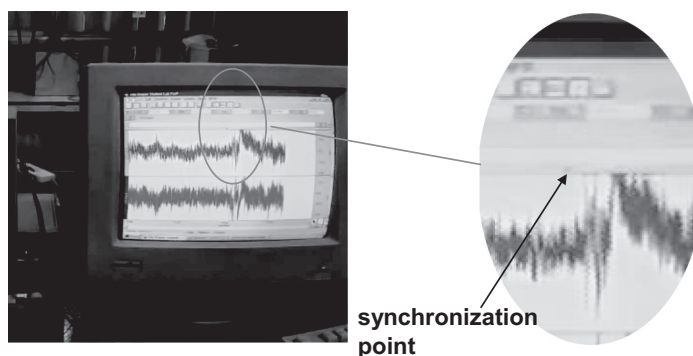
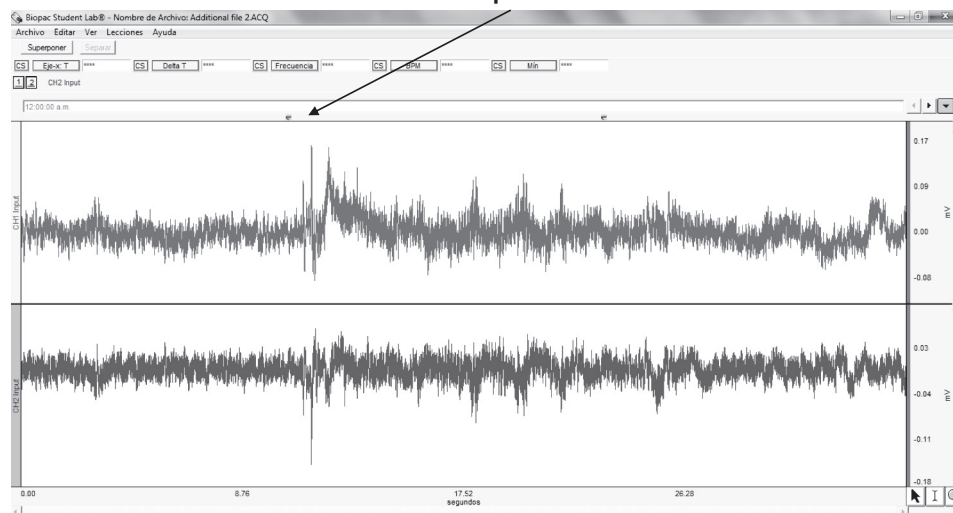


Fig. 1. Experimental setup for the electrocorticographic (ECoG) recordings. The synchronization point was at 10.55 s in the ECoG recording.



Thus, the spectrogram of ECoG signals was calculated using the following parameters: a Hamming window length of 250 samples, an overlap between segments of 96% (240 samples), and number of discrete Fourier transform points of 1,000.

Finally, a qualitative analysis of the spectrograms was required. *C. Identification of ECoG  $\beta$ -oscillations during the gait.* Students determined the duration and temporal location of  $\beta$ -oscillations from ECoG recordings (Fig. 4, A and B). For this, the instructor provided a

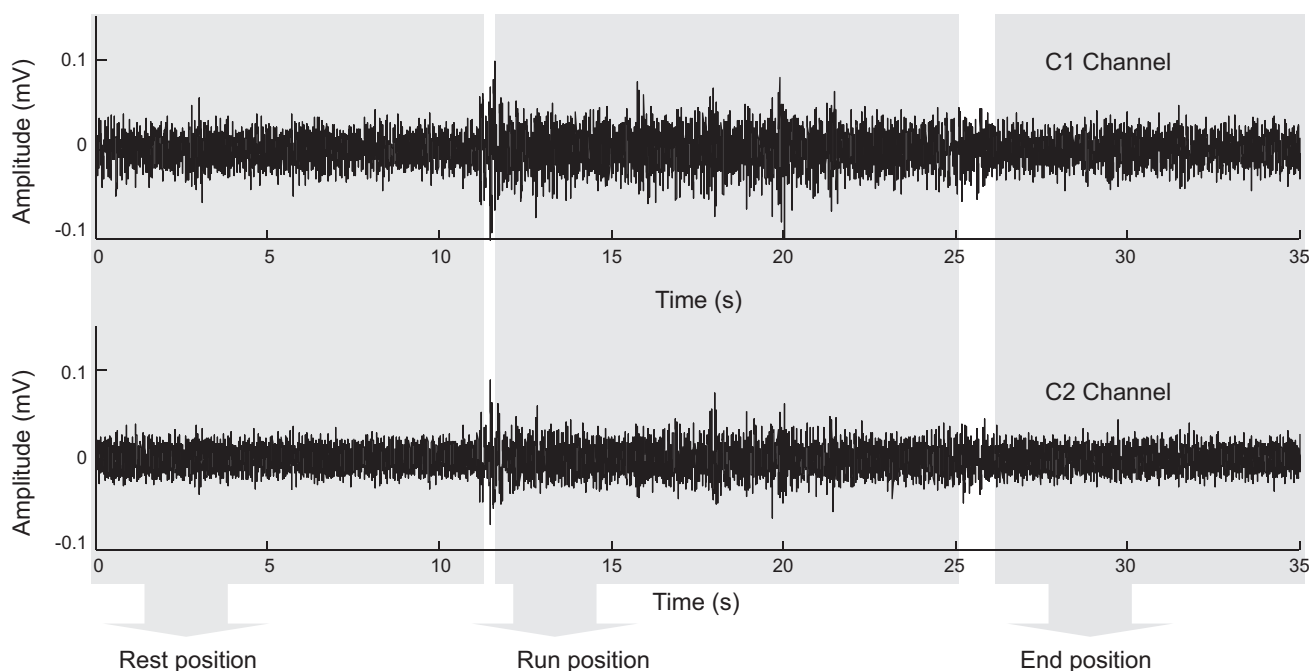


Fig. 2. ECoG recordings. Activity was recorded from the cortical motor area of the left hemisphere (superior recording) and right hemisphere (inferior recording).

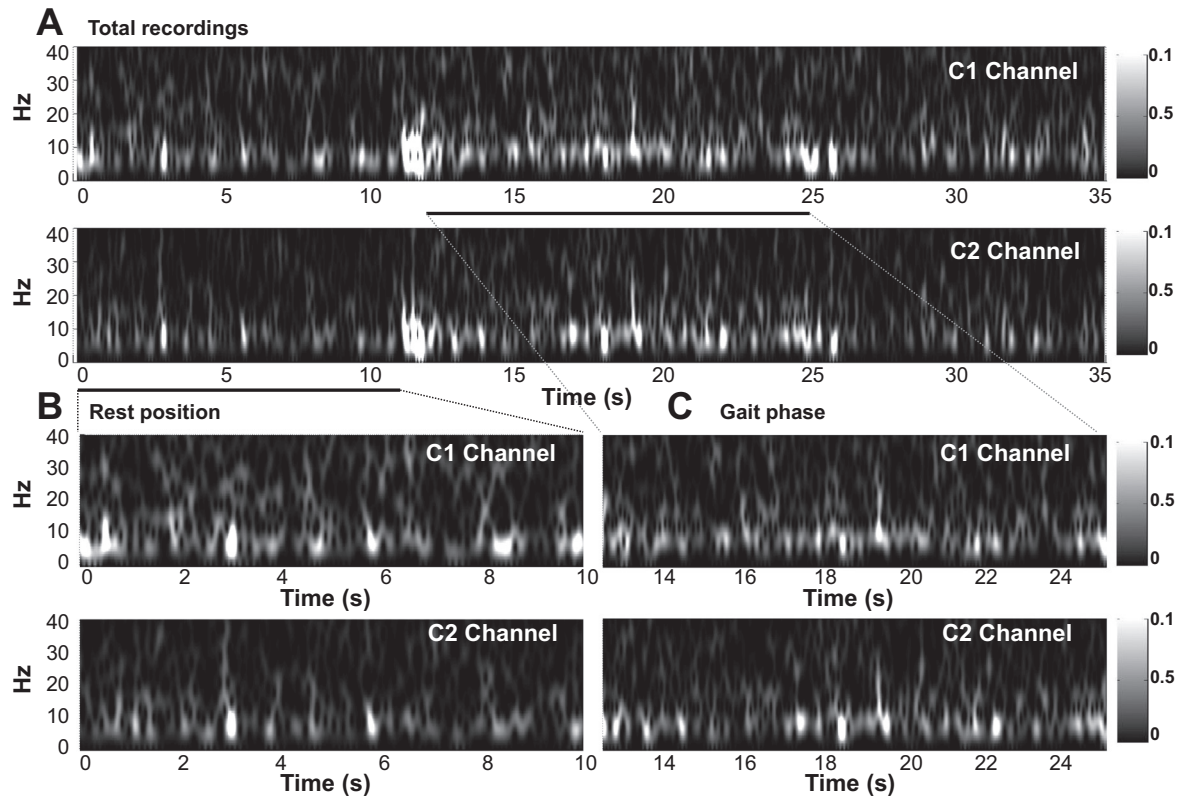


Fig. 3. Time-frequency analysis of ECoG recordings. A. Spectrograms obtained for the two channels recordings. B. Spectrograms corresponding to the rest and running phase of the animal gait.

number of specific tasks:

I. OBTAINING  $\beta$ -OSCILLATIONS FROM THE RAW ECG. For this, it was recommended to use a bandpass digital filter with cutoff frequencies of 15 and 30 Hz (Fig. 4C).

II. OBTAINING THE ENVELOPE OF  $\beta$ -OSCILLATIONS. The instructor provided fundamental concepts of envelope detection and a simple estimation method for its determination. The envelope temporal series was estimated through temporal location of the local maximum of  $\beta$ -oscillations. A linear or nonlinear interpolation between these was then performed (Fig. 4D).

III. DETERMINING THE MEAN AND SD OF THE ENVELOPE TIME SERIES. The calculation of these statistics was performed using the data of the envelope time series.

IV. DETERMINING THE DURATION AND TEMPORAL LOCATION OF  $\beta$ -OSCILLATIONS. The  $\beta$ -trigger (start point of one oscillation) was determined with a threshold method in which the threshold is the mean plus SD ( $\mu + \sigma$ ; Fig. 4D). Limits of  $\beta$ -activation (temporal duration) were determined with other threshold method. For this, the threshold was the mean minus SD ( $\mu - \sigma$ ; Fig. 4D).

Instructors encouraged students to perform maneuver data processing with Matlab, Octave, or Mathcad (processing softwares used in previous Biomedical Engineering courses).

## RESULTS AND DISCUSSION

### Student Reports

*Signal processing: the development of specific tasks.* Students had to process and analyze the data obtained from the experiment. They simultaneously recorded the ECoG signal and video images (Fig. 1). The recordings were saved as ACQ file (Biopac's AcqKnowledge data). In this way, ECoG activ-

ities were visualized and preprocessed with several computer programs: AcqKnowledge (a program of Biopac Systems) or another processing program such as Mathcad or Matlab. The video files were saved as AVI files.

A. *Analysis of video recordings.* Video images were analyzed by students to establish the animal positions and their corresponding ECoG recordings. They used a synchronization point between the "video image" and "ECoG recording." This synchronization point was established via an event marker provided by AcqKnowledge software (the acquisition system). The marker was detected in the video image at the time it was placed on the PC screen (Fig. 1). ECoG recordings with the event markers were stored as ACQ file (see Supplemental Material, Supplemental File S1).

ECoG recordings were exported to ASCII data using BSL 3.7.2 software (Biopac Systems) and displayed and preprocessed with Matlab. The reading and viewing of ECoG recordings were performed with the following commands:

```
A = dlmread('data_ECoG.txt'); % open
and load a txt file
t = A(:, 1); % time vector
C1 = A(:, 2); % ECoG data channel 1
C2 = A(:, 3); % ECoG data channel 2
figure; % create figure window
subplot(2, 1, 1); plot(t, C1, 'k');
```

<sup>1</sup> Supplemental Material for this article is available at the *Advances in Physiology Education* website.

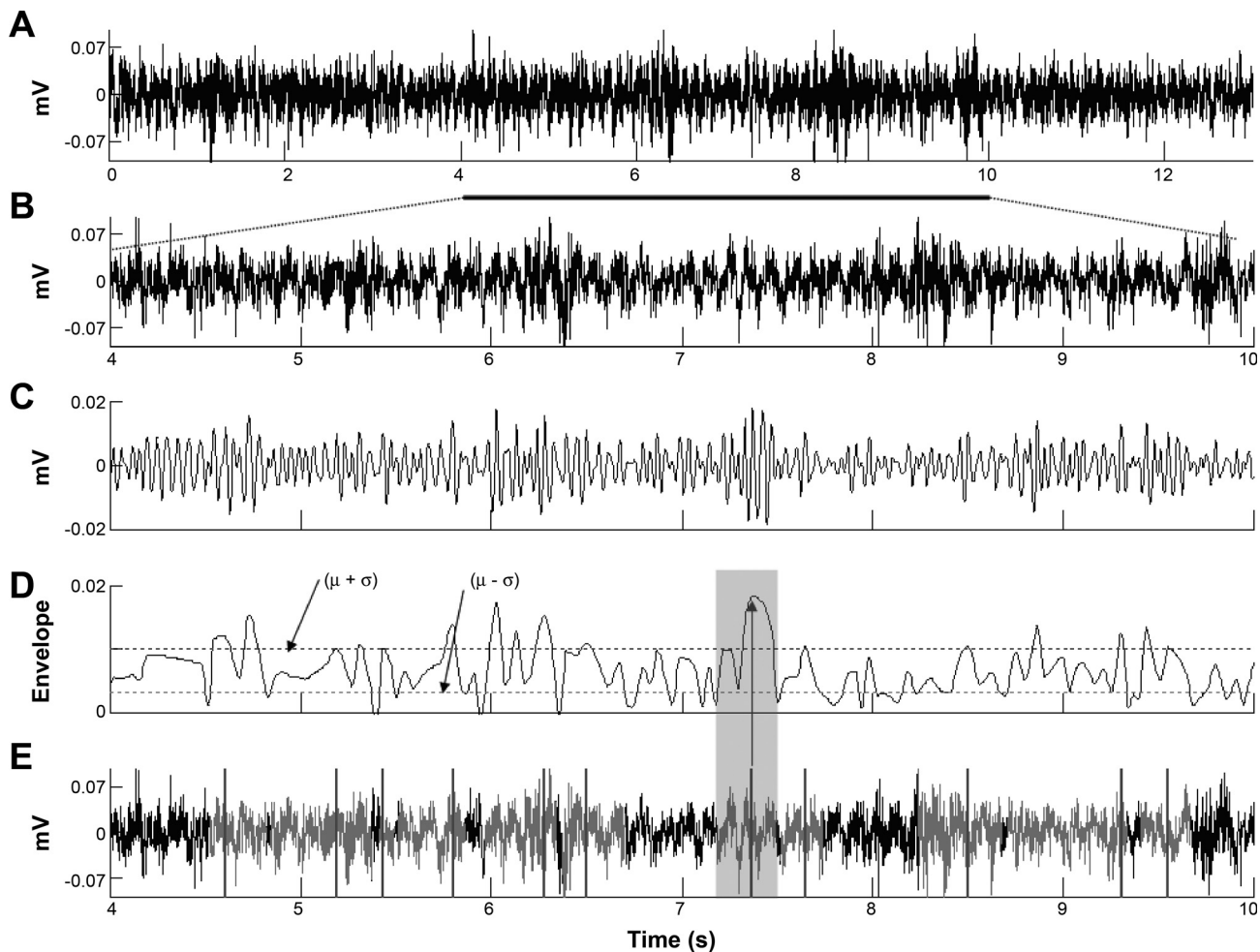


Fig. 4. ECoG recording and analysis of the signal. *A*: a section of a 13-s ECoG signal recorded during the animal gait. *B*: 6 s extracted and displayed from *A*. *C*: signal filtered with a bandpass digital filter (15 and 30 Hz). *D*: the envelope temporal series of  $\beta$ -oscillations obtained. The mean and SD are indicated by two horizontal dashed lines. *E*: temporal location and duration of  $\beta$ -oscillations (in gray) and the  $\beta$ -trigger (vertical gray lines).

```
% plots vector time versus vector C1
subplot(2,1,2);plot(t,C2,'k');
% plots vector time versus vector C2
```

**B. Time-frequency analysis of ECoG recordings.** Students performed a time-frequency analysis by calculating and displaying ECoG spectrograms (Fig. 3). These were calculated using the “spectrogram.m” Matlab function, which is part of the Signal Processing toolbox (Spectral Analysis). This function allows the ECoG spectrogram to be obtained using short-time Fourier transform and is implemented as follows:

```
fs = 1000;% sample frequency
[S1, F1, T1, P1]
= spectrogram(C1, 250, 240, 2000, fs);
% spectrogram of C1 data
[S2, F2, T2, P2]
= spectrogram(C2, 250, 240, 2000, fs);
% spectrogram of C2 data
figure;subplot(2,1,1);
surf(T1, F1, P1, 'edgecolor', 'none');
```

```
axis tight;view(0,90);
xlabel('Time(Sec)');
ylabel('Frequency(Hz)');
subplot(2,1,2)
surf(T2, F2, P2, 'edgecolor', 'none');
axis tight;
view(0,90);xlabel('Time(Sec)');
ylabel('Frequency(Hz)');
```

The input variables of the spectrogram function were as follows: C1 and C2, ECoG *channel 1* and *channel 2*, respectively; “250” was the Hamming window length; “240” was the number of samples that each segment overlaps; “2000” was the fast Fourier transform length; and fs was the sampling frequency. The output variables of spectrogram function were as follows: S, short-time Fourier transform, returned as a matrix; F, frequencies, returned as a vector; T, time instants, returned as a vector, and P, power spectral density or power spectrum, returned as a matrix.

ECoG spectrograms of two channels were calculated and visualized with the instructions described above (Fig. 3). Stu-

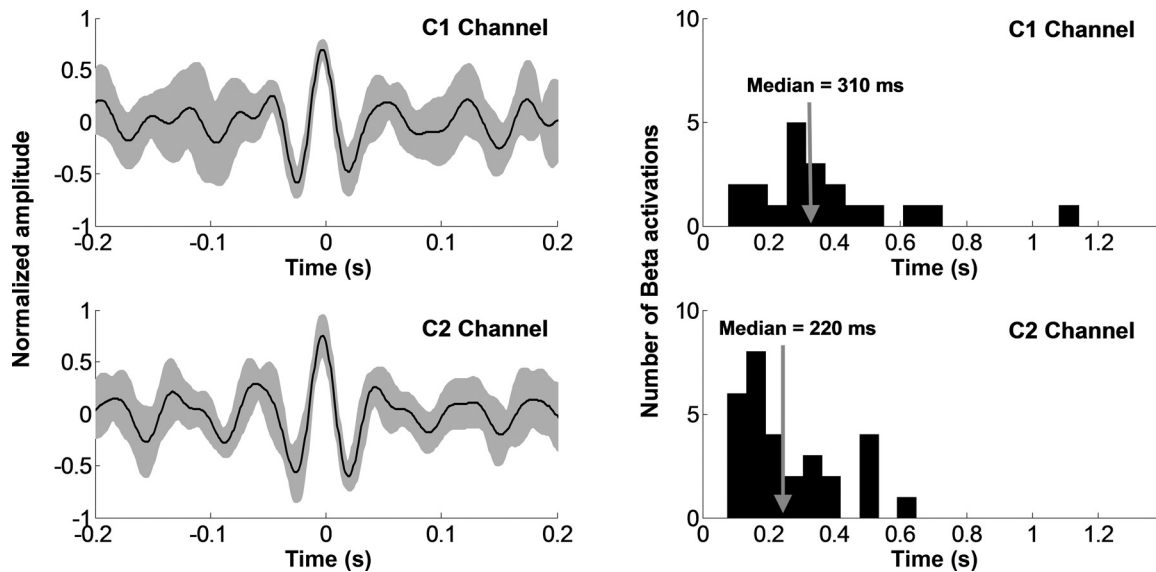


Fig. 5. Temporal duration of  $\beta$ -activations for *channel 1* and *channel 2*. The histograms (*right*) show the number and duration of  $\beta$ -oscillations detected for the two hemispheres.

dents visualized the spectral component changes due to animal position changes. From the analysis of video recordings (*item A*), students established the following: the animal was at rest from 0 to 11 s and remained in a dynamic gait state from 12 to 25 s. In this way, students established correlations between the oscillation frequency band obtained from the spectrogram and animal behavior.

The spectrogram showed frequency contents related to the rhythmic activation of the  $\mu$ -band (8–12 Hz) that spread out over the recording time extracted. The apparent absence of  $\beta$ -band oscillations in this context probably created confusion among students considering that the electrodes were placed over the M1 cortex. However, instructors clarified these results, explaining some technical and physiological concepts.

The results could be explained by the placement of electrodes very near the somatosensory area (S1); the electrodes received signals from the S1 contiguous area to the M1 area. The instructors explained to students that although the  $\beta$ -band oscillation (13–30 Hz) was not visible in the spectrogram, this does not mean that it was absent. It is probably that the  $\beta$ -rhythm remains hidden under the higher power of the  $\mu$ -band oscillations. The instructors recommended that students use other signal processing techniques to visualize higher-frequency bands. This is explained below.

*C. Identification of ECoG  $\beta$ -oscillations during animal behavior.*  $\beta$ -Oscillations were extracted from ECoG recordings using a bandpass digital Butterworth filter with cutoff frequencies of 15 and 30 Hz (Fig. 4B). This implementation was made with the “butter” function of Matlab software. The “butter” function designs an order  $N$  digital Butterworth filter with a normalized cutoff frequency of  $W_n$ .

$$H(z) = \frac{b(1) + b(2) \times z^{-1} + \dots + b(n+1) \times z^{-n}}{1 + a(2) \times z^{-1} + \dots + a(n+1) \times z^{-n}} \quad (3)$$

This function returns the filter coefficients in length  $n + 1$  row vectors  $b$  and  $a$ , with coefficients in descending powers of  $z$  (Eq. 3). It can be implemented as follows:

```
[b, a] = butter (5, [15 / 500 30 / 500]);
% the normalized cutoff frequencies
is
% defined as fc / (fs / 2) .
where fc is cutoff frequency in Hz
and fs is
sample
% rate, and (fs / 2) is the Nyquist rate.
beta_C1 = filter (b, a, C1);
beta_C2 = filter (b, a, C2);
```

Thus, students were able to generate Fig. 4C, which shows the  $\beta$ -synchronization periods. To determine the duration and temporal location of  $\beta$ -oscillations, students calculated the envelope of the signal (*task ii*).

The  $\beta$ -oscillation envelope was performed using concepts provided by the instructor, and students implemented it with the following commands:

```
upperind
= find (diff(sign(diff(bets_C1))) < 0)
+ 1; % determines the
% local maxima of beta_C1
upperind
= [1; upperind; length (beta_C1)];
% The numbers of initial and
% final samples of beta_C1 are added
to upper in dvector.
f = 1; l = length (beta_C1);
xi = f : l; % sample vector
env_C1
```

```
= interp1 (upperind, sig(upperind),
xi, method, 'cubic');
% this last command performs an
interpolation 'cubic'
% between consecutive local maxima
and it generates the envelope
% vector.
```

These procedures allowed students to obtain Fig. 4D. The mean and SD of the envelope were then obtained using the “mean.m” and “std.m” Matlab functions (*task iii*). The  $\beta$ -oscillation trigger was determined as follows:

```
%Local Peaks detection
% A threshold value is set to
mean value
plus a standard deviation
m1 = mean (env_C1(100 : end - 100));
% mean of envelope vector
s1 = std (env_c1(100 : end - 100));
% standard deviation of envelope vector
%Detection of values that exceed the
mean + std
p1 = find (env_C1 > m1 + s1);
% find indices of elements that are
greater
% thn mean + std
% A binary time series is generated
d1 = zeros (length(env_C1), 1);
d1(p1) = 1;
%Then, location of the peak maxima
is searched
aux1 = diff(d1); % edge detection
as1 = find (aux1 == 1); del = find (aux1
== 1);
fori = 1 : length(as1)
[aux, I] = max(env_C1(as1(i) : del(i)))
max1(i) = I + as1(i);
end
```

Similar procedures were used to determine the  $\beta$ -trigger of channel 2.

The temporal duration of  $\beta$ -activations was determined as follows:

```
%Temporal duration of beta activations
% A threshold
value is set to mean
value minus standard deviation.
%Detection of values that exceed
the mean - std
p1 = find (env_C1 > m1 - s1);
% find indices of elements
that are greater
%than mean - std
```

```
% A binary time serie is generated
d1 = zeros (length(env_C1), 1);
d1(p1) = 1;
% Then, rising and falling edges are
detected
aux1 = diff(aux1 == 1); %Edge detection
as1 = find (aux1 == 1); del = find(aux1 =
= -1);
%finally, temporal durations of beta
oscillations is calculated
fori = 1 : length(as1)
t1(i) = (as1(i) - del(i)) / fs;
end
```

The *t1* vector contains the temporal duration values of  $\beta$ -oscillations detected. Thus, students were able to create Figs. 4E and 5. The global temporal duration of  $\beta$ -oscillations in channel C1 was 310 ms, whereas that in channel C2 was 220 ms (median values of temporal durations; Fig. 5).

In this activity, students compared and analyzed the distributions of temporal durations of  $\beta$ -activations during the animal gait. Distributions of two channels were expected to be similar. However, the differences observed could be influenced by possible differences in electrode placements; i.e., the electrode in *channel 2* could be better positioned in relation to the area activated by hindlimb movements.

### Conclusions

The inclusion of this laboratory experience in the undergraduate program of Biomedical Engineering was a novel approach that generated a great interest in neurophysiology research. Despite student inexperience in electrophysiological recordings, they were excited to apply theoretical understanding of the underlying physiological processes discussed during the classes.

The most important goal of this course was that students develop basic insights into ECoG data processing and also explore alternative digital processing strategies for extracting specific features of biological signals. During the program students were trained in signal processing, but this was the first time they used them in a realistic electrophysiology experiment. In the laboratory experiment, students visualized ECoG signals and analyzed the different band activities evoked by different behavioral states to correctly make physiological interpretations. This also introduced students to animal behavior analysis with the help of video recordings techniques.

The results obtained from data processing analysis were discussed with students during the class to avoid misinterpretations of the electrophysiological findings. Although students attended the theoretical classes, the electrophysiological experiments involve technical difficulties and challenging interpretations that were guided by the instructor.

At the end of the course, students elaborated individual reports that were evaluated as evidence of their general comprehension of the concepts covered in this laboratory.

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#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

#### AUTHOR CONTRIBUTIONS

A.L.A., M.C., and P.T. performed experiments; A.L.A., F.D.F., and C.J.F. interpreted results of experiments; A.L.A. and F.D.F. drafted manuscript; A.L.A. edited and revised manuscript; F.D.F., M.C., and C.J.F. analyzed data; F.D.F. prepared figures.

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