jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

AUTHOR's COPY

BrainBeats: an open-source EEGLAB plugin to jointly analyze EEG and cardiovascular (ECG/PPG) signals

Cédric Cannard[1,2], Helané Wahbeh[2], Arnaud Delorme[1,2,3]

[1] Centre de Recherche Cerveau et Cognition (CerCo), CNRS, Toulouse III University, France
[2] Institute of Noetic Sciences (IONS), Novato, California, USA.
[3] Swartz Center of Computational Neuroscience (SCCN), INC, UCSD, La Jolla, USA

KEYWORDS: open-source, EEGLAB, EEG, ECG, PPG, HRV, qEEG, feature-analysis, HEP/HEO, brain-heart interplay, heart artifact removal.

SUMMARY: The BrainBeats toolbox is an open-source EEGLAB plugin designed to analyze EEG and cardiovascular (ECG/PPG) signals jointly. It offers three main protocols: heartbeat-evoked potentials assessment, feature-based analysis, and heart artifact extraction from EEG signals. It should aid researchers and clinicians in studying brain-heart interactions with enhanced reproducibility and accessibility.

ABSTRACT: The link between the cortical and cardiovascular systems is garnering increased attention due to their potential to offer valuable insights into brain and heart function coupling. EEG and ECG/PPG provide non-invasive, cost-effective, and portable solutions for capturing brain-heart interactions in laboratory, clinical, or real-world settings. However, scalable applications in this domain are limited due to technical challenges and the lack of guidelines. Existing tools often lack statistical methodologies, easy-to-use user interface, or automated capability for large datasets, crucial for reproducibility. A further void exists in standardized quantitative EEG (qEEG) and heart-rate variability (HRV) feature extraction methods, undermining clinical diagnostics or the robustness of machine learning (ML) models. In response to these challenges, we introduce the BrainBeats toolbox, implemented as an open-source EEGLAB plugin providing a suite of signal processing and feature-extraction functions. The toolbox integrates three main protocols: 1) Heartbeat-evoked potentials (HEP) and oscillations (HEO) for assessing time-locked brain-heart interplay at the millisecond accuracy; 2) qEEG and HRV feature extraction for examining associations between various brain and heart metrics or building robust feature-based ML models; 3) Automated extraction of heart artifacts from EEG signals to remove any potential cardiovascular contamination while conducting EEG analysis. We provide a step-by-step tutorial for performing these three methods on an open-source dataset containing simultaneous 64-channel EEG, ECG, and PPG. A range of parameters can be tuned to tailor unique research needs via either the graphical user interface (GUI) or the command line. BrainBeats should make brain-heart interplay research more accessible and reproducible.

INTRODUCTION:

For a long time, the reductionist approach has dominated scientific inquiry in human physiology and cognition. This approach involved dissecting complex bodily and mental processes into smaller, more manageable components, allowing researchers to focus on individual systems in isolation. This strategy arose due to the immense challenge of studying the intricate and interconnected nature of the human body and mind[1]. Reductionism has been instrumental in understanding individual subsystems in isolation, such as elucidating the role of ion channels and action potentials for neural[2] or cardiac[3] communication. However, a significant gap remains in understanding how these isolated systems interact on a larger spatial and temporal scale. The multimodal (integrative or ecological) framework considers the human body a complex multidimensional system, where "the mind is seen not as a product of the brain but as an activity of the living being; an activity which integrates the brain within the everyday functions of the human body."[4] The multimodal and reductionist approaches are not exclusive, just like we cannot study one neuron without the whole brain or the whole brain without understanding individual neuron properties. Together, they pave the way for a more comprehensive, synergetic understanding of human health, pathology, cognition, psychology, and consciousness. The present method aims to ease the multimodal investigation of the interplay between the brain and the heart by providing joint analysis of electroencephalography (EEG) and cardiovascular signals, namely electrocardiography (ECG) and photoplethysmography (PPG). This toolbox, implemented as an EEGLAB plugin in MATLAB, addresses existing methodological limitations and is made open source to facilitate accessibility and reproducibility in the scientific area. It implements the latest guidelines and recommendations into its design and default parameters to encourage users to follow known best practices. The proposed toolbox should be a valuable resource for researchers and clinicians interested in 1) extracting features from EEG and ECG/PPG signals, 2) studying heartbeat-evoked potentials, or 3) removing heart artifacts from EEG signals.

Heart-brain research: which measures?

The relationship between the heart and the brain has been historically studied via neuroimaging methods such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). Using these tools, researchers highlighted some brain regions associated with cardiovascular control (e.g., manipulation of heart rate and blood pressure[5]), showed the influence of heart rate on the BOLD signal[6], or identified potential brain-body pathways contributing to coronary heart disease (i.e., stress-evoked blood pressure[7]). While these studies have significantly advanced our understanding of the complex interplay between the central nervous system (CNS) and cardiovascular function, these neuroimaging techniques are expensive, have limited availability, and are confined to controlled laboratory settings, which restricts their practicality for real-world and large-scale applications.

In contrast, EEG and ECG/PPG are more affordable and portable tools that offer the potential for studying brain-heart interactions in more diverse settings and populations or over longer periods, providing new opportunities. ECG measures the electrical signals generated by each heartbeat when the heart contracts and relaxes via electrodes placed on the skin (usually on the chest or

arms). PPG measures blood volume changes in the microvascular tissues (i.e., blood flow and pulse rate) using a light source (e.g., LED) and a photodetector (commonly placed on a fingertip, wrist, or forehead), relying on how blood absorbs more light than the surrounding tissue. Both methods provide valuable information about cardiovascular function but serve different purposes and offer distinct data types. Like ECG, EEG records the electrical fields generated by the synchronized activity of thousands of cortical neurons that propagate through the extracellular matrix, tissues, skull, and scalp until they reach the electrodes placed on the scalp's surface. As such, the use of EEG and ECG/PPG holds great promise for advancing our understanding of the physiological, cognitive, and emotional processes underlying brain-heart interactions and their implications for human health and well-being. Therefore, capturing heart-brain interplay from EEG, ECG/PPG signals with the BrainBeats toolbox may be particularly useful for the following scientific areas: clinical diagnostic and forecasting, big data machine learning (ML), real-world self-monitoring[8], and mobile brain/body imaging (MoBI)[9,10].

Two approaches for jointly analyzing these signals

There are two main approaches to studying interactions between EEG and cardiovascular signals:

1) The heartbeat-evoked potentials (HEP) in the time domain (i.e., event-related potentials; ERP) and the heartbeat-evoked oscillations (HEO) in the time-frequency domain (i.e., event-related spectral perturbations; ERSP). This approach examines how the brain processes each heartbeat with millisecond (ms) accuracy, requiring that both time series are time-locked (i.e., synchronized) and that the heartbeats are marked with events in the EEG signals. This approach has gained interest in recent years[11–16].

Feature-based: this approach extracts EEG and heart-rate variability (HRV) features from continuous signals and examines associations between them. This has been done independently for EEG (often termed "quantitative EEG" or qEEG[17]), ECG[18–20], and PPG[21–23]. This approach presents promising applications by capturing both state- and trait-related variables. Note that, for both EEG and cardiovascular signals, the longer the recording, the more dominant the trait variable[24–26]. Thus, the applications depend on the recording parameters. Feature-based analyses are gaining growing interest, providing new quantitative metrics for forecasting the development of mental and neurological disorders, treatment-response, or relapse[27–32]. This approach is especially compelling with large and real-world datasets (e.g., clinic, remote monitoring), which can be more easily obtained thanks to the recent innovations in wearable neurotechnology[8]. A less explored application is the identification of associations between specific brain and heart features, highlighting potential underlying central nervous system dynamics. Heart-rate variability (HRV) can be calculated from both ECG and PPG signals. It provides information about the autonomous nervous system (ANS) by measuring the variations in time intervals between heartbeats (i.e., the normal-to-normal intervals)[24]. Increased sympathetic (SNS) activity (e.g., during stress or exercise) typically reduces HRV, while parasympathetic (PNS) activity (e.g., during relaxation) increases it. A slower breathing rate generally increases HRV due to enhanced PNS activity, especially for short recordings (<10 minutes)[24]. Higher HRV scores generally suggest a more resilient and adaptable ANS, while a lower HRV can indicate stress, fatigue, or underlying

health issues. Long HRV recordings (i.e., at least 24 hours) provide a predictive prognosis for various health conditions, including cardiovascular diseases, stress, anxiety, and some neurological conditions[24]. Measures like blood pressure, heart rate, or cholesterol levels give information about the cardiovascular system's status. In contrast, HRV adds a dynamic aspect, showing how the heart responds to and recovers from stress.

BrainBeats' advantages over existing methods

While many tools exist to process cardiovascular and EEG signals independently from one another, none is currently available for jointly analyzing them. Furthermore, most available means to process cardiovascular signals involve costly licensing, do not allow automated processing (especially beneficial for large datasets), have proprietary algorithms that prevent transparency and reproducibility, or require advanced programming skills by not providing a graphical user interface (GUI). To our knowledge, four open-source MATLAB toolboxes support HEP/HEO analysis with a GUI. The *ecg-kit* toolbox[33], the BeMoBIL pipeline[34], the *HEPLAB* EEGLAB plugin[35], and the *CARE-rCortex* toolbox[36]. While *HEPLAB, BeMoBIL,* and *ecg-kit* facilitate HEP analysis by detecting heartbeats and marking them in the EEG signals, they do not provide statistical analysis or are limited to the time domain (i.e., HEP). The *CARE-rCortex* plugin addressed these issues by supporting ECG and respiratory signals, time-frequency domain analysis, statistics, and advanced baseline normalization and correction methods adapted to HEP/HEO analysis. However, it uses the Bonferroni method for statistical correction of the type 1 error (i.e., false positives), which is too conservative and not physiologically sound for EEG applications, leading to an increase in type II errors (i.e., false negatives)[37]. Furthermore, the toolbox does not offer command-line access for automation. Finally, recent studies recommend against baseline correction methods [38–40], as they reduce the signal-to-noise ratio (SNR) and are "statistically unnecessary and undesirable" (see abstract[38]).

To address these limitations, we introduce the *BrainBeats* toolbox, which is currently implemented as an open-source EEGLAB plugin in the MATLAB environment. It incorporates the following advantages over previous methods:

I) An easy-to-use GUI (for non-programmers) and command-line capabilities (for programmers aiming to perform automated processing).

II) validated algorithms, parameters, and guidelines for processing cardiovascular signals, such as detecting R peaks, interpolating RR artifacts, and computing HRV metrics (e.g., implanting guidelines for windowing, resampling, normalization, etc.[24,41,42]). This is important because Vest et al. (2018) demonstrated how modest differences in these processing steps can lead to divergent results, contributing to the lack of reproducibility and clinical applicability of HRV metrics.

III) validated algorithms, default parameters, and guidelines for processing EEG signals, including filtering and windowing[40,43], re-referencing[44,45], removal of abnormal channels and artifacts[46–48], optimized ICA decomposition and classification of independent components[49–52]. Note: users can fine-tune all preprocessing parameters or even preprocess their EEG data with their preferred method before using the toolbox to match their needs (e.g., with EEGLAB *clean_rawdata* plugin[48], the BeMoBIL pipeline[34], etc.).

IV) Heartbeat-evoked potentials (HEP, i.e., time domain) and oscillations (HEO; event-related

spectral perturbations with wavelet or FFT methods, and inter-trial coherence are available through the standard EEGLAB software) from ECG signals. Parametric and nonparametric statistics with corrections for type 1 errors are available via EEGLAB's standard software. Nonparametric statistics include permutation statistics and spatiotemporal corrections for multiple comparisons (e.g., spatiotemporal clustering or threshold-free cluster enhancement)[53,54]. Users can even use the LIMO-EEG plugin to implement hierarchical linear modeling, which accounts well for within and between-subjects variance and implements an assumption-free mass-univariate approach with robust control for type I and II errors[55,56]. Note: statistical analyses on HEP/HEO data can also be performed in the independent component domain.

V) HEP/HEO analysis from PPG signals for the first time.

VI) Supports the joint extraction of EEG and HRV features for the first time.

VII) The toolbox provides various data visualizations to inspect signals at various necessary processing steps and outputs at the subject level (see Representative results).

Information to help readers decide whether the method is appropriate for them

This toolbox is appropriate for any researcher or clinician with EEG and ECG/PPG data. The plugin does not yet support importing EEG and ECG/PPG signals from separate files (although this feature will be available soon). The toolbox is appropriate for anyone aiming to perform HEP/HEO analysis, extract EEG and/or HRV features with standardized methods, or simply remove heart artifacts from EEG signals.

PROTOCOL

0. BrainBeats requirements

0.1. Install MATLAB and EEGLAB on your computer. EEGLAB can be downloaded at https://github.com/sccn/eeglab and unzipped (or cloned for Git users) anywhere on your computer. See the GitHub page for more details on installation.

0.2. Add the path to the EEGLAB folder in MATLAB's home panel by clicking > "Set Path" button > select the eeglab folder you just unzipped > "Save" > "Close".

0.3. Launch EEGLAB by typing 'eeglab' in MATLAB's command window.

0.4. Install the BrainBeats plugin by clicking "File" > click "Manage EEGLAB extensions" > Type 'brainbeats' in the search bar > select the BrainBeats plugin in the list > click "Install/Update".

0.5. Load the sample dataset into EEGLAB. Click "File" > Click "Load existing data" > Navigate to the EEGLAB folder at the location you unzipped it, go to the "plugins" folder > go to the "BrainBeats" folder > Go to the "sample_data" folder > select the file "dataset.set".
Note: this dataset corresponds to sub-032 (resting state with eyes opened) from the

following open-source dataset available on the NEMAR platform[1]. This dataset was selected because it contains 63 EEG channels, one ECG channel, and one PPG channel (all time-synchronized since they were recorded simultaneously with the same hardware). See the link to the NEMAR page for more details on how these data were collected. For this tutorial, ECG and PPG data were merged with the EEG dataset, the 3D boundary element method (BEM) electrode coordinates were loaded, and all signals were resampled from 1000 Hz to 250 Hz to reduce the weight of the BrainBeats folder and accelerate computing time. Because this dataset did not have any abnormal EEG channel, we artificially modified channel TP9 to illustrate BrainBeats' abnormal channel detection and removal. Similarly, we artificially simulated a large electrode artifact at the beginning of the file and a high-frequency muscle artifact in the temporal channels from 3 to 6 seconds to illustrate BrainBeats' artifact removal feature. Users can find some preprocessing outputs (e.g., signal quality index of the cardiovascular time series, NN intervals, removed EEG channels, segments, and components, etc.) in the EEGLAB structure: EEG.brainbeats.preprocessings. All parameters are also exported in EEG.brainbeats.parameters. We encourage users to report these outputs in scientific publications to increase the replicability of findings. When users keep "Saving outputs" checkbox, everything is saved in the corresponding .set file at the same location as the original file that was loaded into EEGLAB.

1. Method 1: Heartbeat-evoked potentials (HEP) and oscillations (HEO)

1.1. Open BrainBeats' first general user interface (GUI) to select the main parameters: in EEGLAB, click "Tools" > "BrainBeats" > "1st level (subject level)". Select "Heartbeat-evoked potentials HEP" as the analysis to run, "ECG" as the heart data type, and click on the button to display the list of channels to select the ECG channel labeled "ECG" (or type it directly in the text box). Keep the options "Visualize outputs (recommended)" and "Save outputs" selected and click "Ok". **See Figure 1.1.** in the Representative Results section.

1.2. A second GUI window pops up based on the previous choices (i.e., HEP analysis with ECG data). Here, users can select preprocessing parameters for both EEG and ECG signals. Users can turn off preprocessing by unchecking the boxes if they already preprocessed their file before launching Brainbeats (**Figure 1.2.**). In the "Preprocess EEG" section, change "Power line noise" to 50 Hz since these EEG data were recorded at the Rural Federal University, Russia. Click "Ok" to launch.

1.3. A warning message will appear, asking for confirmation to remove the PPG channel that has been detected (**Figure 1.3.**). This is expected since the toolbox is not designed to analyze both ECG and PPG simultaneously (or other auxiliary channels), and keeping it in the dataset will lead to serious errors (e.g., artifact removal, poor ICA decomposition, etc.). Click "Yes".

BrainBeats starts performing some checks and separates the ECG from the EEG data to process it separately. ECG and RR time series are processed using validated algorithms from

[1] https://nemar.org/dataexplorer/detail?dataset_id=ds003838

the Physionet Cardiovascular Signal toolbox[42,57] adapted to fit BrainBeats' data formatting, increase clarity, parameter tuning, and computing time (see references for validation of the algorithms). The ECG signal is bandpass-filtered using a customized, validated filter (1-30 Hz) and scans the signal to identify the QRS complex and R-peaks using the Pan–Tompkins (P&T) method[58], implementing some signal processing operations including differentiation, squaring, integration, and smoothing for best performance. The P&T energy threshold is estimated based on the sample rate and the smoothed ECG values to avoid disruption from large bumps. If the RR interval variability exceeds 1.5 times the median, it conducts a "search back" for missed peaks. The mean R-peak sign is calculated over 30 s segments, and peak points are refined through a refractory period check, also managing flatline conditions and ensuring consistent detection. BrainBeats' *get_RR* function performs all these operations seamlessly and outputs the RR intervals, timestamps, filtered ECG signal, R-peaks indices, and heart rate (HR). Users can tune these parameters via the GUI (**Figure 1.2.**) or command line.

Next, BrainBeats' *clean_rr* function identifies abnormal RR intervals or spikes within RR intervals using a forward-backward search and physiological thresholds. The signal quality index (SQI) is calculated[42], and warnings are generated if more than 20% of the RR times series contains RR artifacts (outside of physiological limits or with SQI below .9). Next, the RR artifacts are interpolated using the shape-preserving piecewise cubic method to obtain the normal-to-normal (NN) intervals (default, but users can use other methods or remove them). When several ECG channels are present, these steps are performed on each of them, and the electrode with the least number of RR artifacts is selected for the following steps. A plot displays the filtered ECG signal, identified R-peaks, NN intervals, and interpolated artifacts (see **Figure 1.4.**). Users can scroll through zoomed-in 30-s windows of the R-peaks for closer inspection by pressing the right/left arrows. If the data contain several ECG/PPG channels, the channel with the best signal quality index will be used for the RR intervals. However, the BrainBeats does not support both ECG and PPG signals simultaneously at the moment (because they are rarely recorded simultaneously). We chose a dataset that has both here for tutorial purposes.

Next, BrainBeats bandpass filters the EEG data at 1-40 Hz using a nonlinear causal minimum phase FIR filter by default to reduce smearing activity between pre- and post-heartbeat periods[40]. This is especially important if users wish to examine the pre-heartbeat period to preserve causality while avoiding undesired phase lags.[40] If the lowpass filter is set to a value above the power line frequency (e.g., 80 Hz lowpass with power line frequency at 50 Hz), a sharp notch filter is applied to remove line noise artifacts. The EEG data are then re-referenced to infinity using the REST algorithm (best suited for HEP analysis[45]) unless less than 30 channels are detected (in which case they cannot be reliably re-referenced, and a warning is generated to let users know). BrainBeats' *clean_eeg* function then detects, removes, and interpolates abnormal channels (**Figure 1.5.**). The default parameters for detecting irregular EEG channels are flat lines greater than 5 s (clean_flatlines algorithm), maximum high-frequency noise standard deviation of 4 (original default; clean_channels algorithm), a window length of 5 seconds (to better capture slow-frequency artifacts relative

to the original default 2 seconds[48]; clean_channels algorithm), a minimum correlation between neighboring channels of .65 (instead of the original default of .85 which can lead to more false positives; clean_channels algorithm), and a maximum tolerated portion of 33% (instead of the original default of 50% that can lead to more false negatives; clean_channels algorithm). To increase convergence and replicability of abnormal channel rejection, we increased the number of RANSAC samples to 500 (instead of 50), although it increases the computation time.

Next, R-peaks are inserted as event markers into the EEG data to mark each heartbeat, and the data are segmented around these markers without baseline removal (per guidelines[39,59]; **Figure 1.6.**). Here, because NN intervals have different lengths and EEG must be segmented at a constant length, we estimate the minimum epoch size cutoff following R-peak events using the 5th percentile of the interbeat-interval (IBI) data (i.e., the value below which 5% of the shortest IBIs fall, displayed as a dashed red line on a histogram; see **Figure 1.7.**). This 5th percentile value is a good compromise to preserve as many epochs as possible while making sure they are not too short since the period of interest for HEP/HEO analysis is +200-600 ms[45,60]. Thus, EEG data are segmented from -300 ms before the R-peaks to the 5th percentile value post-R-peak, with the R-peak at time 0. We then remove epochs shorter than 550 ms per guidelines[45,60], or containing more than one R-peak (which would bias the ERP/ERSP). Epochs containing large EEG artifacts are detected using root-mean-square (RMS) and signal-to-noise ratio (SNR) metrics and MATLAB's *isoutlier* function ('*grubbs'* method by default; **Figure 1.8.**). Blind source separation is performed suing the preconditioned independent component analysis (ICA) algorithm for fast computation[49] and accounting for data rank for best performance[51]). The independent components are automatically classified by the *ICLabel* plugin[52] to extract non-brain artifacts (ocular and muscular components are removed with 90% confidence, line noise and channel noise with 99% confidence; **Figure 1.9.**). If desired, users may choose the ICA Infomax algorithm with special parameters for generating replicable IC maps by choosing the option "Long but replicable" for the field "ICA method to extract artifactual components" (note: this method involves much longer computation times).

Finally, since we selected "Visualize outputs" in the first GUI window, a scroll plot displays the final signal, and three plots display the average HEP for each electrode with the possibility to click each electrode to look closer (**Figure 1.10**.) the grand average HEP (**Figure 1.11. top**) and over time (**Figure 1.11. bottom**), the grand average HEO (i.e. ERSP) showing a typical effect at 150-400 ms post-heartbeat in frequencies 8-15 Hz at frontocentral site Fz (permutation statistics, $p<0.05$, FDR-corrected; **Figure 1.12. top**). The inter-trial phase coherence (ITC) shows no effect after correction for family-wise error (**Figure 1.12. bottom**). ERSP is computed using a default 3-cycle wavelet (with a Hanning-tapered window applied, pad ratio of 2) and with the number of cycles in the wavelets used for higher frequencies expanding slowly up to 20% of the number of cycles in the equivalent FFT window at its highest frequency (1 minus 0.8). This controls the shapes of the individual windows measured by the function and their shapes in the resulting time/frequency panes. The baseline is removed for illustration purposes, and ERSP is computed for frequencies 7-25 Hz

to capture the typical HEO effect described in the literature. Lower frequencies cannot be estimated due to the short epoch size due to the interbeat intervals. Note that these plots are generated for tutorial purposes or within-subject analysis. Statistics at the group level are not currently available. Future developments will address the issue of epoch length differing across subjects due to different heart rates.

Notes: Preprocessing plots are generated to visualize the different steps, but this option can be turned off by unchecking the box "Visualize preprocessings." The final EEG data (cleaned and segmented around the R-peaks) do not include the ECG data since it would bias the ERP/ERSP analysis (although users can preserve the heart channel by checking the box "Keep heart channel in final output" if they wish). Users can pause before processing the next file (next condition or participant). Finally, BrainBeats supports EEGLAB's history function: at the end of all operations, users can type "eegh" in MATLAB's command window to print the command line to repeat all the above steps via a single command line, with the parameters that wee selected manually in the GUI, allowing easy automation.

Advanced users can perform all the above steps with default parameters with the following command lines (see the tutorial script in the BrainBeats repository for more options):

```
eeglab; close; % Launch EEGLAB without the GUI
main_path = fileparts(which('eegplugin_BrainBeats.m')); cd(main_path);
EEG     =     pop_loadset('filename','dataset.set','filepath',fullfile(main_path,
'sample_data'));  % Load the sample dataset
EEG = brainbeats_process(EEG,'analysis','hep','heart_signal','ECG', ...
    'heart_channels',{'ECG'},'clean_eeg',true);  % Launch BrainBeats 1st level to
process the file for HEP analysis with default parameters
```

The previous steps performed HEP/HEO from the ECG signal. The following steps are similar but for the PPG signal.

1.4. Load the same dataset again (see **step 0.3.**) since it was overwritten by our operations and perform step 1.1. Again, select PPG for heart signal type and select the PPG channel in the list of electrode labels (**Figure 1.13.**). Click "Ok".

1.5. The second GUI window pops up in a manner similar to **step 1.2.** Except for the preprocessing parameters for cardiovascular signals, they are now adapted to the PPG signal (**Figure 1.14.**). Click "Ok" to run with the default parameters.

1.6. A warning message appears, asking for confirmation to remove the extra ECG channel that has been detected. Again, this is expected. Click "Yes".

By default, the toolbox will preprocess the PPG signal, detect the pulse waves to obtain the RR intervals, identify RR artifacts, if any, and interpolate them (**Figure 1.15.**). EEG preprocessing is the same as above. The same HEP/HEO outputs are generated (see **Figures 1.16., 1.17., and 1.18.**), displaying slightly different results.

Advanced users can perform all the above steps with default parameters with the following

command lines (see the tutorial script in the BrainBeats repository for more options):

```
eeglab; close; % Launch EEGLAB without the GUI
main_path = fileparts(which('eegplugin_BrainBeats.m')); cd(main_path);
EEG     =     pop_loadset('filename','dataset.set','filepath',fullfile(main_path,
'sample_data'));  % Load the sample dataset
EEG = brainbeats_process(EEG,'analysis','hep','heart_signal','PPG', ...
'heart_channels',{'PPG'},'clean_eeg',true);  % Launch BrainBeats 1st level to
process the file for HEP analysis with default parameters
```

2. METHOD 2: Extract EEG and HRV features

2.1. Load the same dataset again (see step 0.3) since it was overwritten by our operations and perform step 1.1. to open the main GUI window again (**Figure 1.1.**), select "Extract EEG & HRV features" for the analysis type, "ECG" for the heart signal type, and select "ECG" in the list of electrode labels. Click "Ok". See **Figure 2.1**.

2.2. The second GUI window pops up like **step 1.2.** but with some differences in EEG preprocessing and for extracting HRV and EEG features (**Figure 2.2.**). Click "Ok" to launch with default parameters.

2.3. The same warning message appears, asking for confirmation to remove the extra PPG channel that has been detected. Again, this is expected. Click "Yes".

BrainBeats will start to preprocess ECG data and extract the NN intervals identically as in METHOD 1 (see **step 1.3.**). Then, it extracts heart-rate variability (HRV) features from the NN intervals in the time (SDNN, RMSSD, pNN50), frequency (ULF, VLF, LF, HF, LF:HF ratio, total power), and nonlinear (Poincare, sample entropy, fractal dimension, phase-rectified signal averaging) domains. Frequency-domain HRV features are computed by default using the Lomb-Scargle periodogram, which does not require resampling (therefore better preserving original information) and best deals with non-uniformly sampled data, missing data, and noise (typical with NN intervals)[61]. The normalized version is selected by default (although users can choose the standard version) and scales the power by the variance of the signal. This provides results that are less sensitive to the overall amplitude of the signal and more focused on the relative strength of periodic components. This is best suited when the signal might have varying noise levels or to make results more comparable across different recordings or subjects. The standard version (no normalization) and Welch and the Fast Fourier Transform (FFT) methods are also available (although not recommended), with resampling automatically performed to create the necessary regularly sampled time series. Users can select between these methods by clicking on the button "freq. options." They can also fine-tune the window overlap (default set to 25%) and perform a second-level normalization (i.e., the power of each frequency band is divided by the total power to provide a more intuitive measure of the relative contribution of each frequency component to the overall power (disabled by default since this option is most meaningful when all four bands are available, requiring at least 24 hours of signal). These algorithms are adapted from

the Physionet Cardiovascular signal processing toolbox[42]. Note that BrainBeats automatically checks the file length to ensure that minimum requirements are met (e.g., ULF-HRV power requires 24 hours of data) and sends warning messages if they aren't and does not export these features to prevent unreliable estimations. BrainBeats follows guidelines and recommendations for estimating HRV metrics[24,41].

Next, BrainBeats preprocesses the EEG data. The re-referencing, filtering, and removal of abnormal channels is the same as for the HEP method (see **step 1.3.** and **Figure 1.5.**). Then, large artifacts are detected automatically in the continuous data using the artifact subspace reconstruction (ASR) algorithm (default SD criterion set to 30 and using 80% of available RAM to increase speed). These parameters can be adjusted in the GUI, fields "Threshold to reject bad segments with ASR" and "Available RAM to use for ASR". Note that for the features mode, the EEG and cardiovascular time series do NOT need to be time synchronized since the features are estimated on each signal separately. Thus, EEG artifacts can be removed directly from the EEG data (in red, **Figure 2.3.**), unlike for the HEP mode, where we rejected epochs containing artifacts for both time series because time synchronization at millisecond accuracy is essential for that method. Then, fast ICA is performed in the same way as for HEP (see **step 1.3.**), except that heart components are removed this time if detected by ICLabel with 99% confidence (it was preserved for HEP/HEO since we do not want to remove relevant heart-related signals). The classified components are slightly different this time since ICA was performed on continuous data (instead of segmented for HEP). Two components were removed, one with 99.9% confidence and the other with 97.7% confidence.

Next, BrainBeats extracts some EEG features in the frequency and nonlinear domains. Frequency domain features currently include the average PSD for the delta (1-3 Hz), theta (3-7 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (30+ Hz) frequency bands. By default, the PSD is normalized to decibels (dB) as it facilitates the comparison of results across recordings and subjects. However, users can export standard PSD (in $\mu V^2/Hz$) or normalize by the total power (to capture each band's contribution to total power). Additionally, users can choose whether the frequency bounds should be defined using the conventional (predefined) bounds (e.g., 8-13 Hz for the alpha frequency band) or individualized bounds, where the bounds are detected from the distribution of the power spectral density (e.g., 7.3-12.6 Hz), accounting for inter-individual differences[62]. The second frequency-domain feature is the individual alpha frequency (IAF), using the alpha center of gravity (better deals with split peaks or ambiguous peaks than the "peak alpha frequency" method). See Corcoran et al. (2018)[62] for more details on the algorithms and rationale for estimating individualized frequency bands and the IAF. The third frequency-domain feature is alpha asymmetry on all available (symmetric) electrode pairs. The pairs are obtained using the theta distances, requiring the EEG data to contain electrode coordinates. Alpha asymmetry is computed following guidelines (2-s hamming window with 50% overlap, the logarithm of alpha power from the left channel minus logarithm of alpha power from the right channel)[43]. Hence, positive values indicate greater left-than-right alpha power and vice versa. Another option in the "freq. options" is to normalize alpha asymmetry scores by dividing alpha power from

each electrode by total power.[43] Other frequency-domain parameters that can be fine-tuned by clicking on the button "freq. options" include the overall frequency range on which to compute the PSD (default = 1-40 Hz), the window type (default = 'hamming'), the window overlap (default = 50%), and the window size (default = 2 s).

Nonlinear-domain entropy features include fuzzy entropy and fractal dimension. These features are thought to capture nonlinear, complex dynamics of the brain that are missed by spectral measures and show particular promise for investigating interactions between various body systems [63–65]. Fuzzy entropy is more reliable and robust than its alternatives (sample and approximate entropy) but requires longer computation times (especially with long EEG time series with high sampling rates). To address this issue, when EEG signals are longer than 2 minutes long with a sampling rate greater than 100 Hz, they are automatically downsampled (or decimated when the factor is not an integer) to 90 Hz (i.e., corresponding to a Nyquist frequency of 45 Hz, to match our default lowpass filter and avoid line noise artifacts as much as possible). Furthermore, parallel computing is activated by default when estimating EEG features, which accelerates computing time, especially when many EEG channels are available. Finally, because we selected "Visualize outputs" in the first GUI, BrainBeats generates a plot displaying the power spectral density (PSD) for HRV and EEG data (**Figure 2.4.**) along with some topography displaying some EEG features (**Figure 2.5.**).

Notes: All features are exported into the EEGLAB structure: EEG.brainbeats.features and saved in a .mat file in the same folder where the dataset was loaded (except if the option was turned off in the first GUI window). Users can also find some preprocessing outputs in EEG.brainbeats.preprocessing and all the parameters used in EEG.brainbeats.parameters. We encourage users to report these outputs in scientific publications to increase the replicability of findings. When users keep the "Saving outputs" box checked in the first GUI window, everything is saved in an EEGLAB .set file at the same location as the original file that was loaded into EEGLAB. Finally, BrainBeats supports EEGLAB's history function: at the end of all operations, users can type "eegh" in MATLAB's command window to print the command line to repeat all the above steps via a single command line, with the parameters that were selected manually in the GUI, allowing easy automation.

Advanced users can perform all the steps above with the following command:

```
eeglab; close; % Launch EEGLAB without the GUI
main_path = fileparts(which('eegplugin_BrainBeats.m')); cd(main_path);
EEG     =     pop_loadset('filename','dataset.set','filepath',fullfile(main_path,
'sample_data'));  % Load the sample dataset
EEG = brainbeats_process(EEG,'analysis','features','heart_signal','ECG', ...
    'heart_channels',{'ECG'},'clean_eeg',true);
```

The previous steps extracted HRV features from ECG signal. The following steps are similar but for PPG signal.

2.4. Load the same dataset again (see **step 0.3**.) since it was overwritten by our operations and open the main GUI again (see step 2.1.). Select "Extract EEG & HRV features" for the analysis and select "PPG" for the heart signal type and the channel name. Click "Ok".

2.5. The 2nd GUI window now shows the parameters for preprocessing PPG and for extracting the HRV and EEG features. Click "Ok" to run with the default parameters.

2.6. A warning message will appear, asking for confirmation to remove the detected ECG channel. This is to be expected since the toolbox is not designed to analyze both ECG and PPG simultaneously (or other auxiliary channels), and keeping it in the dataset will lead to serious errors (e.g., artifact removal, poor ICA decomposition, etc.). Click "Yes".

BrainBeats preprocesses the PPG signal and estimates the NN intervals as in **step 1.5.** It then extracts the HRV features from the NN intervals, the same as in **step 2.2.** except that the NN intervals have now been obtained from the PPG signal. The EEG signals are preprocessed as in step 2.2., and BrainBeats plots the PSD (the only difference here is the PSD estimated from the NN intervals obtained from the PPG signals, as compared to ECG, **Figure 2.6.**).

Advanced users can perform all the steps above with the following command:

```
eeglab; close; % Launch EEGLAB without the GUI
main_path = fileparts(which('eegplugin_BrainBeats.m')); cd(main_path);
EEG     =     pop_loadset('filename','dataset.set','filepath',fullfile(main_path,
'sample_data'));  % Load the sample dataset
EEG = brainbeats_process(EEG,'analysis','features','heart_signal','PPG', ...
    'heart_channels',{'PPG'},'clean_eeg',true);
```

3. Method 3: Extract heart artifacts from EEG signals.

3.1. Load the sample dataset (see **step 0.3**.)

3.2. Open the main GUI window by clicking "Tools" > "BrainBeats" > "1st level (subject level)" (**Figure 1.1.**), selecting "Extract heart artifacts from EEG signals" for the analysis type, "ECG" for heart signal type and select "ECG" in the list of electrode labels. Click "Ok" to launch with default settings.

3.3. The 2nd GUI window (**Figure 3.1.**) now shows the parameters to preprocess the EEG data. Click "Ok" to run with the default parameters since the sample dataset is not preprocessed.

3.4. A warning message will appear, asking for confirmation to remove the ECG channel that has been detected. This is to be expected since the toolbox is not designed to analyze both ECG and PPG simultaneously (or other auxiliary channels), and keeping it in the dataset will lead to serious errors (e.g., artifact removal, poor ICA decomposition, etc.). Click "Yes".

The ECG signal is bandpass-filtered to remove slow frequency drifts below 1 Hz and high-frequency noise above 20 Hz (with a noncausal zero-phase FIR filter). The EEG signals are preprocessed as in METHOD 2 (see **step 2.3.**).

Independent component analysis (ICA) is performed using the "preconditioned ICA for real data" algorithm (PICARD), although users can choose the standard Infomax algorithm or modified Infomax algorithm for replication from the GUI in **step 3.2.** Then, ICLabel classifies all the independent components. If a component is classified as a heart component with 80% confidence, it is, by default, detected and extracted automatically from the EEG data. Users can edit this confidence level in the GUI (**step 3.2.**). By default, the boost mode smears ECG signal into the EEG signals, increasing classification performance. Instead of 6 heart components, one component is extracted from the EEG signals (although the boost mode can be disabled in the GUI). A final plot displays what part of the EEG signals has been extracted using the method (in red; **Figure 3.3.**). Note that the ECG signal is red, confirming the extraction of ECG-related components. The ECG channel is removed after this step since it no longer contains any relevant information.

For advanced users, these steps can be performed using the following command lines:

```
eeglab; close; % Launch EEGLAB without the GUI
main_path = fileparts(which('eegplugin_BrainBeats.m')); cd(main_path);
EEG =
pop_loadset('filename','dataset.set','filepath',fullfile(main_path,'sample_data')
);
EEG = brainbeats_process(EEG,'analysis','rm_heart','heart_signal','ECG', ...
    'heart_channels',{'ECG'},'clean_eeg',true,'vis_cleaning',false);
```

REPRESENTATIVE RESULTS:

METHOD 1: Heartbeat-evoked potentials (HEP) and oscillations (HEO)

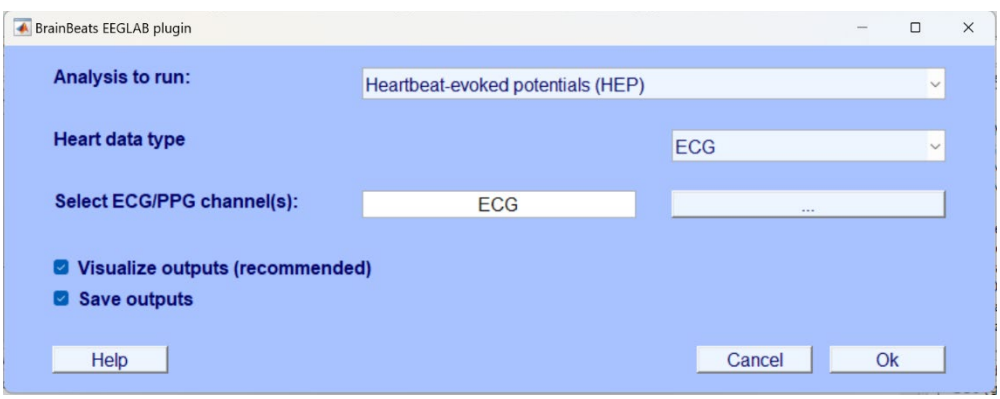

FIGURE 1.1. *Selecting general parameters from the main general user interface (GUI) to process the sample dataset for HEP/HEO analysis.*

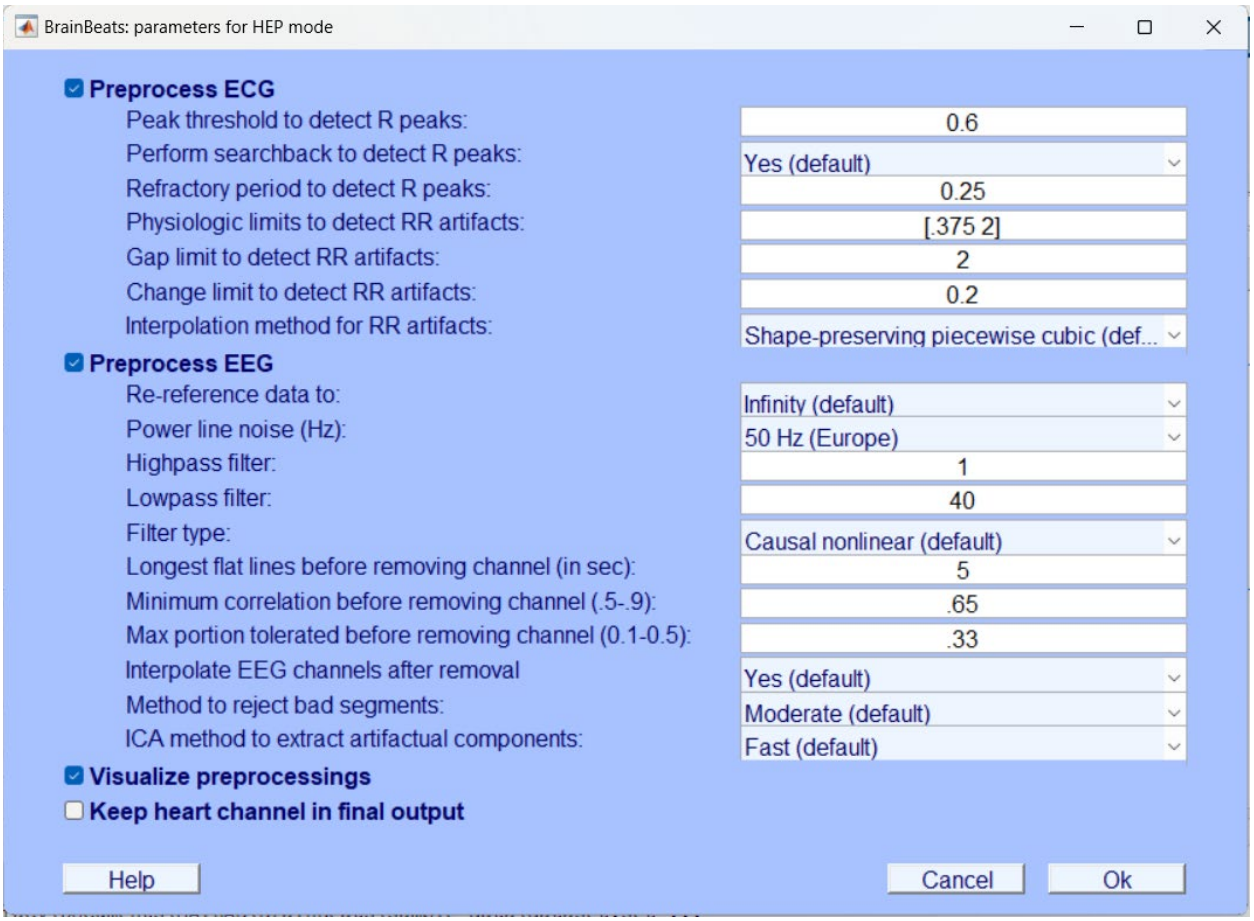

FIGURE 1.2. *ECG and EEG preprocessing parameters for HEP/HEO analysis. This graphic user interface (GUI) allows users to fine-tune the main preprocessing parameters to: 1) obtain the RR intervals from ECG signals, detect and interpolate RR artifacts if any to obtain the NN intervals; 2) filter and re-reference EEG data, detect and interpolate abnormal EEG channels if any, extract non-brain artifacts using ICA; 3) choose if wish to visualize preprocessing steps (for fine-tuning), or keep the cardiovascular channel in the final output (not recommended for HEP/HEO).*

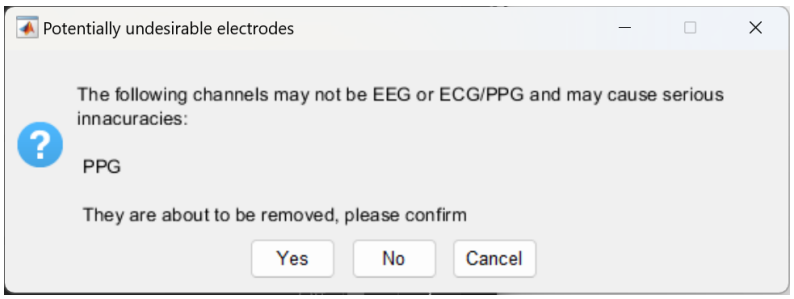

FIGURE 1.3. *A warning message is asking for user confirmation to remove the extra PPG channel detected in this dataset. This is to be expected, because the toolbox is not currently designed to process both ECG and PPG signals at the same time.*

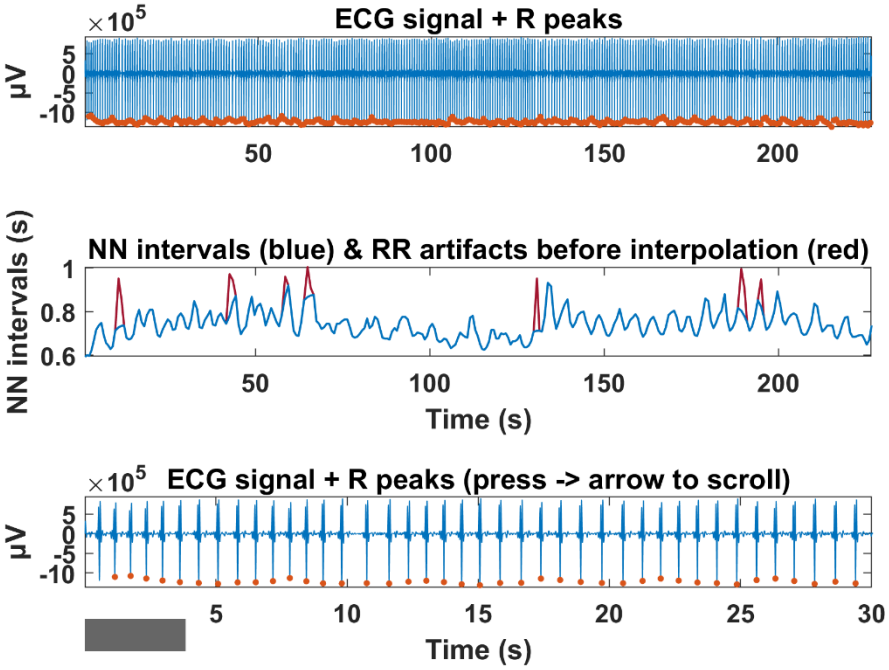

FIGURE 1.4. Top*: preprocessed ECG signal (blue) with the R-peaks detected by BrainBeats (orange dots, i.e. RR intervals).* ***Middle****: Normal-to-Normal (NN) intervals (in blue) after interpolation of the RR artifacts (in red).* ***Bottom****: Same as top plot but zoomed in (30-s window) to inspect the R-peaks more closely with a scrolling feature by pressing the left/right arrows. Note: the get_RR algorithm detected the negative polarity of the ECG peaks and reports in MATLAB's command window that 11/305 (3.61%) of the heartbeats were flagged as artifacts and interpolated, as well as the average signal quality index (SQI) of the RR intervals (before interpolation) with a value of 1, which is the highest value. Low signal quality corresponds to 20% or more of the RR series being flagged or with SQI<0.9.*

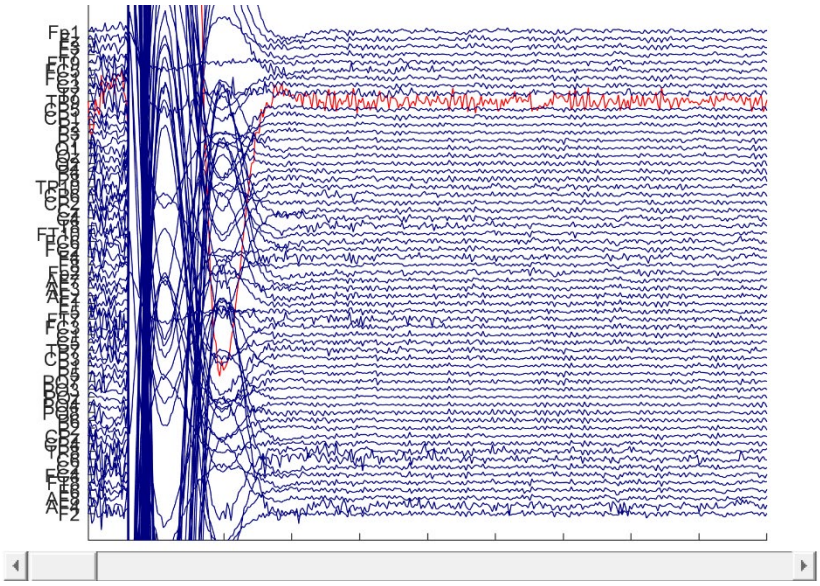

FIGURE 1.5. *Visualization of the abnormal EEG channel that was automatically detected and removed from the dataset. Note: the large artifact is dealt with in a further step. EEG data were bandpass-filtered (1-40 Hz) and re-referenced to infinity.*

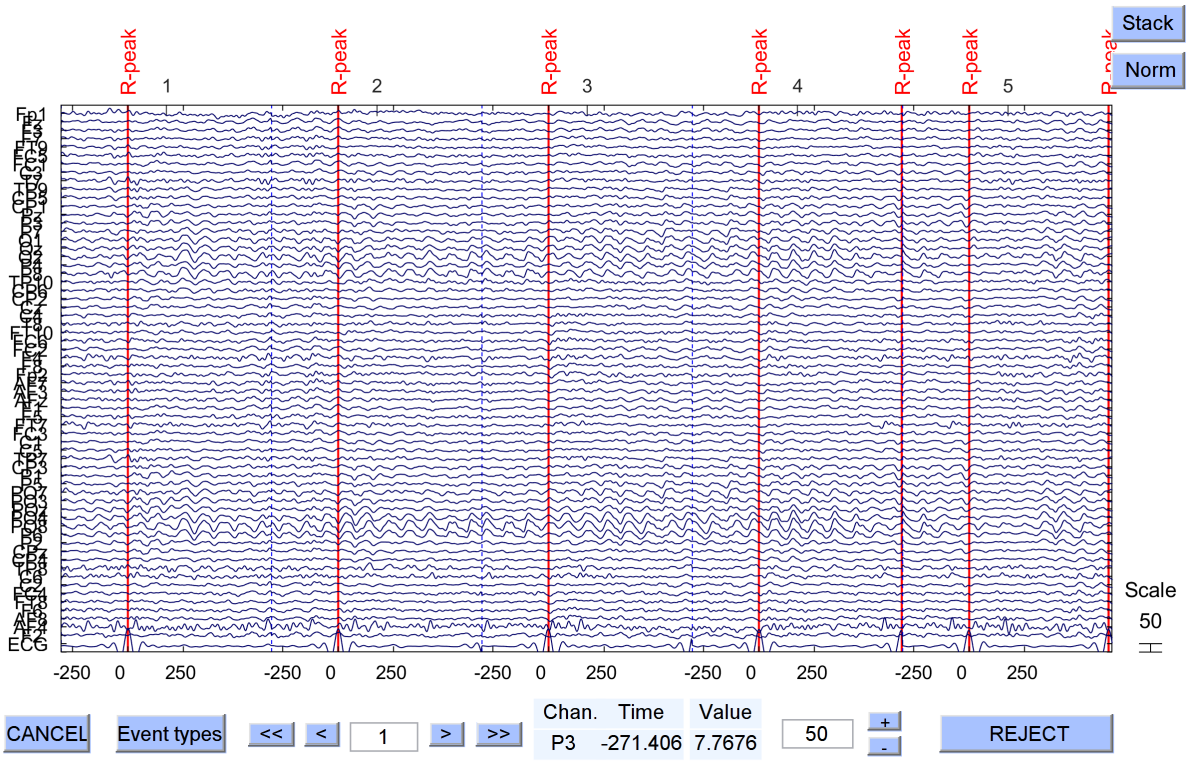

FIGURE 1.6. *Visualization 64-channel EEG data after preprocessing and inserting the R-peaks as events in the signal to mark each R-peak with millisecond accuracy. The ECG signal is included in the plot at the bottom for visual confirmation.*

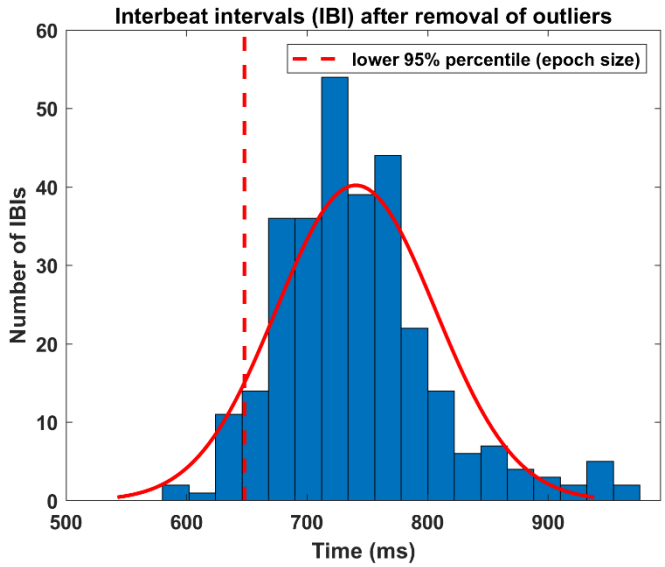

Figure 1.7. *Histogram of the interbeat intervals (IBI) with fitted normal density (red line) and the corresponding 5% percentile (red dashed line) used as the upper cutoff value at which the EEG data are segmented (i.e., 650 ms after the R-peak for this file) since epochs of different sizes cannot be used for analysis. Note: BrainBeats also reports in MATLAB's command window that one epoch was shorter than 550 ms and removed, and two outlier IBI were removed.*

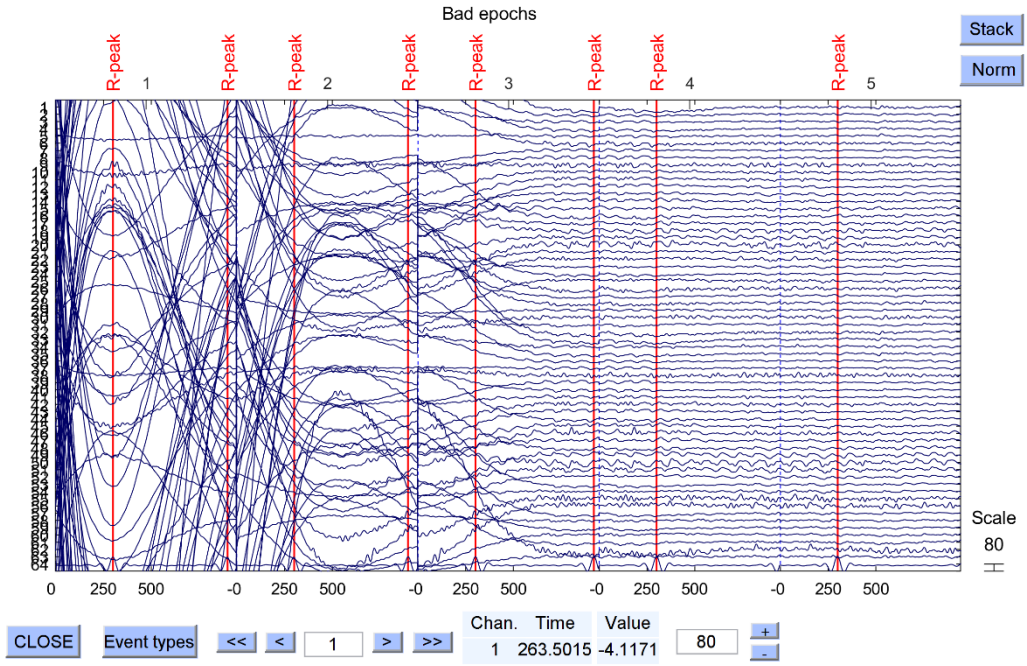

Figure 1.8. *Visualization of the 15 EEG outlier epochs (i.e., containing artifacts) that were detected and removed prior to performing independent component analysis (ICA).*

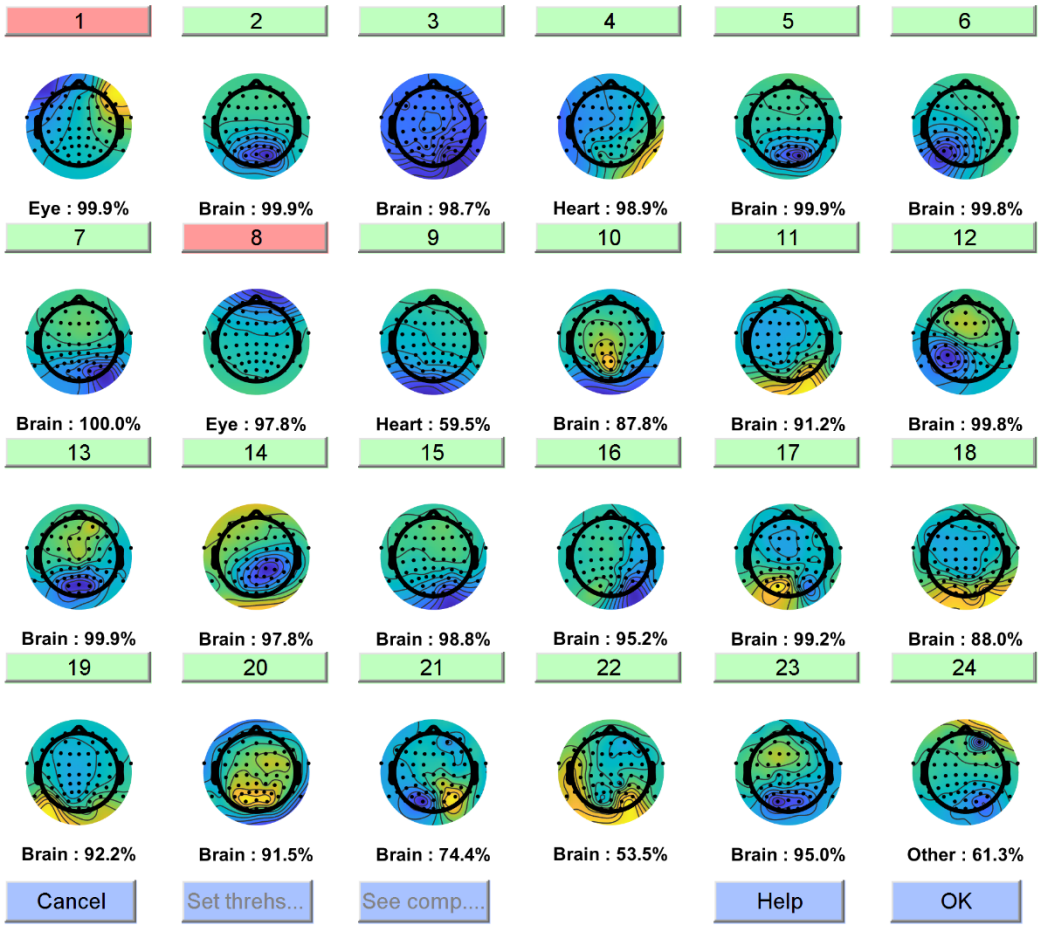

Figure 1.9. *After performing blind source separation to obtain the independent components of our EEG data, the ICLabel plugin is used to classify them and flag the non-brain components for extraction automatically. Here, we can see two ocular components flagged for removal with 99.9% confidence and 97.8% confidence, respectively.*

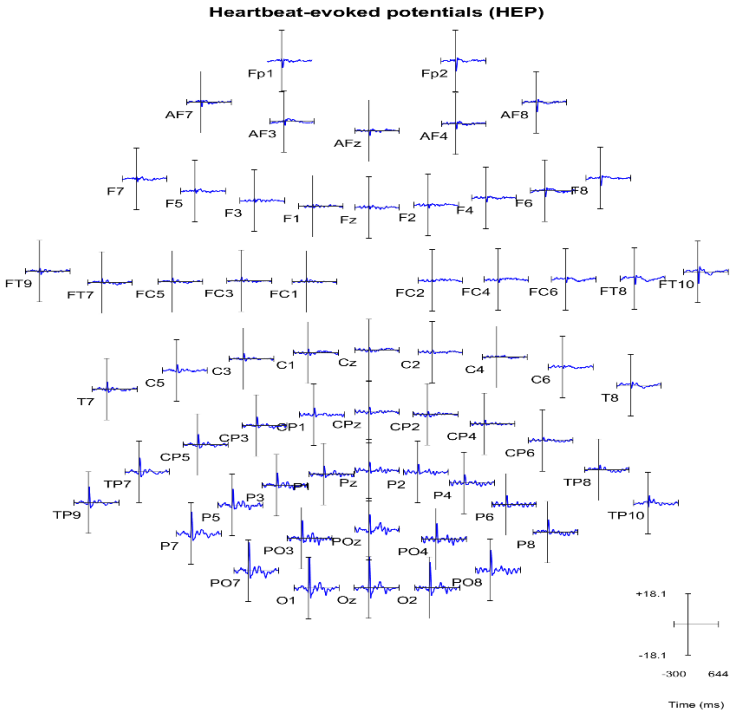

Figure 1.10. *Visualization of the heartbeat-evoked potentials (HEP) obtained from the ECG signal for each EEG channel. Users can click on each channel to inspect it more closely.*

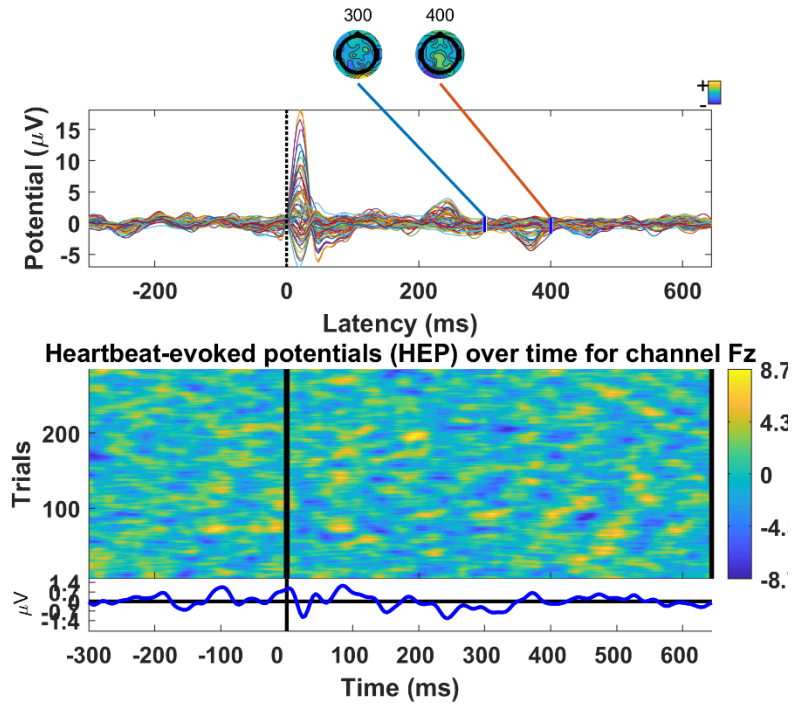

Figure 1.11. Top: Grand average heartbeat-evoked potentials (HEP). All electrodes are superimposed in the time domain, with scalp topographies showing amplitude distribution in the period of interest (200-500 ms after heartbeat). **Bottom**: HEP evolution over time (each "trial" corresponds to a heartbeat).

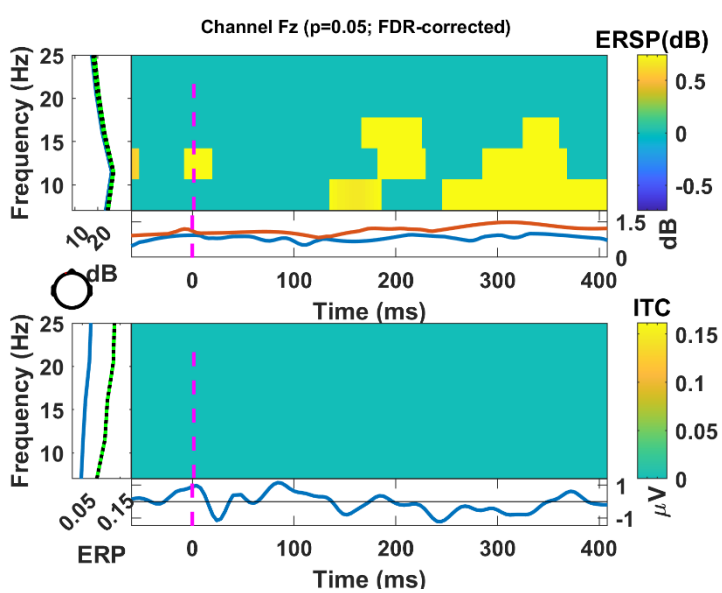

Figure 1.12. Top: *Heartbeat-evoked oscillations (HEO) for EEG channel Fz (frontocentral region) after permutation statistics (1000-iterations) and corrected for false discovery rate (FDR) at the 95% confidence level (p<0.05), displaying a significant effect at time 0 (i.e., R-peak), and from 150*

to 400 ms after the heartbeat in the frequencies 7-16 Hz (consistent with previous findings[60,66]). ***Bottom****: Inter-trial coherence displayed no significant effect after FDR correction, suggesting that the EEG phase for Fz is random with respect to the heartbeats (no phase-locking or resetting as with experimental stimuli). Note: this result is at the subject level.*

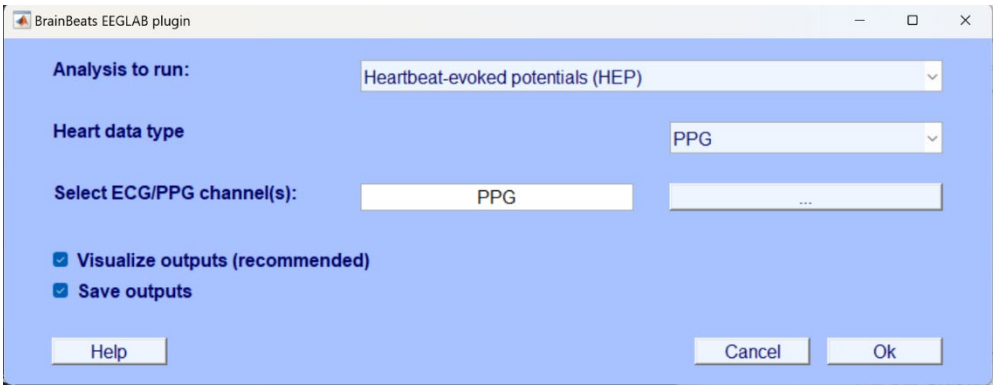

Figure 1.13. *Selecting the general parameters for HEP/HEO analysis with PPG signals (as opposed to ECG signals above).*

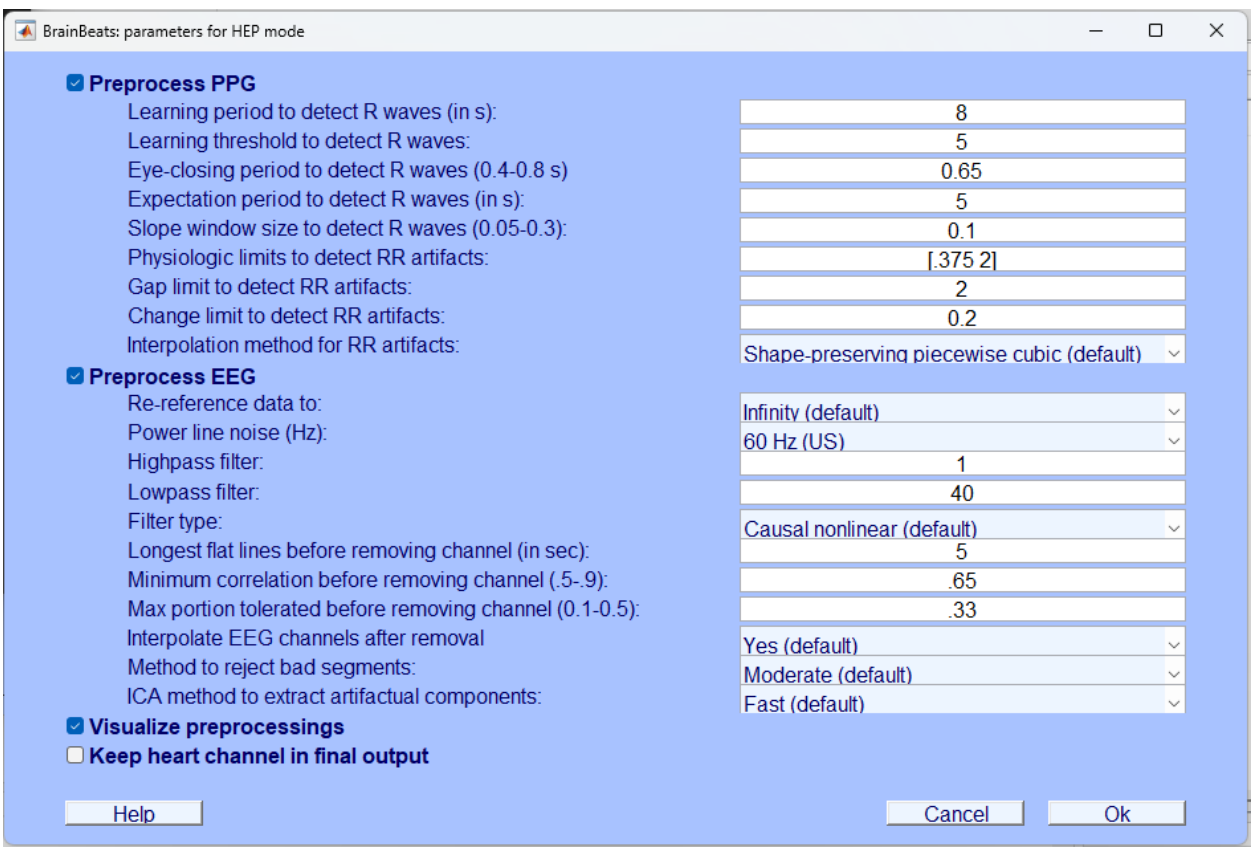

Figure 1.14. *Preprocessing parameters for PPG and EEG signals. The graphical user interface (GUI) is the same as Figure 1.2 for EEG but has different parameters for estimating the RR intervals from*

the PPG signals.

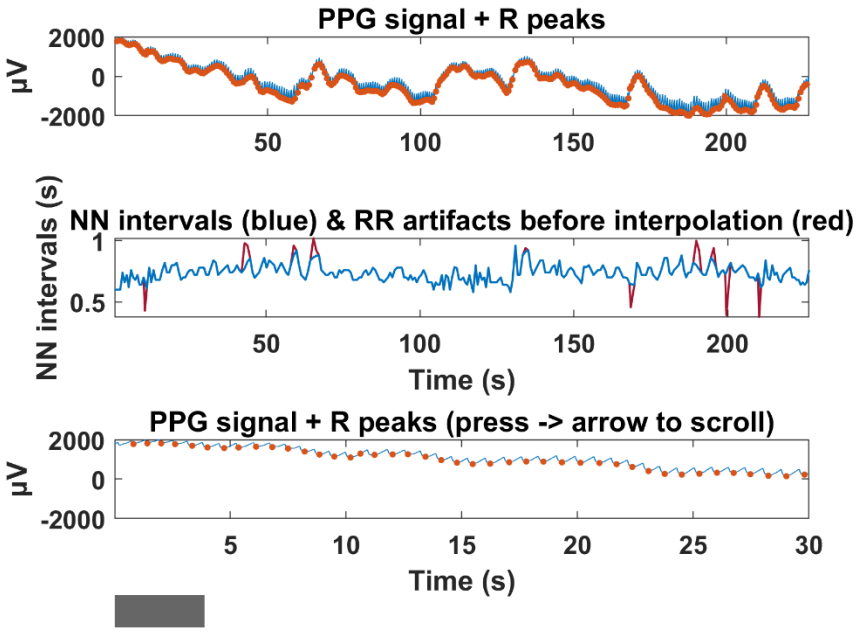

FIGURE 1.15. *Same as Figure 1.4. but for PPG instead of ECG.* ***Top****: preprocessed PPG signal (blue) with the pulse waves detected by BrainBeats (orange dots, i.e. RR intervals).* ***Middle****: Normal-to-Normal (NN) intervals (in blue) after interpolation of the RR artifacts (in red).* ***Bottom****: Same as top plot but zoomed in (30-s window) to inspect the pulse waves more closely with a scrolling feature by pressing the left/right arrows. Note: the get_RR algorithm successfully detected the pulse waves and interpolated and reports in MATLAB's command window that 11/305 (3.61%) of the heartbeats were flagged as artifacts and interpolated, as well as the average signal quality index (SQI) of the RR intervals (before interpolation) with a value of 1, which is the highest value. Low signal quality corresponds to 20% or more of the RR series being flagged or with SQI<0.9.*

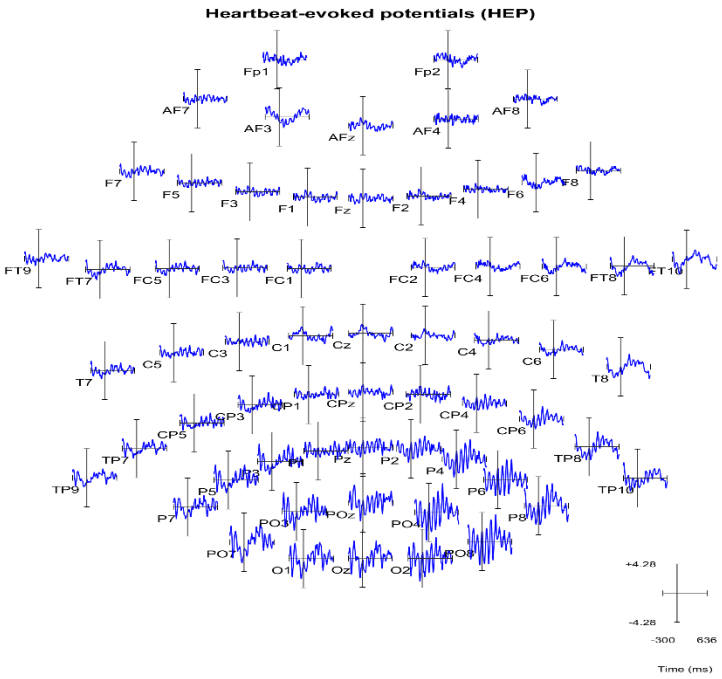

Figure 1.16. *Visualization of the heartbeat-evoked potentials (HEP) obtained from the PPG signal for each EEG channel. Users can click on each channel to inspect it more closely. Same figure as Figure 1.10. but from PPG as opposed to ECG. Note: this result is at the subject level. HEP/HEO has never been performed from PPG signals before, so it is unclear whether this is expected or not. Future research is required to interpret this result. See the discussion for more details on the interpretations of HEP/HEO from PPG signals.*

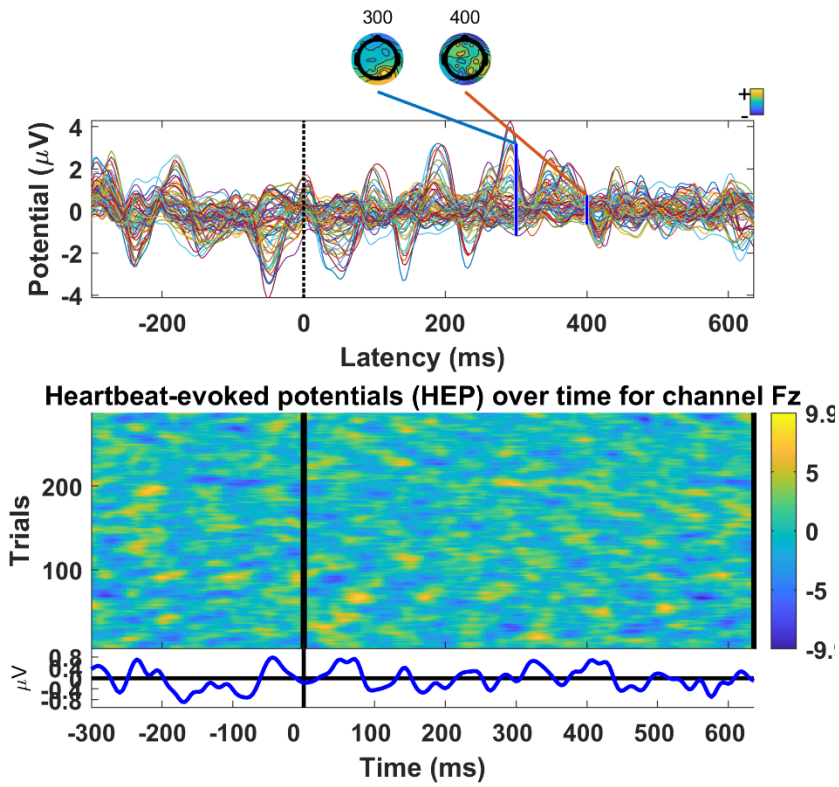

Figure 1.17. Same as Figure 1.11. but obtained with PPG signal. **Top**: Grand average heartbeat-evoked potentials (HEP). All electrodes are superimposed in the time domain, with scalp topographies showing amplitude distribution in the period of interest (200-500 ms after heartbeat). **Bottom**: HEP evolution over time (each "trial" corresponds to a pulse wave). We observe some oscillations in the EEG signals but starting before time 0 (i.e., valley of the pulse waves), suggesting *EEG phase is probably random with respect to the heartbeats. Note: this result is at the subject level. HEP/HEO has never been performed from PPG signals before, so it is unclear whether this is expected or not. Future research is required to interpret this result. See the discussion for more details on the interpretations of HEP/HEO from PPG signals.*

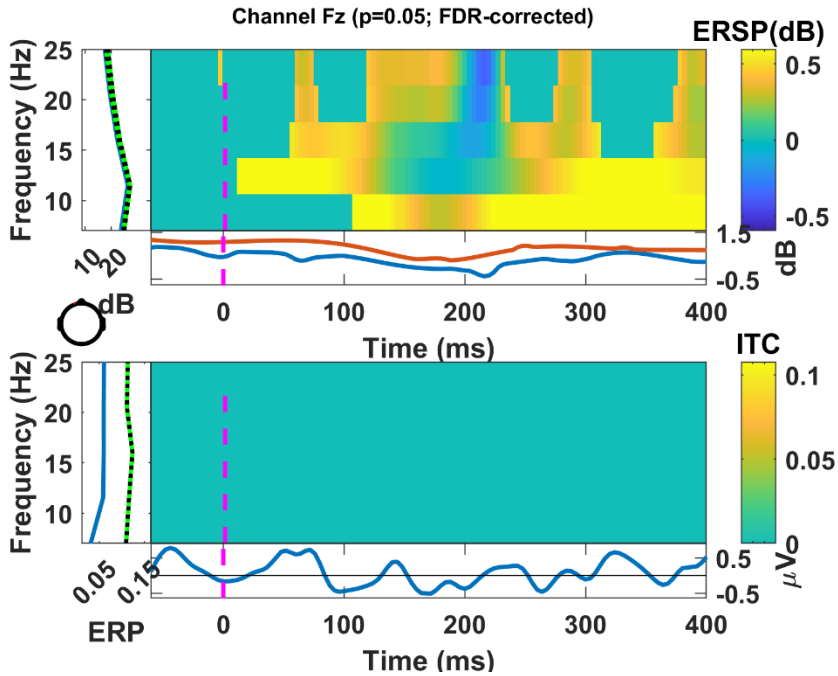

Figure 1.18. *Same as Figure 1.12. but using PPG signal instead of ECG signal.* ***Top:*** *Heartbeat-evoked oscillations (HEO) for EEG channel Fz (frontocentral region) after permutation statistics (1000-iterations) and corrected for false discovery rate (FDR) at the 95% confidence level (p<0.05), displaying a significant effect in almost the whole window..* ***Bottom****: Inter-trial coherence (ITC) displays no significant effect after FDR correction, suggesting that EEG phase for Fz is random with respect to the pulse waves (no phase-locking or resetting as with experimental stimuli). Note: this result is at the subject level. HEP/HEO has never been performed from PPG signals before, so it is unclear whether this is expected or not. Future research is required to interpret this result. See the discussion for more details on the interpretations of HEP/HEO from PPG signals.*

<u>METHOD 2: Extracting HRV and EEG features</u>

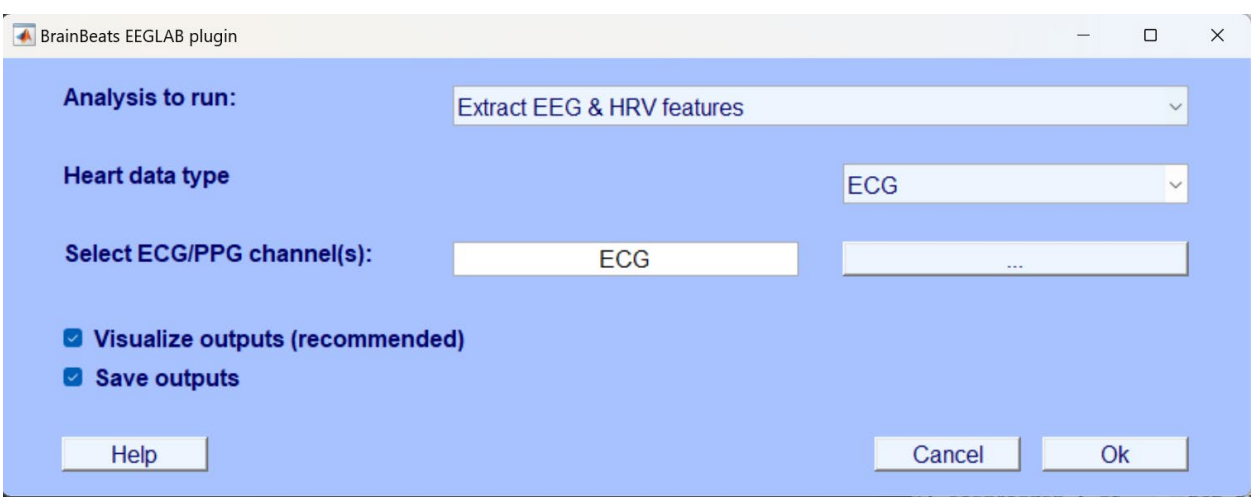

Figure 2.1. *First graphical user interface (GUI) to select the type of analysis to run (i.e., extracting HRV and EEG features), the type of cardiovascular signal to use (i.e., ECG), select the electrode label from the list (i.e., "ECG"), choose to plot and save outputs or not.*

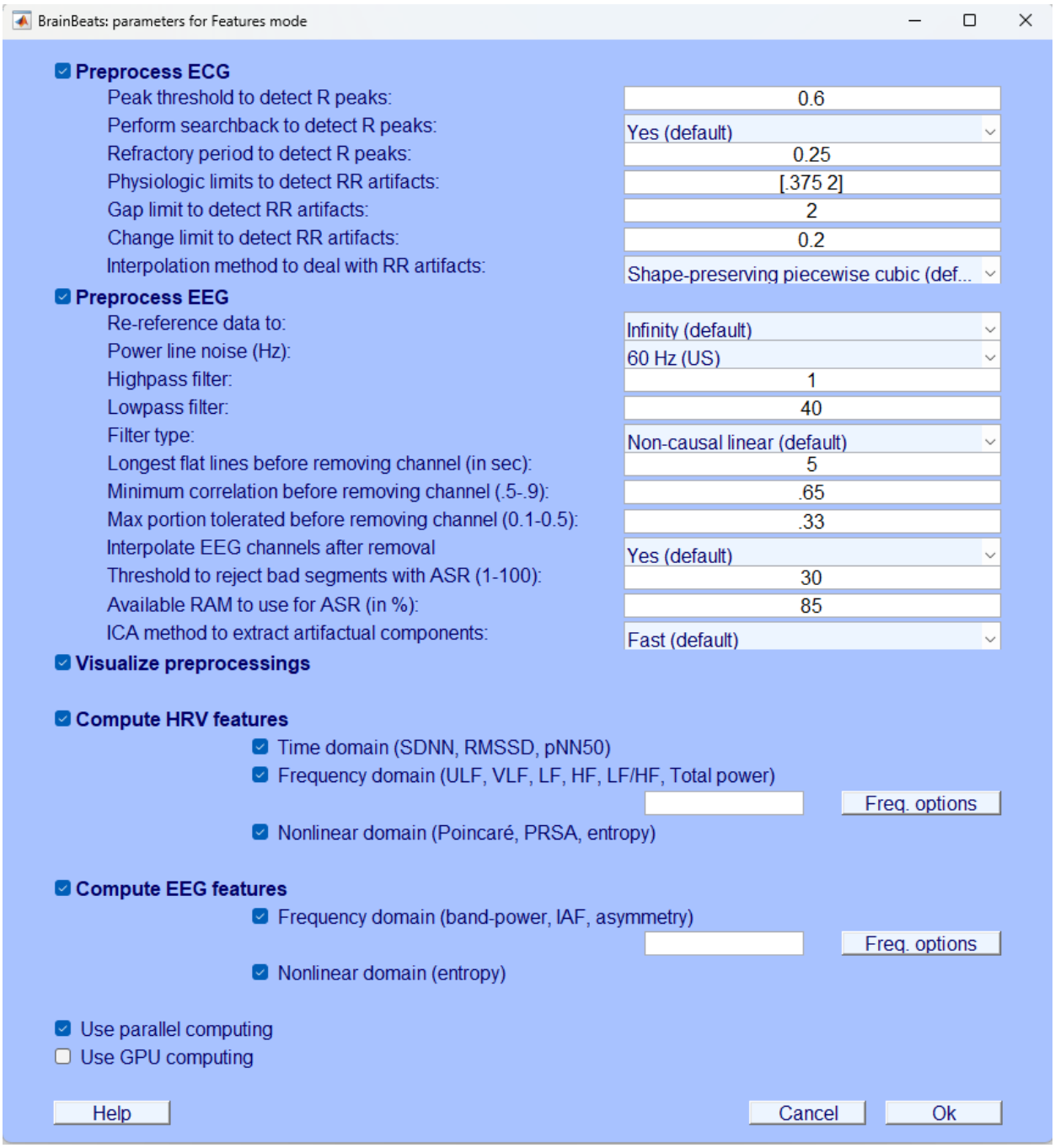

Figure 2.2. *Second graphical user interface (GUI) to fine-tune parameters to 1) obtain the RR intervals from ECG signals, detect and interpolate the RR artifacts (if any) to obtain the NN intervals; 2) filter and re-reference EEG data, detect and interpolate abnormal EEG channels (if any), extract non-brain artifacts using ICA; 3) to visualize preprocessing steps; 4) compute heart-*

rate variability (HRV) features and in which domain (with additional parameters for the frequency domain by clicking on "freq. options"); 5) compute EEG features and in which domain (with additional parameters for the frequency domain by clicking on "freq. options"); 6) use parallel or GPU computing for faster computation time. By clicking "Ok", default recommended parameters are automatically selected.

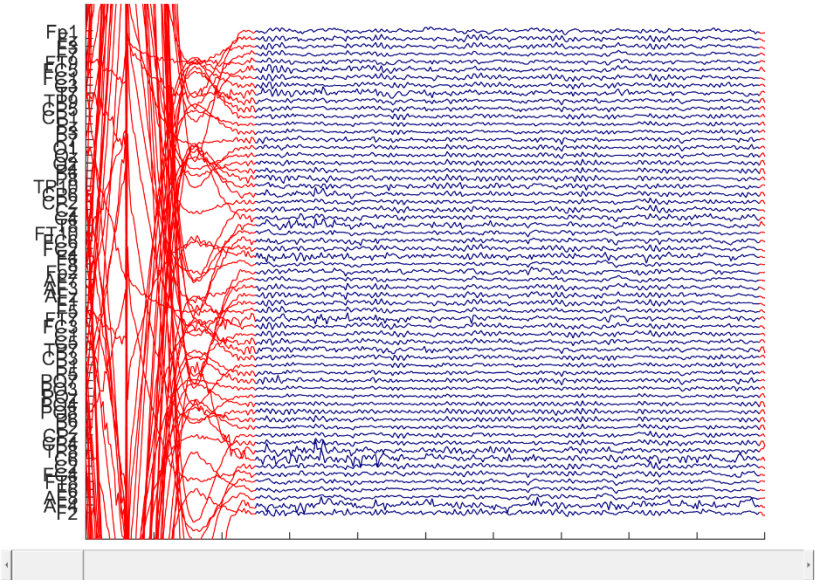

Figure 2.3. *Illustration of a large EEG artifact detected and removed by the artifact subspace reconstruction (ASR) algorithm.*

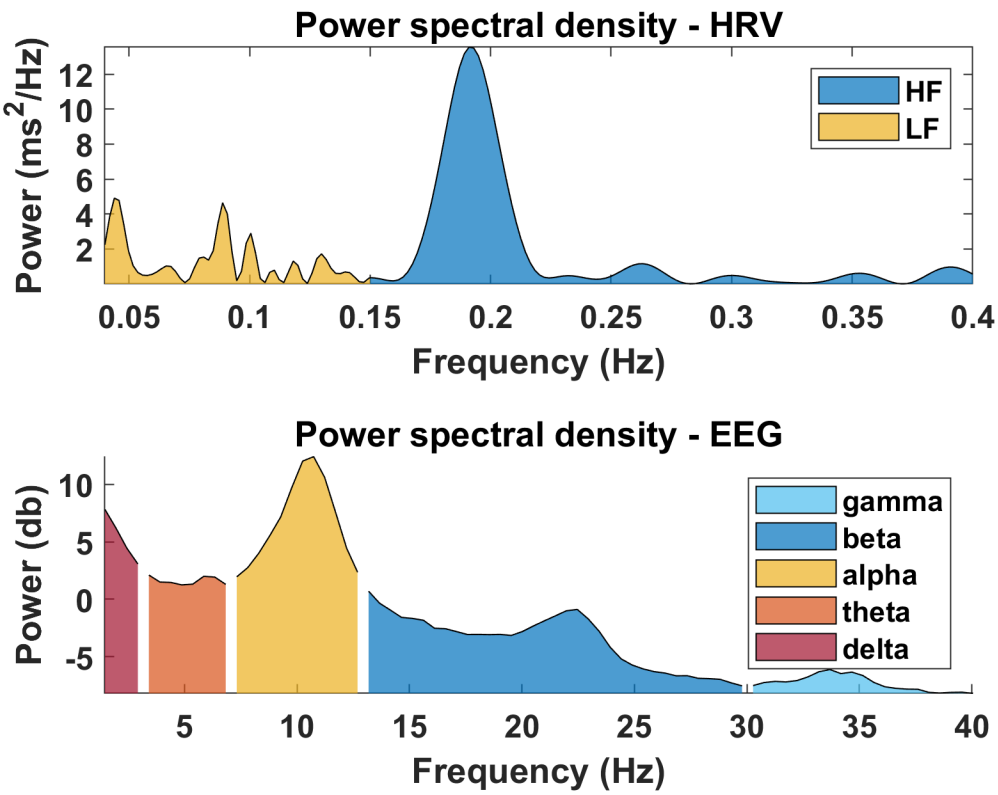

Figure 2.4. *Visualization of the power spectral density (PSD) extracted from the EEG and ECG signals.* ***Top****: Heart-rate variability (HRV) power in the low-frequency (LF;* 0.04-0.15 Hz*) and high-frequency (HF*; 0.15-0.40 Hz*) bands, estimated using the normalized Lomb-Scargle periodogram from the NN intervals. We observe a spectral peak at ~0.19 Hz within the HF frequency band.* ***Bottom****: PSD normalized to decibels (dB) estimated from the continuous EEG data, averaged across all channels for visualization. We observe a typical peak in the alpha frequency band at ~10.5 Hz.*

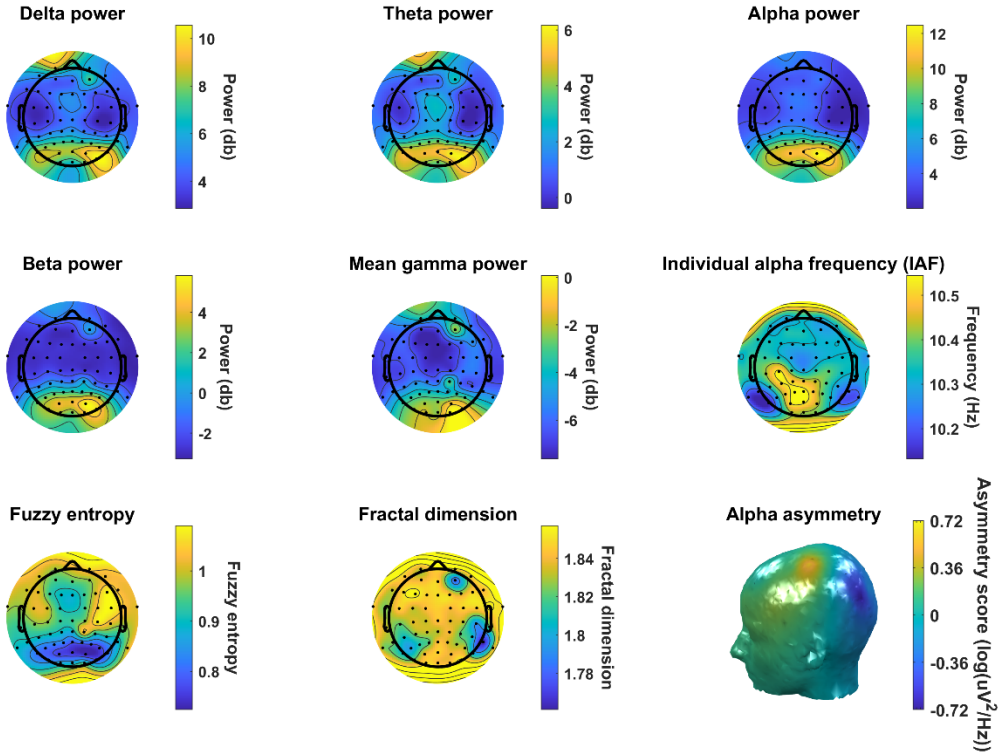

Figure 2.5. *Scalp topographies illustrating the distribution of the main EEG features extracted by BrainBeats from the 64-channel EEG data, including spectral power in the delta (1-3 Hz), theta (3-7 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (30-40 Hz) frequency bands, the individual alpha frequency (IAF), fuzzy entropy, fractal dimension, and alpha asymmetry. Notes:*
Greater fuzzy entropy values reflect higher complexity in predictability and regularity, whereas the fractal dimension reflects greater complexity in terms of geometry and fractal characteristics. *Alpha asymmetry was calculated on 16 symmetric pairs. Positive values reflect greater left-than-right alpha power, typically associated with greater left-than-right inhibition of local cortical regions.*

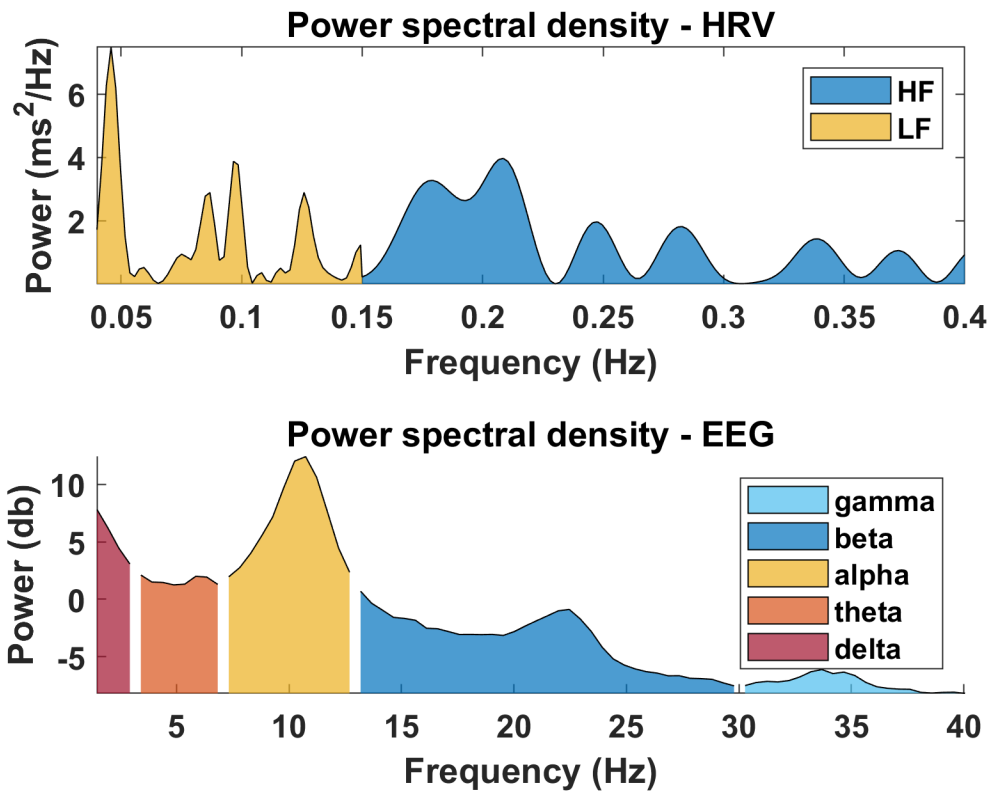

Figure 2.5. *Same as Figure 2.4. except that this time, the HRV power spectral density (PSD) was calculated on NN intervals obtained with the PPG signal. The EEG PSD is the same.*

METHOD 3: Extracting heart artifacts from EEG signals

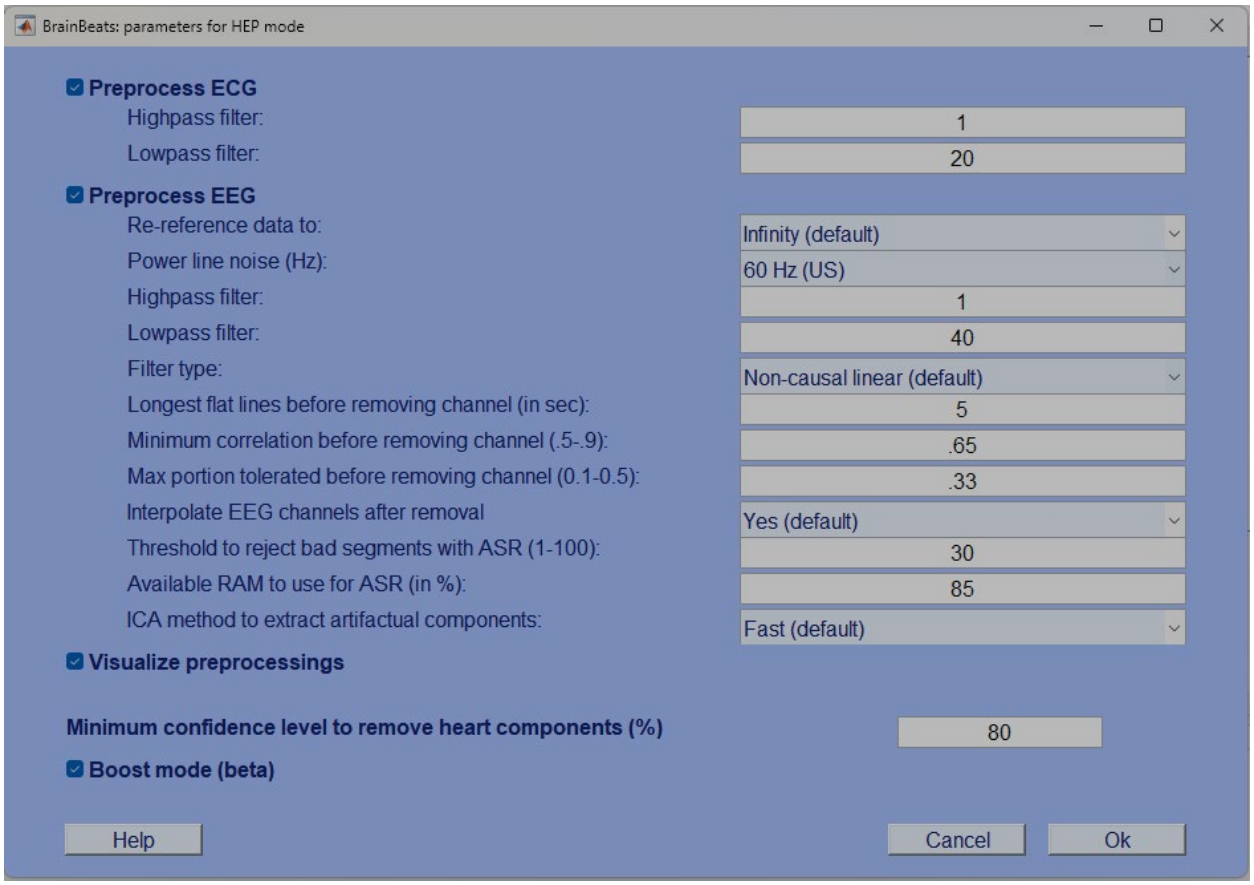

Figure 3.1. *2nd GUI to select preprocessing parameters prior to removing heart components from EEG signals (Method 3). Data cleaning can dramatically affect the performance of this method. The confidence level to classify and remove heart artifacts is set to 80% by default. The boost mode increases performance by smearing ECG signal across the EEG signals, but it can be turned off.*

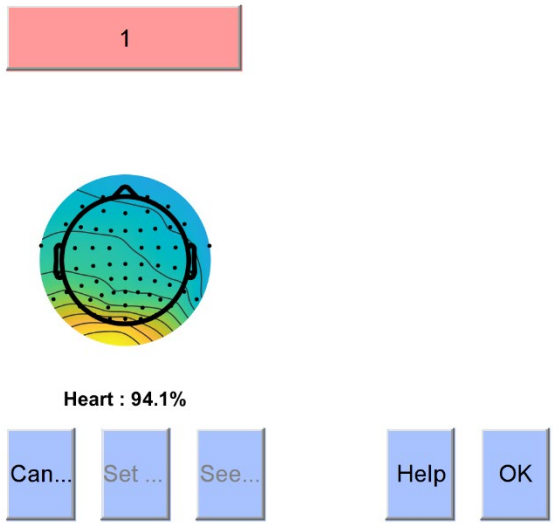

Figure 3.2. *Visualization of the heart component detected and removed by BrainBeats, with 94.1% confidence. Note: Without the "boost" mode selected by default, six components were removed for the same dataset to achieve similar performance.*

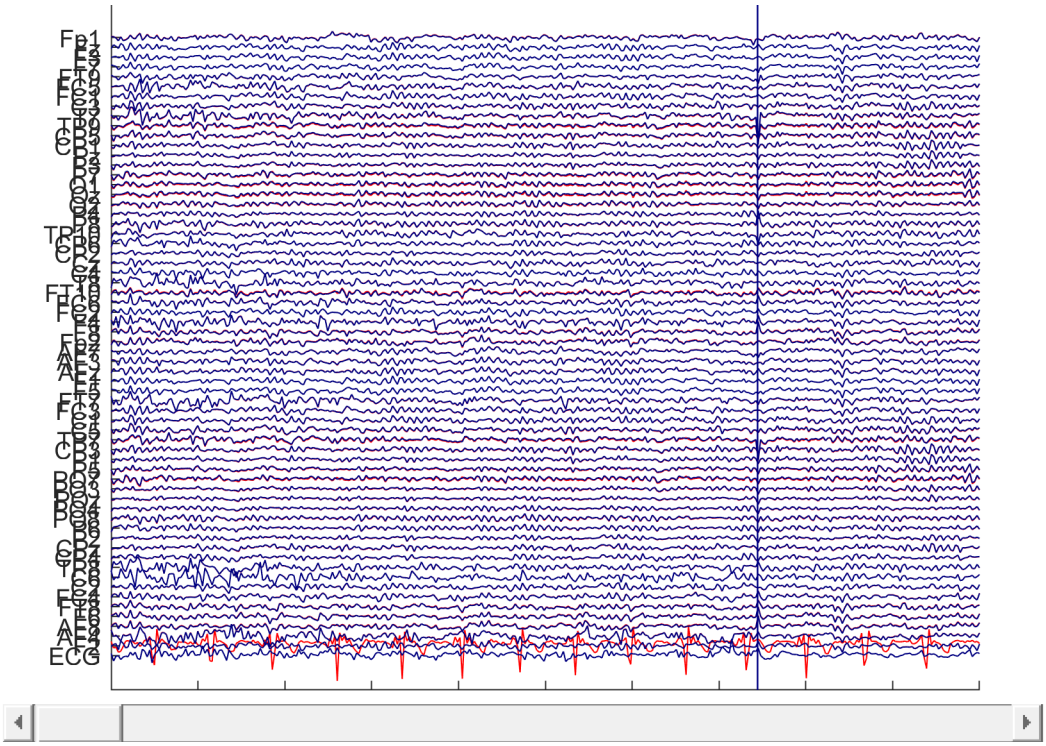

Figure 3.3. *Visualization of the final output: the EEG signals (in blue) after extracting the ECG component (in red).*

DISCUSSION:

Critical steps in the protocol

Critical requirements are described in **steps 0.1-0.3.** Warnings and error messages are implemented at various places in the toolbox to help users understand why they may encounter issues (e.g., file length being too short for calculating a reliable measure of ultra-low frequency HRV, signal quality is too low for any reliable analysis, missing electrode locations, etc.). For advanced users, each function is documented, and the parameters can be easily fine-tuned (recommended parameters and typical ranges are documented). Users can access this information using the MATLAB "help" feature (followed by the function's name).

Limitations of the method

Users must possess a dataset that includes EEG and cardiovascular (ECG or PPG) within the same

file, or know how to merge them independently. A future version will allow to automatically load BIDS datasets with any amount of files directly into BrainBeats, merge the EEG and cardiovascular signals available, and deal with potential time synchronicity issues (e.g., different sampling rates). Data importation is currently not automated because various plugins must be installed to account for the many different EEG data formats available (e.g., .csv, .edf, .bdf, .vhdr, etc.).

Entropy features are particularly promising for capturing complex, bidirectional interactions between cardiovascular, subcortical, and cortical systems that may be hidden in nonlinear feedback loop dynamics[24,67,68]. However, they are very computationally heavy and can take a long time to compute from EEG signals, especially with high sampling rates. While improvements were made (parallel computing, signal downsampling/decimation), future work will aim to reduce these computational costs. While statistics are provided for Method 1 and 3 (at the subject and group levels using the standard EEGLAB STUDY), no statistical analysis is currently provided for Method 2 to assess interactions between HRV and EEG features (although it will be available soon). Meanwhile, users may still use Method 2 to extract features easily and reliably, perform statistics with any standard statistical software, or build ML models with their own method of choice.

ECG offers a direct assessment of cardiac electrophysiology by capturing the heart's electric fields, whereas PPG measures blood volume changes in microvascular beds, reflecting cardiac activity more indirectly through blood flow dynamics. ECG-based HEP/HEO directly correlates with cardiac electrical activity, whereas peripheral vascular dynamics influence PPG-based HEPs more. Identifying the R-peak in ECG is straightforward due to its clear manifestation in the QRS complex. However, in PPG, the analogous peak aligns with the maximum blood volume point (pulse wave peak) rather than the valley, representing the lowest blood volume point between pulses and not corresponding to the ECG's R-peak. This difference in signal characteristics results in timing disparities between ECG and PPG signals. The delay in PPG relative to ECG, due to the underlying physiological processes and their measurement site's distance from the heart, can influence the temporal aspects of observed HEPs. Consequently, these variations impact HEP/HEO analysis interpretations. Clinically, this divergence necessitates careful consideration in selecting the appropriate modality for HEP analysis, with ECG preferred for direct cardiac electrophysiological studies and PPG offering benefits in ease of use and patient comfort for long-term monitoring. While ECG and PPG can facilitate HEP analysis, their differing signal natures and physiological implications suggest that their inferences are not directly interchangeable. The choice between ECG and PPG for HEP analysis should be tailored to the specific objectives and needs of the study or clinical application.

For HEP/HEO analysis, epochs are defined using thresholds based on the distribution of the individuals' interbeat intervals (IBI; ~600-1000 ms). This leads to different epoch lengths across subjects and errors for group analysis. Furthermore, the short epoch length resulting from IBIs, relative to conventional EEG studies (stimuli are typically spaced in several seconds to allow the brain to return to baseline), leads to potential undesired edge effects or prevents users from examining slow frequencies. Time-frequency decomposition typically requires epochs to extend up to 3 cycles in the lowest frequency of interest beyond the window of interest. For HEO, the

window of interest is 200-500 ms. Thus, one would require, for example, an additional 600 ms before and after the window (i.e., -400 to 900 ms) for examining frequencies as low as 5 Hz. If one wanted to examine frequencies as low as 1 Hz, an additional 3 seconds is needed before and after the window of interest. This is necessary for obtaining correct time and frequency resolution while avoiding edge effects.

Significance of the method with respect to existing methods

Overall, BrainBeats provides state-of-the-art signal processing techniques for both EEG and cardiovascular signals, with fine-tuning capabilities via command line and graphical user interface (GUI).

Method 1 can be performed using advanced hierarchical linear modeling and statistical testing and analyzing brain-evoked response by heartbeats using ERP, ERSP, and ITC measures. Furthermore, analyses can be performed at the EEG channel level as well as at the independent component level.

Method 2 does not currently have a pre-existing alternative. To our knowledge, HEP/HEO has never been performed using PPG signals and is now easily available. While this is currently a limitation regarding interpretations (see above), it will allow users to elucidate the distinctions between their different representations.

Method 3 allows quick and easy removal of heart components from the EEG signals in a fully automated manner. While this is already possible in EEGLAB with the ICLabel plugin, it requires users to perform a series of steps and choice of parameters (e.g., highpass filtering the signals, running ICA, running ICLabel, tuning parameters, subtracting the heart components from the EEG signals, and removing the ECG channels) that can easily lead to errors (e.g., ghost ICs[51]). Furthermore, we introduce a novel "boost" method that increases the performance of this method using the cardiovascular signal (generally not included in these operations).

Additionally, the toolbox implements computing performance improvements to accelerate the estimation of EEG features (mainly multiscale entropy measures), including both GPU and parallel computing. Note that these options are only as beneficial as the users' hardware (i.e., graphic card and number of processors and threads).

Future directions

The toolbox will continue to be modified and improved by the authors in the long term to implement the latest guidelines and recommendations by field experts and fix any errors that may arise.

Additional features and methods will be added to assess interactions between EEG and cardiovascular signals. For example, qEEG features such as theta:beta ratio or similar spectral ratios thought to capture relevant clinical or cognitive information can easily be added to the

toolbox. New methods will include, for example, EEG-ECG direct and partial coherence, the "time-resolved directional brain/heart interplay measurement"[69], or classification of HEP or feature data using machine learning (e.g., decision trees, random forest, naïve Bayes, SVM, KNN, long short-term memory networks, etc.)[14].

To address the short epoch issue (see limitations above), we aim to implement in future versions the "reflection" method, which mirrors the signal from the window of interest (i.e., backward version of the signal) before and after the window of interest to expand it. This provides smooth transitions and removes the undesired edge effects. The mirrored sections are then removed.

ACKNOWLEDGMENTS:

The Institute of Noetic Sciences supported this research. We thank the developers of the open-source algorithms that were adapted to develop BrainBeats.

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Bertalanffy, L. von. *General system theory : foundations, development, applications*. (G. Braziller, 1968).
2. Hodgkin, A. L. & Huxley, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544 (1952).
3. Bean, B. P. Nitrendipine block of cardiac calcium channels: high-affinity binding to the inactivated state. *Proc. Natl. Acad. Sci. U. S. A.* **81**, 6388–6392 (1984).
4. Fuchs, T. *Ecology of the Brain: The phenomenology and biology of the embodied mind*. (Oxford University Press, 2017).
5. Napadow, V. *et al.* Brain correlates of autonomic modulation: Combining heart rate variability with fMRI. *NeuroImage* **42**, 169–177 (2008).
6. Chang, C., Cunningham, J. P. & Glover, G. H. Influence of heart rate on the BOLD signal: The cardiac response function. *NeuroImage* **44**, 857–869 (2009).
7. Gianaros, P. J. & Sheu, L. K. A review of neuroimaging studies of stressor-evoked blood pressure reactivity: Emerging evidence for a brain-body pathway to coronary heart disease risk. *NeuroImage* **47**, 922–936 (2009).
8. Cannard, C., Brandmeyer, T., Wahbeh, H. & Delorme, A. Self-health monitoring and wearable neurotechnologies. *Handb. Clin. Neurol.* **168**, 207–232 (2020).
9. Gramann, K., Ferris, D. P., Gwin, J. & Makeig, S. Imaging natural cognition in action. *Int. J. Psychophysiol.* **91**, 22–29 (2014).
10. Jungnickel, E., Gehrke, L., Klug, M. & Gramann, K. Chapter 10 - MoBI—Mobile Brain/Body Imaging. in *Neuroergonomics* (eds. Ayaz, H. & Dehais, F.) 59–63 (Academic Press, 2019). doi:10.1016/B978-0-12-811926-6.00010-5.
11. Al, E. *et al.* Heart–brain interactions shape somatosensory perception and evoked potentials. *Proc. Natl. Acad. Sci.* **117**, 10575–10584 (2020).
12. Banellis, L. & Cruse, D. Skipping a Beat: Heartbeat-Evoked Potentials Reflect Predictions during Interoceptive-Exteroceptive Integration. *Cereb. Cortex Commun.* **1**, tgaa060 (2020).
13. Baranauskas, M., Grabauskaitė, A., Griškova-Bulanova, I., Lataitytė-Šimkevičienė, B. & Stanikūnas, R. Heartbeat evoked potentials (HEP) capture brain activity affecting subsequent heartbeat. *Biomed. Signal Process. Control* **68**, 102731 (2021).
14. Candia-Rivera, D. *et al.* Neural Responses to Heartbeats Detect Residual Signs of Consciousness during Resting State in Postcomatose Patients. *J. Neurosci.* **41**, 5251–5262 (2021).
15. Jiang, H. *et al.* Brain–Heart Interactions Underlying Traditional Tibetan Buddhist Meditation. *Cereb. Cortex* **30**, 439–450 (2020).
16. Kumral, D. *et al.* Attenuation of the Heartbeat-Evoked Potential in Patients With Atrial Fibrillation. *JACC Clin. Electrophysiol.* **8**, 1219–1230 (2022).

17. Thakor, N. V. & Tong, S. Advances in quantitative electroencephalogram analysis methods. *Annu. Rev. Biomed. Eng.* **6**, 453–495 (2004).
18. Thayer, J. F., Åhs, F., Fredrikson, M., Sollers, J. J. & Wager, T. D. A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* **36**, 747–756 (2012).
19. Mather, M. & Thayer, J. F. How heart rate variability affects emotion regulation brain networks. *Curr. Opin. Behav. Sci.* **19**, 98–104 (2018).
20. Kemp, A. H. & Quintana, D. S. The relationship between mental and physical health: Insights from the study of heart rate variability. *Int. J. Psychophysiol.* **89**, 288–296 (2013).
21. Daneshi Kohan, M., Motie Nasrabadi, A., Shamsollahi, M. B. & Sharifi, A. EEG/PPG effective connectivity fusion for analyzing deception in interview. *Signal Image Video Process.* **14**, 907–914 (2020).
22. Übeyli, E. D., Cvetkovic, D. & Cosic, I. Analysis of human PPG, ECG and EEG signals by eigenvector methods. *Digit. Signal Process.* **20**, 956–963 (2010).
23. Zambrana-Vinaroz, D., Vicente-Samper, J. M., Manrique-Cordoba, J. & Sabater-Navarro, J. M. Wearable Epileptic Seizure Prediction System Based on Machine Learning Techniques Using ECG, PPG and EEG Signals. *Sensors* **22**, 9372 (2022).
24. Shaffer, F. & Ginsberg, J. P. An Overview of Heart Rate Variability Metrics and Norms. *Front. Public Health* **5**, 258 (2017).
25. Coan, J. A. & Allen, J. J. B. The state and trait nature of frontal EEG asymmetry in emotion. in *The asymmetrical brain* 565–615 (MIT Press, 2003).
26. Hagemann, D., Hewig, J., Seifert, J., Naumann, E. & Bartussek, D. The latent state-trait structure of resting EEG asymmetry: replication and extension. *Psychophysiology* **42**, 740–52 (2005).
27. Widge, A. S. *et al.* Electroencephalographic Biomarkers for Treatment Response Prediction in Major Depressive Illness: A Meta-Analysis. *Am. J. Psychiatry* **176**, 44–56 (2019).
28. Olbrich, S. & Arns, M. EEG biomarkers in major depressive disorder: Discriminative power and prediction of treatment response. *Int. Rev. Psychiatry* **25**, 604–618 (2013).
29. Kumar, Y., Dewal, M. L. & Anand, R. S. Epileptic seizures detection in EEG using DWT-based ApEn and artificial neural network. *Signal Image Video Process.* **8**, 1323–1334 (2014).
30. Acharya, U. R. *et al.* Automated diagnosis of epileptic EEG using entropies. *Biomed. Signal Process. Control* **7**, 401–408 (2012).
31. de Aguiar Neto, F. S. & Rosa, J. L. G. Depression biomarkers using non-invasive EEG: A review. *Neurosci. Biobehav. Rev.* **105**, 83–93 (2019).
32. Cannard, C., Wahbeh, H. & Delorme, A. Electroencephalography correlates of well-being using a low-cost wearable system. *Front. Hum. Neurosci.* **15**, 736 (2021).
33. Demski, A. J. & Soria, M. L. ecg-kit: a Matlab Toolbox for Cardiovascular Signal Processing. **4**, e8 (2016).
34. Klug, M. *et al. The BeMoBIL Pipeline for automated analyses of multimodal mobile brain and body imaging data*. http://biorxiv.org/lookup/doi/10.1101/2022.09.29.510051 (2022) doi:10.1101/2022.09.29.510051.
35. Perakakis, P. HEPLAB. (2023).
36. Grosselin, F., Navarro-Sune, X., Raux, M., Similowski, T. & Chavez, M. CARE-rCortex: A Matlab toolbox for the analysis of CArdio-REspiratory-related activity in the Cortex. *J. Neurosci. Methods* **308**, 309–316 (2018).
37. Luck, S. J. & Gaspelin, N. How to get statistically significant effects in any ERP experiment (and why you shouldn't). *Psychophysiology* **54**, 146–157 (2017).
38. Alday, P. M. How much baseline correction do we need in ERP research? Extended GLM model can replace baseline correction while lifting its limits. *Psychophysiology* **56**, e13451 (2019).

39. Delorme, A. EEG is better left alone. *Sci. Rep.* **13**, 2372 (2023).
40. Widmann, A., Schröger, E. & Maess, B. Digital filter design for electrophysiological data – a practical approach. *J. Neurosci. Methods* **250**, 34–46 (2015).
41. Pham, T., Lau, Z. J., Chen, S. H. A. & Makowski, D. Heart Rate Variability in Psychology: A Review of HRV Indices and an Analysis Tutorial. *Sensors* **21**, 3998 (2021).
42. Vest, A. N. *et al.* An open source benchmarked toolbox for cardiovascular waveform and interval analysis. *Physiol. Meas.* **39**, 105004 (2018).
43. Smith, E. E., Reznik, S. J., Stewart, J. L. & Allen, J. J. B. Assessing and Conceptualizing Frontal EEG Asymmetry: An Updated Primer on Recording, Processing, Analyzing, and Interpreting Frontal Alpha Asymmetry. *Int. J. Psychophysiol. Off. J. Int. Organ. Psychophysiol.* **111**, 98–114 (2017).
44. Dong, L. *et al.* MATLAB Toolboxes for Reference Electrode Standardization Technique (REST) of Scalp EEG. *Front. Neurosci.* **11**, 601 (2017).
45. Candia-Rivera, D., Catrambone, V. & Valenza, G. The role of electroencephalography electrical reference in the assessment of functional brain–heart interplay: From methodology to user guidelines. *J. Neurosci. Methods* **360**, 109269 (2021).
46. Mullen *et al.* Real-time Neuroimaging and Cognitive Monitoring Using Wearable Dry EEG. *IEEE Trans. Biomed. Eng. Spec. Issue Wearable Technol.* **62**, 2553–67 (2015).
47. Chang, C.-Y., Hsu, S.-H., Pion-Tonachini, L. & Jung, T.-P. Evaluation of Artifact Subspace Reconstruction for Automatic EEG Artifact Removal. in *2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)* 1242–1245 (2018). doi:10.1109/EMBC.2018.8512547.
48. Miyakoshi, M. Artifact subspace reconstruction: a candidate for a dream solution for EEG studies, sleep or awake. *Sleep* **46**, zsad241 (2023).
49. Ablin, P., Cardoso, J.-F. & Gramfort, A. Faster independent component analysis by preconditioning with Hessian approximations. *IEEE Trans. Signal Process.* **66**, 4040–4049 (2018).
50. Frank, G., Makeig, S. & Delorme, A. A Framework to Evaluate Independent Component Analysis applied to EEG signal: testing on the Picard algorithm. Preprint at http://arxiv.org/abs/2210.08409 (2022).
51. Kim, H. *et al.* ICA's bug: How ghost ICs emerge from effective rank deficiency caused by EEG electrode interpolation and incorrect re-referencing. *Front. Signal Process.* **3**, (2023).
52. Pion-Tonachini, L., Kreutz-Delgado, K. & Makeig, S. ICLabel: An automated electroencephalographic independent component classifier, dataset, and website. *NeuroImage* **198**, 181–197 (2019).
53. Maris, E. & Oostenveld, R. Nonparametric statistical testing of EEG- and MEG-data. *J Neurosci Methods* **164**, 177–90 (2007).
54. Pernet, C. R., Latinus, M., Nichols, T. E. & Rousselet, G. A. Cluster-based computational methods for mass univariate analyses of event-related brain potentials/fields: A simulation study. *J Neurosci Methods* (2015) doi:10.1016/j.jneumeth.2014.08.003.
55. Pernet, C. R., Chauveau, N., Gaspar, C. & Rousselet, G. A. LIMO EEG: A Toolbox for Hierarchical LInear MOdeling of ElectroEncephaloGraphic Data. *Comput. Intell. Neurosci.* **2011**, (2011).
56. Pernet, C. *et al.* Electroencephalography robust statistical linear modelling using a single weight per trial. *Aperture Neuro* **2022**, (2022).
57. Clifford, G. Signal processing methods for heart rate variability. (Oxford University, UK, 2002).
58. Pan, J. & Tompkins, W. J. A Real-Time QRS Detection Algorithm. *IEEE Trans. Biomed. Eng.* **BME-32**, 230–236 (1985).
59. Maess, B., Schröger, E. & Widmann, A. High-pass filters and baseline correction in M/EEG analysis. Commentary on: "How inappropriate high-pass filters can produce artefacts and incorrect conclusions in ERP studies of language and cognition". *J. Neurosci. Methods* **266**, 164–165 (2016).

60. Park, H.-D. & Blanke, O. Heartbeat-evoked cortical responses: Underlying mechanisms, functional roles, and methodological considerations. *NeuroImage* **197**, 502–511 (2019).
61. Lomb, N. R. Least-squares frequency analysis of unequally spaced data. *Astrophys. Space Sci.* **39**, 447–462 (1976).
62. Corcoran, A. W., Alday, P. M., Schlesewsky, M. & Bornkessel-Schlesewsky, I. Toward a reliable, automated method of individual alpha frequency (IAF) quantification. *Psychophysiology* **55**, e13064 (2018).
63. Chen, W., Zhuang, J., Yu, W. & Wang, Z. Measuring complexity using FuzzyEn, ApEn, and SampEn. *Med. Eng. Phys.* **31**, 61–68 (2009).
64. Cannard, C. & Delorme, A. An open-source EEGLAB plugin for computing entropy-based measures on MEEG signals. (2022) doi:10.31234/osf.io/xwmyk.
65. Lau, Z. J., Pham, T., Chen, S. H. A. & Makowski, D. Brain entropy, fractal dimensions and predictability: A review of complexity measures for EEG in healthy and neuropsychiatric populations. *Eur. J. Neurosci.* **56**, 5047–5069 (2022).
66. Luft, C. D. B. & Bhattacharya, J. Aroused with heart: Modulation of heartbeat evoked potential by arousal induction and its oscillatory correlates. *Sci. Rep.* **5**, 15717 (2015).
67. Costa, M., Goldberger, A. L. & Peng, C.-K. Multiscale entropy analysis of biological signals. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **71**, 021906 (2005).
68. Humeau-Heurtier, A. Multiscale Entropy Approaches and Their Applications. *Entropy* **22**, 644 (2020).
69. Catrambone, V., Greco, A., Vanello, N., Scilingo, E. P. & Valenza, G. Time-Resolved Directional Brain–Heart Interplay Measurement Through Synthetic Data Generation Models. *Ann. Biomed. Eng.* **47**, 1479–1489 (2019).