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A software package for the study of REM-sleep microstructure

1 Introduction

This note describes the software package, written in the Matlab/Octave language, that we have applied in our laboratory to quantitatively determine the REM-sleep microstructure by analyzing the ElectroOculoGraphic (EOG) signal recorded during sleep.

The REM-sleep microstructure consists in the alternation of four sub-stages inside a REM-sleep period. This alternation is due to two important phenomena reported in the literature:

1) Rapid Eye Movements (REMs) are significantly present only in “phasic” sub-periods of REM-sleep periods, and are absent or poorly present during “tonic” sub-periods.

2) In addition to REMs, also Slow Eye Movements (SEMs) occur during REM-sleep periods.

The sub-stages are respectively characterized by:

No enhancement either of SEMs or REMs (sub-stage 1);

Selective enhancement of SEMs (sub-stage 2);

Selective enhancement of REMs (sub-stage 3);

Enhancement of both SEMs and REMs (sub-stage 4).

For each REM-sleep period, the analysis is carried out in four steps:

1) Two band-pass filters (0.2-0.6 Hz, and 1-3 Hz) are applied to the EOG signal to obtain the SEM component and the REM component, respectively.

2) The time course of the average absolute value over a moving window lasting 2.5 seconds is calculated for both components. The difference between this average and the average of the absolute value over the entire REM-sleep period is then divided by the average amplitude over the entire period. In other words, a comparison is made between

the quasi-instantaneous amplitude and the background amplitude of both components. Two non-dimensional descriptors are thus obtained.

3) A sub-period of the REM-sleep period is considered as characterized by an enhancement of one of the two components if the relative descriptor is greater than 0 over the entire sub-period and greater than 1 at least in one instant of the sub-period.

4) Finally, each instant of the REM-sleep period is assigned to one of the four sub-stages.

The numerical values of the parameters (cutoff frequencies, time length of the moving window, and thresholds applied to the descriptors) have been suggested by the literature, including the literature about the NREM microstructure.

The package consists of seven functions.

The functions “component_calc.m”, “average_calc.m”, “transient_simpl.m”, and “segmentation.m” are successively executed for the analysis of a single REM-sleep period. All of them are called by “microstr.m”, which thus provides the thorough characterization of the microstructure of a REM-sleep period.

In turn, a thorough analysis of all the REM-sleep periods of a night sleep is obtained by running “root_function.m”. In fact, this function calls the function “epoca.m” to extract the portions of the EOG signal that correspond to the REM-sleep periods, and then calls “microstr.m” for each period.

The package can be downloaded here:
https://github.com/massimomagrini/rem_microstructure

2 Description of the package functions

component_calc.m

```
[epoch_comp] = component_calc (epoch, f1, f2, sf)
```

This function applies an ideal rectangular filter in the frequency domain to the input signal *epoch* (the portion of the EOG signal corresponding to the considered REM-sleep period). The cutoff frequencies of the filter are *f1* and *f2* (in Hz) (f1 = 0.2 for SEMs and 1.0 for REMS; f2 = 0.6 for SEMs and 3.0 for REMs). *sf* is the sampling rate (in Hz). For each REM-sleep period, this function is applied twice, to respectively obtain the SEM component and the REM component.

average_calc.m

```
[moving_average] = average_calc (epoch_comp, time_window, sf)
```

The input *epoch_comp* is the SEM (and then the REM) component of the EOG signal over a REM-sleep period (the output of “component_calc.m”). The output of “average_calc.m” is the convolution of the absolute value of the component with a rectangular window lasting *time_window* (for our analyses, time_window = 2.5 s).

transient_simpl.m

```
[initial_time, final_time] = transient_simpl (moving_average,
thresh0, thresh1,sf)
```

The input *moving_average* is the output of the function “average_calc.m” applied first to the SEM component and then to the REM component. For our analyses, thresh0 = 0 and thresh1 = 1.
The output *initial_time* is a vector whose components are the initial times (in seconds) of the sub_epochs that are characterized by an enhancement of the EOG component. The output *final_time* is a vector whose components are the end times (in seconds) of the sub_epochs that are characterized by an enhancement of the EOG component. All these times are given with respect to the beginning of the recording.

segmentation

```
[epoch_iss, epoch_sub_perc] = segmentation (epoch, SEM_initial,
SEM_final, REM_initial, REM_final, sf)
```

The input vectors *SEM_initial*, *SEM_final*, *REM_initial*, and *REM_final* are the outputs of the function “transient_simpl.m” respectively applied to the moving average relative to the SEM component and to the moving average relative to the REM component.
The output *epoch_iss* (instantaneous sub-stage) gives the value of the sub-stage (equal to 1, or 2, or 3, or 4) for each instant of the considered REM-sleep epoch. The output

epoch_sub_perc gives the time percentage for each sub-stage in the considered REM-sleep epoch.

microstr.m

```
[epoch_iss, epoch_sub_perc] = microstr (epoch, sf)
```

This function has the same output of “segmentation.m”. It simply allows to orderly execute the analysis of the considered EOG portion (input *epoch*).
The code of this function, which is here reported, provides a clear description of the rationale of the analysis method.

```
[SEM_comp] = component_calc (epoch, 0.2, 0.6, sf);
[REM_comp] = component_calc (epoch, 1., 3., sf);
[SEM_moving_average] = average_calc (SEM_comp, 2.5, sf);
[REM_moving_average] = average_calc (REM_comp, 2.5, sf);
[SEM_initial, SEM_final] = transient_simpl (SEM_moving average, 0., 1., sf);
[REM_initial, REM_final] = transient_simpl (REM_moving_average, 0., 1., sf);
[epoch_iss, epoch_sub_perc] =
        segmentation (epoch, SEM_initial, SEM_final, REM_initial, REM_final, sf);
```

epoca.m

```
[epoch] = epoca (trace, start_time, time_length, sf)
```

This function is applied to obtain the portions of the EOG signal that correspond to REM-sleep periods. The output *epoch* is the portion of *trace* (the EOG signal over the entire night recording) corresponding to the epoch lasting *time_length* (in seconds) starting from *start_time* (in seconds). The values of s*tart_time* and *time_length* are those given by sleep-stage scoring.

root_function.m

```
[csi, iss, sub_perc, epochs, duration] = root_function
(filename, start_time, end_time, sf)
```

While the vectors *epoch_iss* and *epoch_sub_perc,* which are the output of “microstr.m”, are related to a single REM-sleep epoch, *iss* and *sub_perch* are matrices that are related to all the REM-sleep epochs of the night sleep. In fact, *sub_perc* (n, k) indicates the percentage of sub-stage *k* (k = 1, 2, 3,4) for the nth REM-sleep period, and *iss* (n, i) indicates the instantaneous values of sub-stage *k* for the nth REM-sleep period (the index *i* corresponds to the instant (i/sf – 1) (in seconds), *sf* being the sampling rate (in Hz).
The matrix *epochs* (n, i) gives the values of the EOG portion corresponding to the nth REM-sleep period at the instant (i/sf – 1), in the interval from i = 1 to i = duration (n) * sf.
The output *csi* gives the number of sampled points in the EOG signal recorded during

the entire night. The input variable *filename* indicates the ascii file containing this EOG signal. The output vector *duration* (n) indicates the duration of the nth REM-sleep period.

3 Example

Figure 1 gives an example of analysis. The analyzed EOG signal is relative to the second REM period (lasting 510 s) of the polygraphic recording labeled as "n2" that we have downloaded from the website *physionet.org* (see reference below)
The aim of this example is to illustrate the intermediate steps that allow the final calculation of the instantaneous sub-stages. To build the six graphs of the figure, the function was applied whose code we report below. The file "n2_RocLoc.ascii" was extracted by file "n2.edf" in the database, as well as the value of the sampling frequency and the formula for expressing the signal values in μV. The values of the initial time and of the final time of the second REM-sleep period were drawn from the stage scoring reported in the database. The output vectors of the function are the six vectors plotted in the figure. The code is the following:

```
function [g1, g2, g3, g4, g5, g6] = example_graphs
a = load ('n2_RocLoc.ascii', '-ascii'); % The eog file (subject n2) has
been loaded
a = a';
for i = 1: size (a,2)
   a(i) = (a(i) + 10922) * 0.0305191356894342 - 333.33;
endfor % The eog values are now expressed in microvolt
sf = 128.; % Sampling frequency (Hz)
start_time_2 = 23790; % Start time (in seconds) of the second REM-sleep
period
end_time_2 = 24300; % End time (in seconds) of the second REM-sleep
period
time_length_2 = end_time_2 - start_time_2;
[g1] = epoca (a, start_time_2, time_length_2, sf); % eog signal, 2nd
period
[g2] = component_calc (g1, 0.2, 0.6, sf); % SEM component
[g3] = component_calc (g1, 1., 3., sf); % REM component
[SEM_average] = average_calc (g2, 2.5, sf); % moving average (SEMs)
[REM_average] = average_calc (g3, 2.5, sf); % moving average (REMs)
SEM_average_mean = mean (SEM_average);
REM_average_mean = mean (REM_average);
g4 = SEM_average ./ SEM_average_mean; % SEM non-dimensional descriptor
g5 = REM_average ./ REM_average_mean; % REM non-dimensional descriptor
% The two following instructions provide the initial and final times
% of the sub-periods respectively characterized by SEM and REM
% enhancement
[SEM_initial, SEM_final] = transient_simpl (SEM_average, 0., 1., sf);
[REM_initial, REM_final] = transient_simpl (REM_average, 0., 1., sf);
% The output g6 of the next instruction gives the final segmentation of
% the considered REM-sleep period into sub-stages
[g6, epoch_sub_perc] = segmentation (g1, SEM_initial, SEM_final,

REM_initial, REM_final, sf);
```

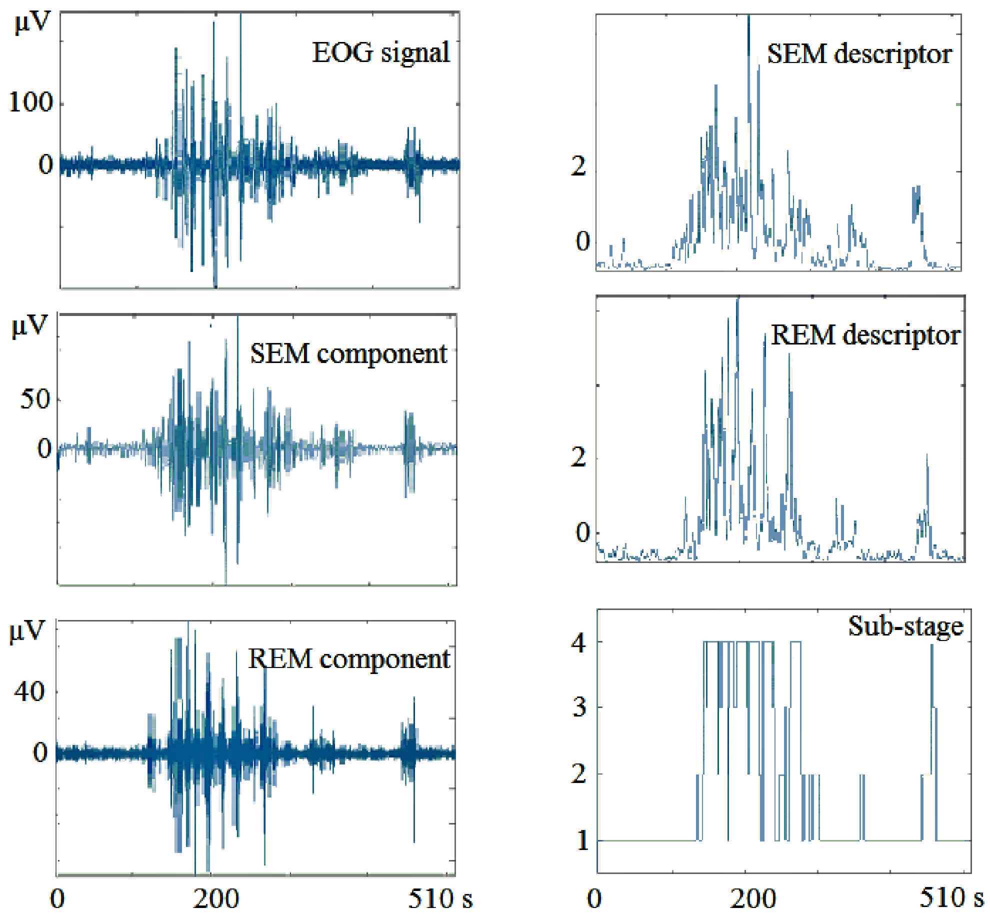

Figure 1. Analysis of the EOG signal relative to the second REM period (lasting 510 s) of the polygraphic recording labeled as "n2" (see reference below).
From top: Left column: EOG signal, SEM component, REM component; Right column: SEM non-dimensional descriptor, REM non-dimensional descriptor, instantaneous sub_stage.

The EOG data used in the example have been downloaded at this web address: *https://physionet.org/content/capslpdb/1.0.0/.*

The standard citations for this site are:

[1] MG Terzano, L Parrino, A Sherieri, R Chervin, S Chokroverty, C Guilleminault, M Hirshkowitz, M Mahowald, H Moldofsky, A Rosa, R Thomas, A Walters. Atlas, rules, and recording techniques for the scoring of cyclic alternating pattern (CAP) in human sleep. Sleep Med 2001 Nov; 2(6):537-553.
[2] Goldberger, A., Amaral, L., Glass, L., Hausdorff, J., Ivanov, P. C., Mark, R., ... & Stanley, H. E. (2000). PhysioBank, PhysioToolkit, and PhysioNet: Components of a new research resource for complex physiologic signals. Circulation [Online]. 101 (23), pp. e215–e220.