**TITLE:** *(150 characters maximum)*

An open-source EEGLAB plugin for jointly analyzing EEG and cardiovascular (ECG/PPG) signals.

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**KEYWORDS:** *(6 minimum, 12 maximum)*

open-source, EEGLAB, EEG, ECG, PPG, HRV, feature-analysis, heartbeat-event potentials (HEP) and oscillations (HEO), heart component removal.

**SUMMARY:** *(10-word minimum, 50-word maximum)*

The summary should clearly state the goal of the protocol. It may include a general description of the method and its applications. This description should focus on the protocol, not the results obtained by the method.

**ABSTRACT:** *(150-word minimum, 300-word maximum)*

The abstract should focus on the method being presented rather than the results of a specific experiment. Include a statement about the purpose of the method. A more detailed overview of the method and a summary of its advantages, limitations, and applications is appropriate. Please focus on the general types of results acquired. Do not include references here.

The interaction between the cortical and cardiovascular systems has been the subject of increasing interest, as it can capture useful information about the coupling of brain and heart function. These systems are often analyzed jointly using neuroimaging methods that are invasive (PET) or very costly (MEG, fMRI). EEG and ECG/PPG are non-invasive, low-cost, and highly mobile techniques for monitoring brain and cardiovascular activity. These advantages provide the ability to collect data in real-world settings and in larger and more diverse samples. However, analyzing the interaction between these biosignals is challenging due to their noisy, nonstationary, and nonlinear nature (Klonowski 2009). While the investigation of EEG and heart rate variability (HRV) data has been prevalent for several decades, the methods used for preprocessing, windowing, and choosing appropriate parameters lacks consensus among academic and clinical investigators. Moreover, many of the important steps are omitted from publications, or require advanced programming skills, preventing reproducibility.

the lack of tools to easily process them following guidelines (for comparison of results across investigations), and apply appropriate statistical analyses. Mainly, the lack of standardized methods for processing these signals and dealing with nonstationarity and artifacts.

Existing tools for jointly analyzing these biosignals often require a high level of technical expertise and may not provide all of the necessary features for studying the interaction between these signals. Moreover, many existing tools are not open-source, limiting their accessibility, and a consensus towards clear signal processing guidelines is still in development and reproducibility.

We present the BrainBeats toolbox to address these limitations, an open-source EEGLAB plugin for jointly processing and analyzing electroencephalography (EEG) and electrocardiography/photoplethysmography (ECG/PPG) signals.

The proposed toolbox addresses these limitations by providing a comprehensive set of signal processing functions, implementing the latest guidelines as its default parameters, and three main functions: 1) feature-based for continuous data for assessing associations between various features extracted from EEG and HRV signals (time, frequency, and nonlinear features); 2) event-related for epoched data (EEG-ECG coherence, heartbeat-evoked potentials in time, frequency, and time-frequency domains); 3) EEG signal processing (extracts heart components from EEG signals using ICA). Robust statistics are implemented for assessing relationships between these time series at the individual level (e.g., permutation statistics, corrections for type 1 error). Linear and nonlinear features are provided to better capture complex, bidirectional interactions between that take place between the cardiovascular, subcortical, and cortical systems. Additionally, the toolbox can be used simply for extracting heart components from the EEG data using independent component analysis (ICA). This open-source toolbox should provide a valuable resource for researchers and clinicians interested in studying the relationship between the brain and cardiovascular activity and can be easily adapted and extended to meet specific research needs.

**INTRODUCTION:** *(150-word minimum, 1500-word maximum, 2-paragraph minimum)*

* Purpose of this method

The purpose of this method is to ease the investigation of relationships between the brain and the heart by facilitating the joint analysis of electroencephalography (EEG) and cardiovascular signals, namely electrocardiography (ECG) and photoplethysmography (PPG), while implanting the latest guidelines from experts in this field. This tool addresses limitations from existing tools and is made open source to facilitate accessibility and reproducibility in the field. The proposed toolbox should serve as a valuable resource for researchers and clinicians interested in removing cardiac artifacts from EEG signals, in extracting features from EEG and ECG/PPG signals, or in studying the relationship between brain and cardiovascular activity. Ultimately, this toolbox aims to pave the way for more in-depth investigations into the complex interplay between the brain and heart systems.

* The rationale behind the development and/or use of this method + context of the method in the wider body of published literature

**Reductionist vs. multimodal approaches**

For a long time, the reductionist approach has dominated scientific inquiry in the field of 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 (von Bertalanffy, 1968). Reductionism has been instrumental in understanding individual subsystems in isolation, such as elucidating the role of ion channels and action potentials for neural (Hodgkin & Huxley, 1952) and cardiac (Bean et al., 1984) communication. However, a large gap remains in our understanding of how these isolated systems interact together on a larger spatial and temporal scale. Thanks to recent advances in technology, a more integrative approach is now gaining interest. Innovations in hardware, signal processing, data storage, and computing capabilities have enabled researchers to collect and analyze electrophysiological signals from different bodily systems simultaneously (e.g., heart, brain, skin, etc.; Jiang et al. 2019; Insel et al., 2017; Kappenman & Luck, 2010). This integrative – or multimodal – approach will pave the way for a more comprehensive understanding of human physiology, cognition, pathology, and consciousness (Bunge, 2003; Fuchs, 2018; Thayer & Lane, 2009). Consequently, this multimodal approach is now considered an essential component of modern research, complementing traditional reductionist methods and offering novel insights into the synergistic mechanisms governing the human body and mind.

**Heart-brain research with fMRI and PET**

Studying the intricate relationship between the brain and the heart can yield valuable insights into the underlying physiology and anatomy of the human body, ultimately leading to the development of novel diagnostic and therapeutic tools. The relationship between the heart and the brain has been 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; Critchley 2000; Napadow 2008), showed the influence of heart rate on the BOLD signal (Chang et al., 2009; Shmueli 2007), or identified potential brain-body pathways contributing to coronary heart disease (i.e., stress-evoked blood pressure; Gianaros 2009).

**EEG, ECG, and PPG**

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 of time, providing new opportunities for investigating the dynamic relationship between brain and heart function. ECG measures the electrical signals generated by the heart when it contracts and relaxes via the placement of electrodes on the skin (usually on the chest, arms, or legs). PPG measures blood volume changes in the microvascular tissues by using a light source (e.g., LED) and a photodetector placed on the skin (commonly on a fingertip, earlobe, or forehead). Since blood absorbs more light than the surrounding tissue, the PPG signal can be used to estimate blood flow and pulse rate. Both methods provide valuable information about the cardiovascular function, but they serve different purposes and offer distinct types of data. 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. Similar to ECG, EEG records the electrical fields generated by synchronized activity of thousands of cortical neurons by placing electrodes on the scalp.

**The 2 approaches to jointly analyzing EEG and cardiovascular signals**

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

1. Event-related: each heartbeat is marked in the EEG time series to examine with high temporal accuracy the cortical activity processing cardiac signals (Schandry 1981; Pollatos 2005; Montoya 1993; Park & Tallon-Baudry 2014; Couto 2015; Jiang et al. 2019; Dirlich et al., 1998). This method is termed heartbeat-evoked potentials (HEP) and is similar to traditional event-related potential (ERP) studies, which required the two time series to be time-locked, but the events are heartbeats as opposed to stimuli.
2. Feature-based: using continuous data, this approach extracts features from the EEG signals and from the cardiovascular signals and examines associations between them. Cardiovascular features are typically heart-rate variability (HRV) measures in the time, frequency, or nonlinear domains. This has been done with ECG (Thayer et al. 2012; Mather 2018; Kemp 2013) and PPG to a lesser extent (Khosrow-Khavar 2014). This approach provides trait information that can be used for making medical forecasting or classification (e.g., mental or physical health) and finding more general associations relative to HEP that focus on mechanisms at millisecond accuracy.

The advantages over alternative methods with references to relevant studies

While some tools exist to process cardiovascular and EEG (e.g., EEGLAB, Fieldtrip, Brainstorm) signals separately, none is currently available for jointly analyzing them. Furthermore, the tools available to process cardiovascular signals:

* require expensive license purchase and do not allow processing large datasets in batch via command line (Kubios);
* or require advanced programming skills by not providing a graphical user interface (GUI; Physionet Cardiovascular Signal toolbox; [Vest et al. 2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6442742/pdf/nihms-1509712.pdf)).

To our knowledge, three open-source MATLAB toolboxes exist to support HEP analysis with a GUI. The ecg-kit ([REF](https://github.com/marianux/ecg-kit); Demski and Soria, 2016), the HEPLAB EEGLAB plugin ([REF](https://github.com/perakakis/HEPLAB)), and the CARE-rCortex EEGLAB plugin ([Grosselin et al. 2018](https://www.sciencedirect.com/science/article/abs/pii/S0165027018302474)). While HEPLAB and ecg-kit facilitate HEP analysis by detecting heartbeats and marking them in the EEG signals, they do not provide statistical tools for analysis, and are limited to the time domain (i.e., ERP). The CARE-rCortex addressed these issues by supporting both ECG and respiratory signals, time-frequency domain analysis, statistics, and advanced baseline normalization and correction methods specifically adapted to HEP analysis. However, it uses the Bonferoni method for multiple comparisons correction, which is too conservative for EEG analysis (Ref), and it does not offer command-line access for processing and analyzing large datasets. Furthermore, recent studies recommend against baseline correction methods, highlighting how they reduce signal-to-noise ratio (SNR) and are “statistically unnecessary and undesirable” ([Alday 2019](https://onlinelibrary.wiley.com/doi/abs/10.1111/psyp.13451); Delorme 2023).

To address these limitations, this article presents a new open-source MATLAB toolbox, named “BrainBeats”, implemented as an EEGLAB plugin designed to jointly process and analyze EEG and ECG/PPG signals. It incorporates the following advantages over previous methods:

* Easy-to-use GUI (for non-programmers) and command line mode (for programmers aiming to perform automated processing and analysis on large datasets, use more advanced parameters).
* Implements the validated algorithms from the Physionet Cardiovascular Signal toolbox ([Vest et al. 2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6442742/pdf/nihms-1509712.pdf)) for detecting the QRS complexes, estimating a validated signal quality index (SQI) of the RR time series, and removing or interpolating the RR artifacts automatically with various algorithms (e.g., linear, cubic, nearest neighbor, etc.) to otbain the normal-to-normal (NN) intervals.
* Implements validated EEG signal processing, including referencing (to infinity for montages with at least 30; Yao [2001](https://pubmed.ncbi.nlm.nih.gov/11761077/), [200](https://pubmed.ncbi.nlm.nih.gov/15798293/)5; [2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5331115/); [Dong 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5670162/)), detection and interpolation of bad channels, detection and interpolation of EEG artifacts (Mullen 2015; Delorme 2004; 2023; Pion-Tonachini 2019). Note: Users can also use the toolbox with already cleaned data.
* Can be used to automatically extracting heart components from EEG signals using the ICLabel EEGLAB plugin (**Method 1**).
* Supports HEP analysis (**Method 2**) in time, frequency, and time-frequency domains (EEGLAB STUDY mode) and advanced statistics using hierarchical linear modeling provided by the LIMO plugin (Pernet 2011). This statistical approach accounts well for within and between- subjects variance, downweighs artifactual trials using weighted least square (WLS; Pernet 2022) optimization, and applies bootstrap statistics and advanced corrections for multiple comparisons (e.g., spatiotemporal cluster correction). Furthermore, inter-trial coherence analysis can be performed using EEGLAB default statistics.
* Supports for the first time the study of brain-heart associations using a feature-based approach (**Method 3**). HRV features (listed in **Figure 1**) are calculated following guidelines to facilitate standardized and accurate analysis (Shaffer and Ginsberg 2017). The novelty about this approach is that it captures the trait variable and can be used on large continuous EEG datasets collected in the laboratory or in real-world settings using wearable headsets for example (e.g., clinics, patient’s home; Cannard et al., 2020). These features can be analyzed with simple correlation procedures or with machine learning to make classifications or predictions (e.g., detection of depression before symptoms are too advanced; REF).
* Provides various data visualizations to inspect signals (raw, RR intervals, EEG and RR artifacts that have been removed or interpolated) or outputs for each file (e.g., HEP data, power spectra from NN or EEG signals, etc.; see **Figures 2 and 3**).

For example, Vest et al. demonstrated how modest differences in the approach to HRV analysis  
can lead to divergent results, a factor that might have contributed to the lack of repeatability of  
studies and clinical applicability of HRV metrics.

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

This toolbox is appropriate for any researcher or clinician that possesses data containing EEG and ECG/PPG signals. The plugin does not support the importation of EEG and ECG/PPG signals from separate files yet (although this feature will be available soon). The toolbox can be used for simply removing heart artifacts from EEG signals with high confidence, to simply extract EEG or HRV features (for other purposes), or for assessing interactions between these two systems.

**PROTOCOL:** *(1-page minimum, 10-page maximum)*

1. **Method 1: Heartbeat-evoked potentials (HEP)**
   1. Load data into EEGLAB: *File > Load existing dataset > select “sample\_data1.set”*. See **Figure 1.1.** Note: this dataset was collected with a “wet” Biosemi system during a mindwandering (shortened to 5 minutes to facilitate replication of the following steps), and contains 64 EEG channels and 2 ECG channels.

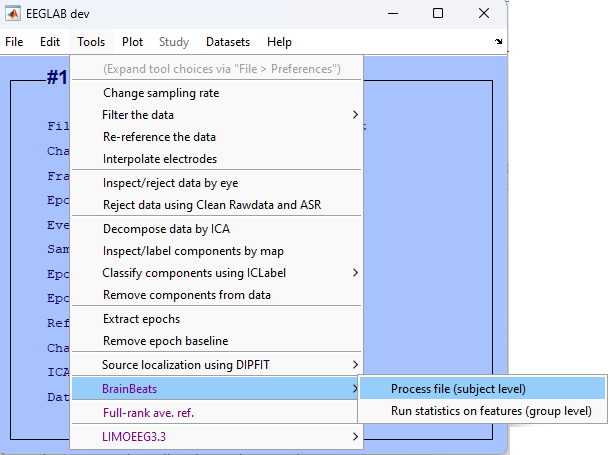


Figure 1.1. Main EEGLAB menu to launch BrainBeats’ general user interface (GUI) to select processing parameters.

* 1. Open the general user interface (GUI) to select parameters: Tools > BrainBeats > Process file (subject level).
  2. Select “Heartbeat-evoked potentials HEP” as analysis to run, “ECG” as heart data type, click on the button to display the list of channels to select the ECG channels labeled “ECG1” and “ECG2” (or type the channel labels directly in the text box next to the button. Select “Shape-preserving piecewise cubic interpolation” as cleaning method of RR artifacts, and clean EEG data. Select “Clean EEG” to process the EEG data, plot and save outputs, and click “Ok” to launch. See overview in **Figure 1.2**. All steps are automated (see **Representative Results**).

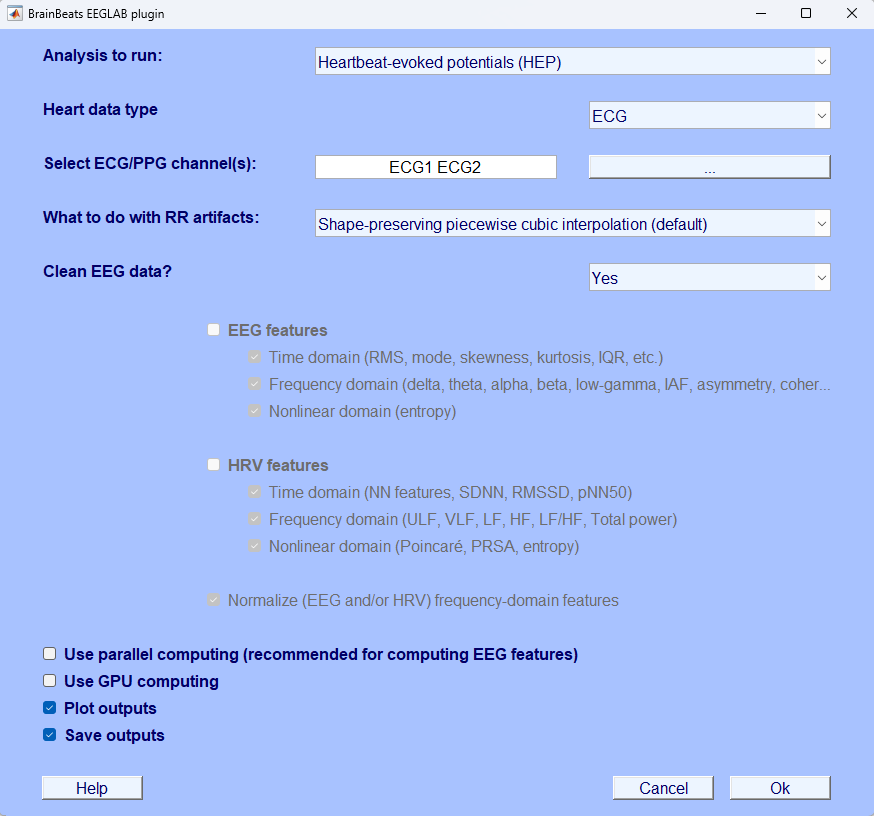


Figure 1.2. Using the GUI to select parameters for preparing data for heartbeat-evoked potentials (HEP) analysis.

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

eeglab; close;

mainDir = fileparts(which('eegplugin\_BrainBeats.m'));

cd(mainDir);

EEG = pop\_loadset('filename','sample\_data1.set','filepath',fullfile(mainDir, 'sample\_data'));

EEG = brainbeats\_process(EEG,'analysis','hep','heart\_signal','ECG', ...

'heart\_channels',{'ECG1' 'ECG2'},'clean\_rr','pchip','clean\_eeg',true, ...

'parpool',false,'gpu',false,'vis',true,'save',true);

Alternatively, as a semi-automated method, the GUI can be launched with the following command:

EEG = brainbeats\_process(EEG);

1. **METHOD 2: Extract EEG and HRV features from continuous data**
   1. Load the dataset and launch the BrainBeats GUI as for METHOD 1. Select “Extract EEG & HRV features from continuous data”, the same parameters for ECG channels, RR interpolation method, and “clean EEG”. Notice that the EEG and HRV feature fields are now unlocked. Check both to extract EEG and HRV features. All domains (time, frequency, nonlinear) are set by default. Check “Use parallel computing” to increase computation speed. “Plot and save outputs” are set by default. Click “Ok” to launch.

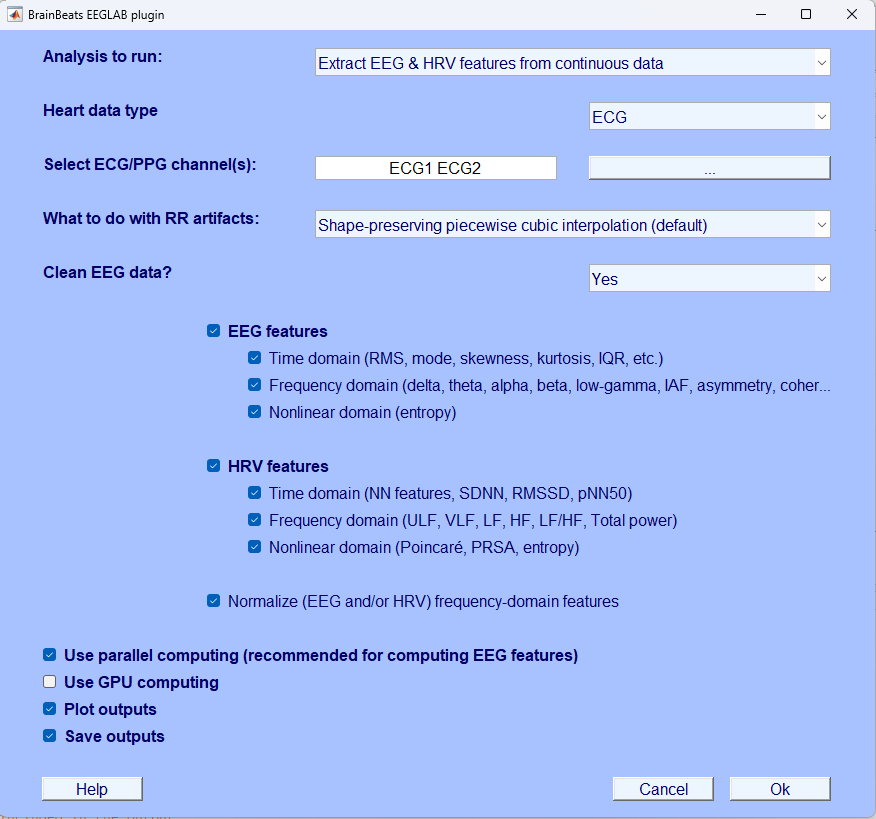


Figure 2.1. BrainBeats GUI to select parameters for extracting HRV and EEG features from continuous data.

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

eeglab; close;

EEG = pop\_loadset('filename','sample\_data2.set','filepath',fullfile(dataDir, 'sample\_data'));

mainDir = fileparts(which('eegplugin\_BrainBeats.m'));

[~, Features] = brainbeats\_process(EEG,'analysis','features','heart\_signal','ECG', 'heart\_channels',{'ECG1' 'ECG2'}, 'clean\_rr','pchip','clean\_eeg',true,'norm',true,...

'eeg\_features', {'time' 'frequency' 'nonlinear'}, ...

'hrv\_features', {'time' 'frequency' 'nonlinear'}, ...

'gpu',false,'parpool',true,'save',true,'vis',true);

1. **Method 3: Remove heart components from EEG signals.**
   1. Load “sample\_data2.set” containing 3 EEG channels and one ECG channel, collected with a “dry” Muse wearable headset.

Open the same BrainBeats GUI (see **Step 1.1**. and **Figure 1.1.**) and set the analysis type as “Remove heart components from EEG signals”, and select the ECG channel from the list of channels. Select “No (already processed)” in the “Clean EEG?” field, since this sample file was already preprocessed. Set “Plot outputs” and “Save outputs” options (set by default). Click “Ok” to launch.

These steps can be run automatically over many files using the following command lines:

eeglab; close; dataDir = fileparts(which('pop\_BrainBeats.m')); cd(mainDir);

EEG = pop\_loadset('filename','sample\_data2.set','filepath',dataDir);

EEG = brainbeats\_process(EEG,'analysis','rm\_heart','heart\_signal','ECG', ...

'heart\_channels',{'ECG'},'clean\_eeg',false,'save',true,'vis',true);

As for Method 1, users can perform statistical analyses using the EEGLAB STUDY mode. Hierarchical linear modeling is supported by the LIMO plugin for advanced analyses and and corrections for type 1 error.

**REPRESENTATIVE RESULTS:**

**METHOD 1**

BrainBeats first separates the ECG from EEG data to process them separately. EEG data are band-passed filtered using a zero-phase non-causal finite impulse response (FIR) filter to remove low-frequency drifts and high-frequency noise (high-pass cutoff frequency = 1 Hz, low-pass cutoff = 45; order = 1650; transition bandwidth = 0.5 Hz). If the data have at least 30 channels and not been referenced, BrainBeats re-references them to infinity/REST (Yao [2001](https://pubmed.ncbi.nlm.nih.gov/11761077/), [200](https://pubmed.ncbi.nlm.nih.gov/15798293/)5; [2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5331115/); [Dong 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5670162/)). Then, BrainBeats uses the *clean\_rawdata* plugin to automatically detect and remove bad EEG channels (flatlinecriterion = 5; ChannelCriterion = .9; LineNoiseCriterion = 5). Removed channels are plotted (**Figure 1.3, in red**) and interpolated using spherical splines (Perrin et al., 1989). CAUTION: These default parameters are implemented for best performance for this sample dataset and in most cases, but we recommend users to clean their datasets before launching BrainBeats to tune parameters for their dataset. For example, abnormal channels may not be reliably detected on low-density EEG montages using this default method.

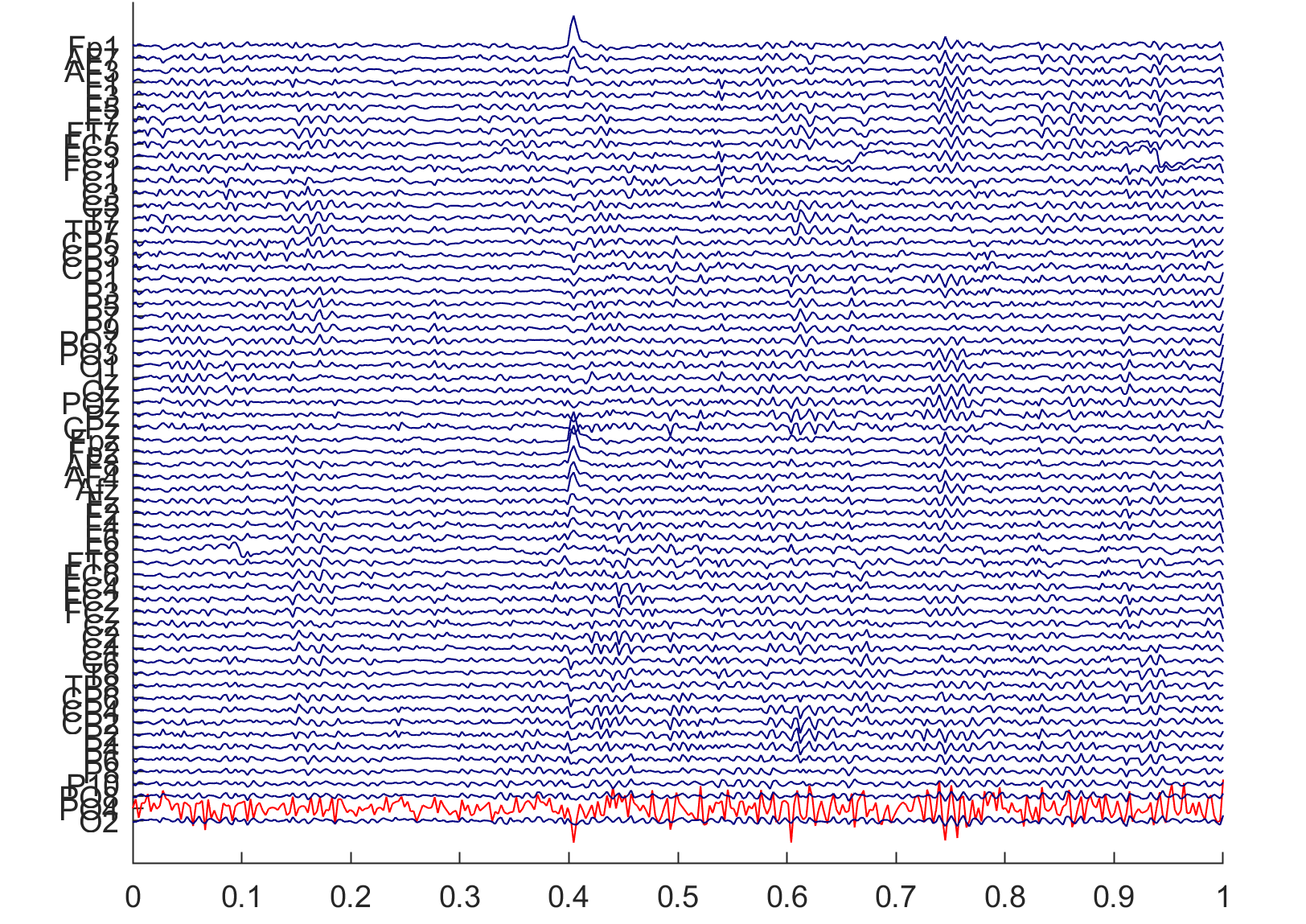


Figure 1.3. Abnormal EEG channels automatically detected and removed by BrainBeats (in red).

Next, BrainBeats detects R-peaks (from QRS complexes) on non-overlapping windows disregarding initial signal artifacts. The signal undergoes bandpass filtering and Pan–Tompkins method (P&T method), including differentiation, squaring, integration, and smoothing ([ref](https://ieeexplore.ieee.org/document/4122029)). The P&T energy threshold is estimated to avoid disruption from large bumps, and if the RR interval variability exceeds 1.5 times the median, it conducts a "search back" for missed peaks. The R-peak's mean sign over 30 seconds determines the QRS complex sign, ensuring consistent detection. QRS detection and search back are conducted based on an energy threshold defined by the signal's sample rate and smoothed ECG values. Each segment's peak sign is ascertained, and peak points are refined through a refractory period check, also managing flatline conditions. The output consists of RR intervals, heart rate (HR), RR interval timestamps, filtered signal, R-peak indices, peak sign, and the estimated P&T energy threshold. The code (*get\_RR* function) is based on methods developed by Behar et al (2014); Johnson et al. (2014).

Next, BrainBeats identifies abnormal RR intervals (e.g., too closely or largely spaced, physiological and non-physiological artifacts), flags them, and interpolates them using the shape-reserving piecewise cubic method (default) to obtain the normal-to-normal (NN) intervals. Spikes within RR intervals are detected using a forward-backward search. Users can choose another interpolation method or to remove them, if desired. Finally, the signal quality index (SQI) of the RR series is calculated. When several ECG channels are present, these steps are performed on each of them, and the RR intervals with the best SQI is selected for the following steps. The filtered ECG signal and identified R-peaks, as well as the NN intervals and interpolated artifacts (from the best electrode) are plotted (see **Figure 1.4.**). Note that users can scroll through the R-peaks more closely using a scroll bar. This code is adapted from the validated algorithms developed for the Physionet Cardiovascular Signal toolbox (Clifford 2002; Vest et al. 2018). See Vest et al. (2018) for validation and performance comparison with other state-of-the-art softwares (e.g., Kubios, ecg-kit).

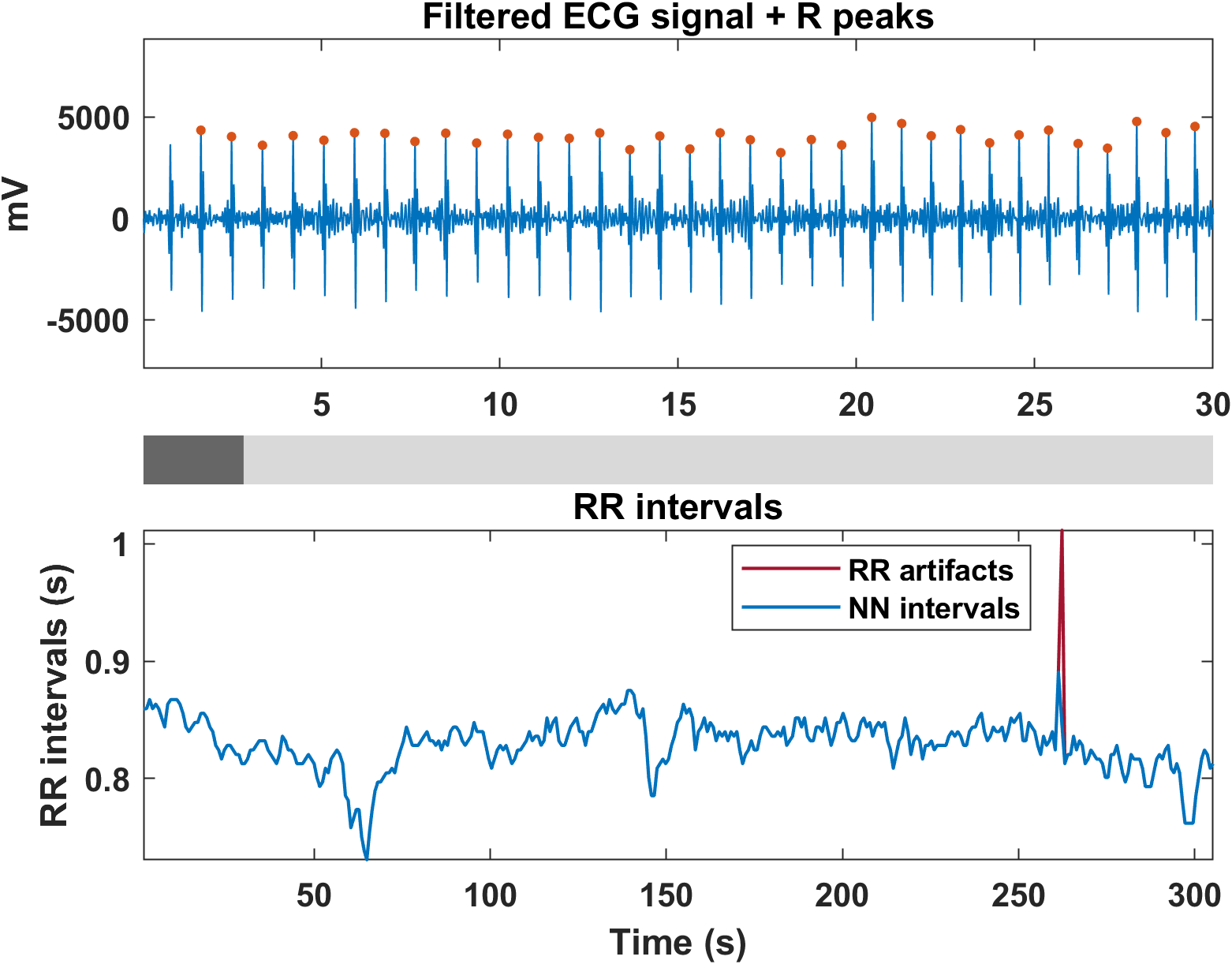


Figure 1.4. Filtered and smoothed ECG signal with identified R-peaks (top panel). Normal-to-normal (NN) intervals with the interpolated RR artifacts (bottom panel).

Next BrainBeats adds R-peak markers to the EEG signals, calculates inter-beat-intervals (IBIs) and removes trials with IBIs less than 550 ms (following recommendations by Candia-Rivera et al., 2021) and Park & Blanke, 2019). The *run\_HEP* function then removes outlier trials (detected by MATLAB’s *isoutlier* function, *‘grubbs’* method) generates a histogram of the resulting IBIs with fitted normal density (see Figure 1.5.). To determine the minimum epoch size cutoff following R-peak events, the 5th percentile of the IBI data is calculated (i.e., value below which 5% of the IBI falls; displayed as a dashed red line in the histogram). This epoch size maximizes the number of epochs and limits overlapping epochs.

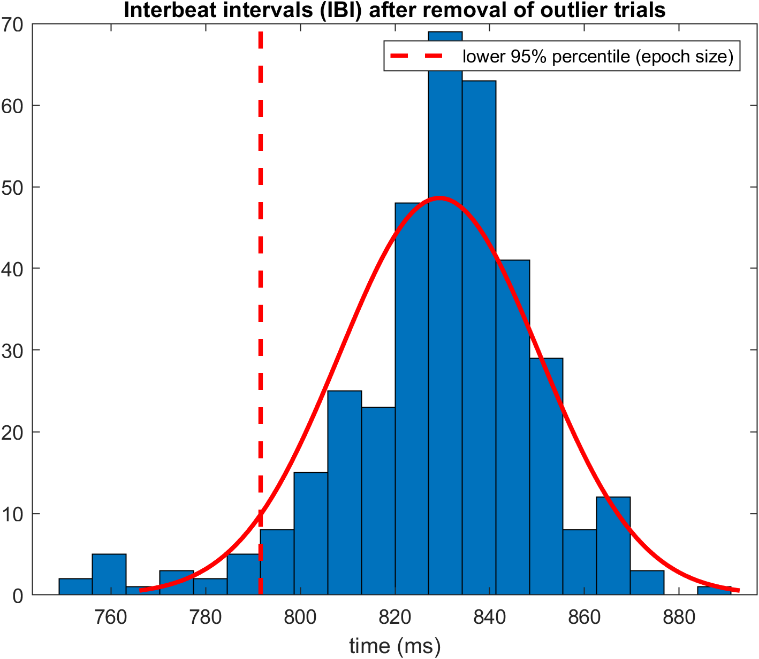


Figure 1.5. Histogram of the interbeat intervals (IBI) with fitted normal density (red line) and the 5% percentile (red dashed line) indicating the minimum cutoff value at which the EEG data are epoched.

BrainBeats then epochs the EEG data from -200 ms to + 5% percentile of IBI and eliminates bad trials (by extracting RMS and signal to noise ratio features for each epoch and identifying outliers) and eye/muscle components with ICA and ICLabel (at least 90% classification confidence for ocular components and 95% confidence for muscular components; see **Figure 1.6.**). These flagged components are then automatically subtracted from the EEG signals while preserving relevant brain signals. Note: if the Picard plugin is already installed in EEGLAB, it used by default for fast computation of ICA (REF), otherwise, the Infomax algorithm is used. Effective data rank is calculated prior to running ICA and PCA-diemnsion reduction is applied when the data are rank-deficient to avoid ghost IC artifacts; Kim et al. 2023). The final output of processed HEP data is plotted for final inspection (see **Figure 1.7.**), and saved in the same directory as the original file loaded by the user (same name with “\_HEP” at the end). Note: it is recommended to follow the BIDS for better organization, replication, and for performing statistics at the group level (EEGLAB STUDY). Users can pause before processing the next file (next condition or participant).

A picture containing colorfulness, aqua, circle, turquoise

Description automatically generated

Figure 1.6. Eye component automatically classified and removed from HEP data to extract ocular artifacts without removing relevant brain signals.

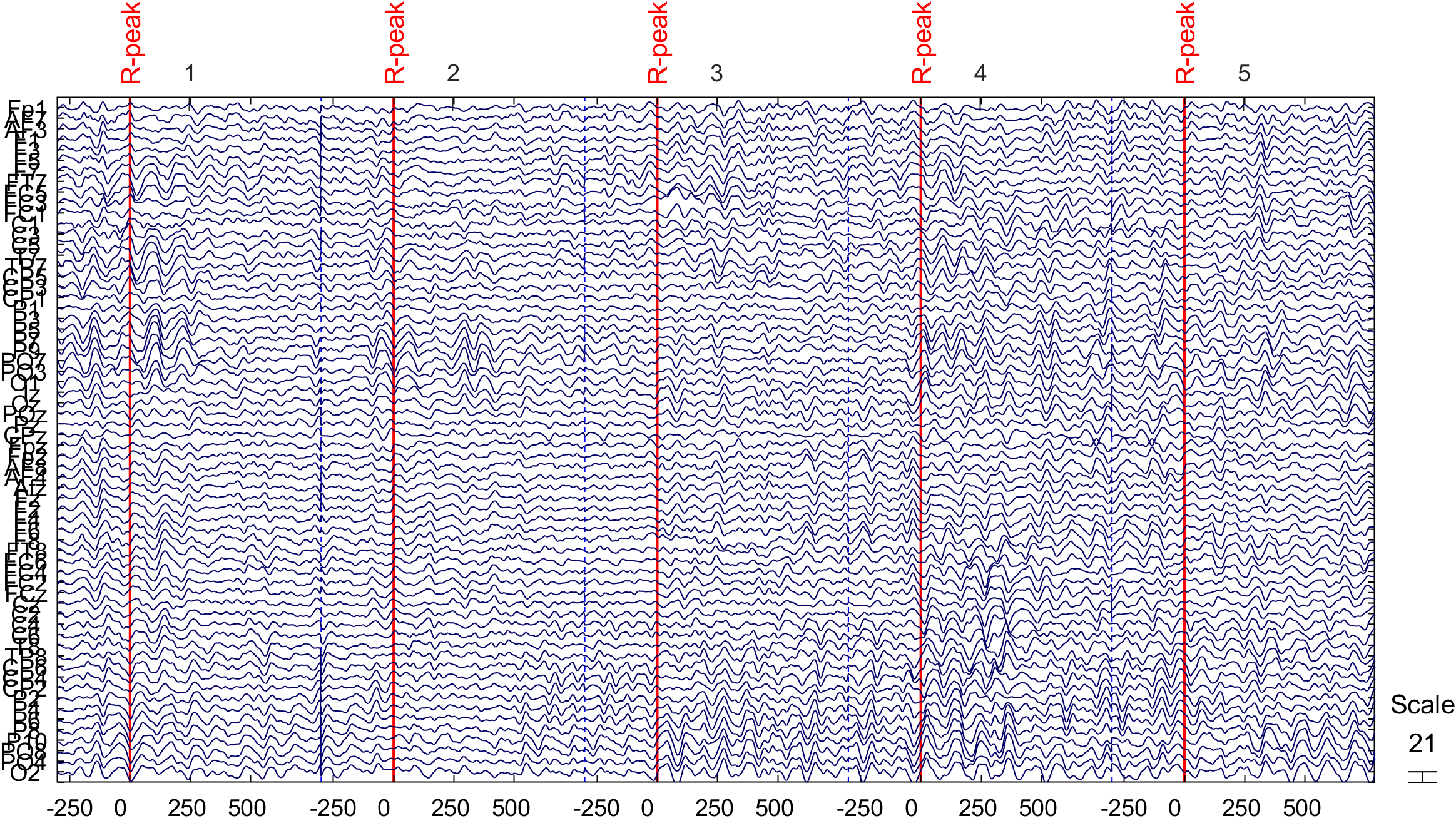


Figure 1.7. Final, cleaned EEG data, ready for HEP analysis.

The HEP averaged across all epochs are plotted as a scalp topography allowing users to inspect each electrode more closely (see Figure 1.8.), superimposed with scalp topography in the region of interest (200-500 ms after heartbeat; **Figure 1.9. top panel**; see Candia-Rivera et al. 2021, and Park & Blanke 2019), and the HEP evolution over time (**Figure 1.9. bottom panel**).

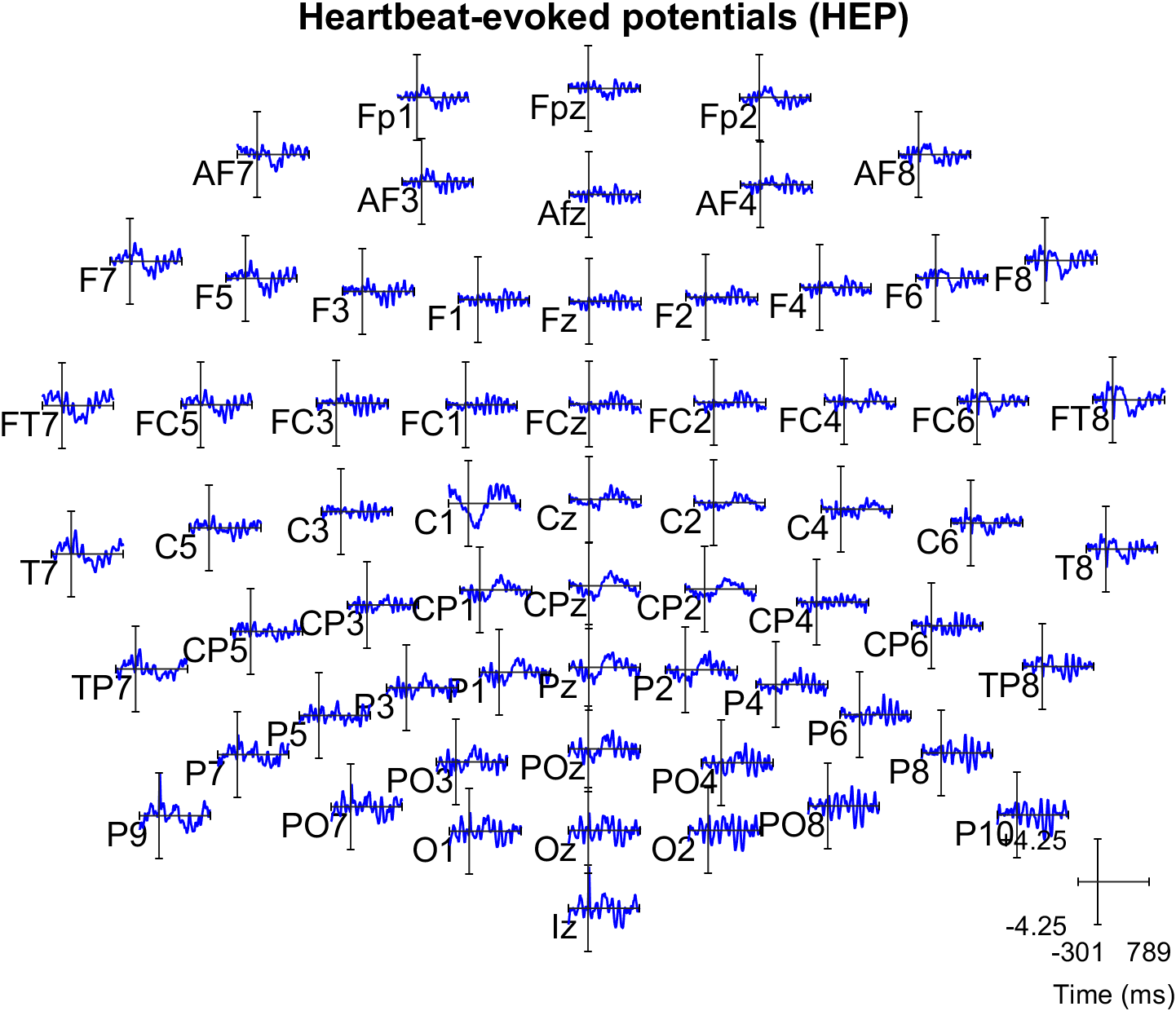


Figure 1.8. HEP averaged across trials for each electrode. Users can click on electrodes of interest for closer inspection.

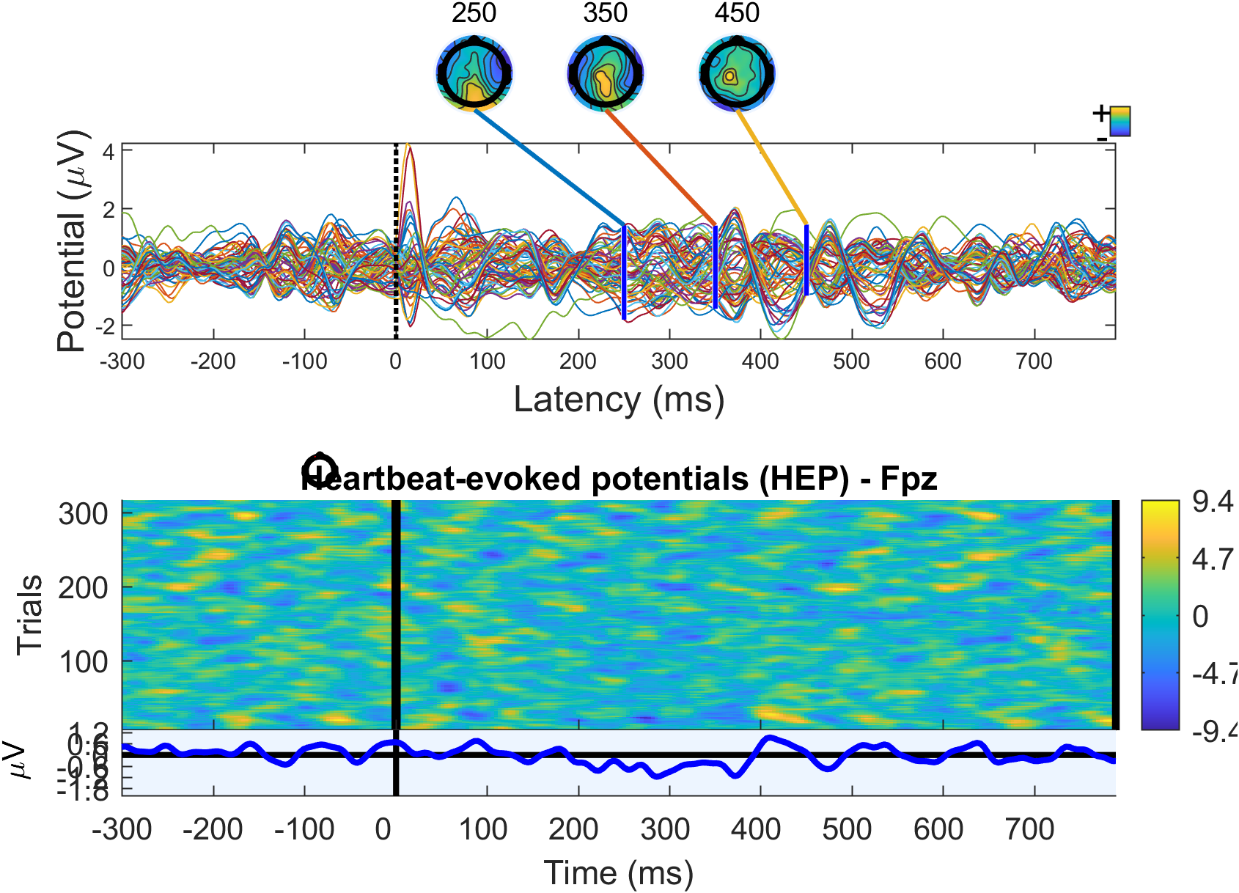
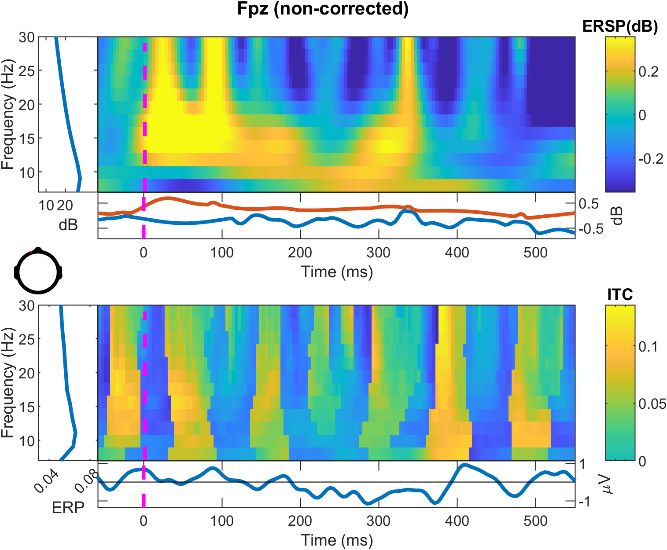
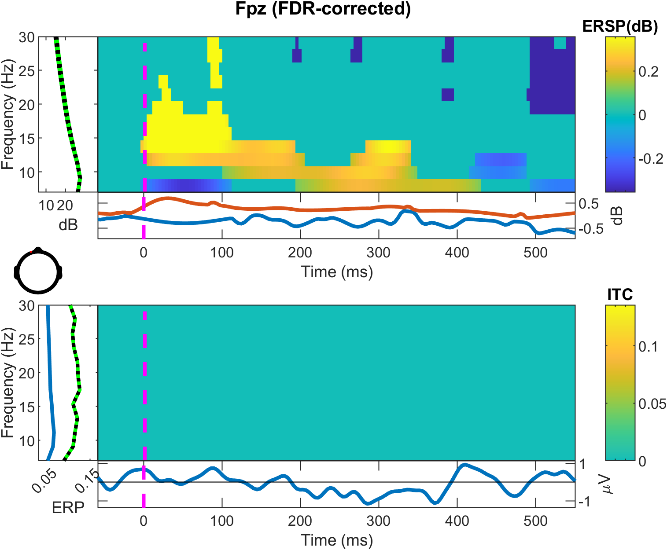


Figure 1.9. Top: HEP averaged across trials, all electrodes superimposed in the time domain, with scalp topographies showing amplitude distribution in the period of interest (200-500 ms after heartbeat). Bottom: HEP evolution over time (each “trial” corresponds to a heartbeat).

The heartbeat evoked oscillations (HEO, or event-related spectral perturbations; ERSP; **Figure 1.10. top panels**), and intertrial coherence (ITC; **Figure 1.10. bottom panels**) are computed for the frontal electrode Fpz using wavelet ([3 0.8] cycles; pre-event baseline removal; pad ratio of 2) for frequencies 7-30 Hz. The same plot is generated but after applying permutation statistics and FDR-correction for controlling for multiple comparisons (type 1 error or family-wise error). After correction (**Figure 1.10. Right**), a significant HEO effect is observed on the sample dataset in the beta band during QRS complex (within 100 ms after R-peak) and in the alpha band during the period of interest (i.e. 200-500 ms, consistent with previous findings; REF). No effect is observed in the ITC data after correction. Note: these plots and results are for replication and illustration only. Low-frequency cannot be estimated due to the short epoch size between the heartbeats.

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When all files are processed, users may import them into an EEGLAB STUDY to compute HEP (i.e., ERP) and HEO (i.e., ERSP and ITC) data on the whole group and run statistics at the group level. We recommend perform hierarchical linear modeling using the LIMO plugin (REF). A full tutorial is available at <https://github.com/LIMO-EEG-Toolbox/limo_tools/wiki>

METHOD 2

RR and NN series obtained as in METHOD 1 (The same **Figures 1.3.** and **1.4.** pop-up). However, EEG data are cleaned differently since EEG and ECG signals do not need to be time-locked with ms accuracy to extract features (unlike for HEP/HEO analysis). Here, instead of removing abnormal epochs, BrainBeats first removes segments with large EEG artifacts using artifact subspace reconstruction (ASR; SD criterion = 30; REF; **Figure 2.1.**). Then, Infomax ICA (or the Picard algorithm if installed) is performed with PCA-dimension reduction to account for effective data rank, and ocular and muscular components are classified and subtracted from the signal using ICLabel (as in Method 1, but on continuous data). One ocular component is removed with 98.6% accuracy.

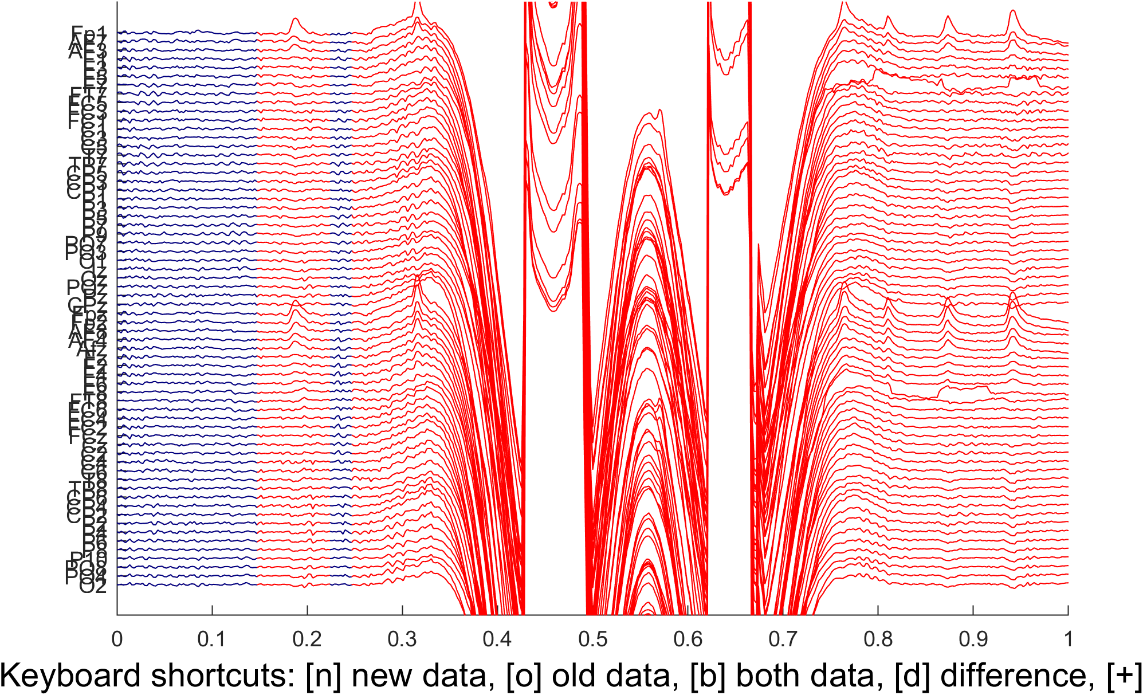


Figure 2.1. EEG artifacts are removed automatically from the continuous EEG data using artifact subspace reconstruction (ASR).

Then, HRV and EEG features are extracted in the time (NN), frequency, and nonlinear domains. See **Table 1** for a list of the features currently available. The Lombscargle-periodogram is the default method to compute frequency HRV as it does not require interpolation or resampling unlike the Welch or FFT methods, better preserving original information. The normalized periodogram is used by default to scale total power to the time series’ variance, better dealing with non-uniformly sampled data (common in NN data), and facilitating comparison of results across different participants. When normalization is set in the GUI (default), each band power is divided by the total power to provide a more intuitive measure of the relative contribution of each band on overall power (Shaffer and Ginsberg, 2017). Note: the Welch and FFT methods, and the required resampling step are also available through command line. EEG spectral power is also normalized to decibels (dB) and to total power (same reasons as for HRV power). Additionally, an algorithm is used to detect individualized frequency bounds and compute band power on the individualized bands (see Corcoran et al. 2018).

Table 1. HRV & EEG features estimated with the BrainBeats plugin

|  |  |  |
| --- | --- | --- |
|  | **HRV** | **EEG** |
| **Time** | NN statistics (mean, mode, variance, skewness, kurtosis, interquartile range), SDNN, RMSSD, pNN50. | Amplitude statistics (RMS, variance, skewness, kurtosis, interquartile range) |
| **Frequency** | Spectral power in the ultra-low frequency (ULF), very-low frequency (VLF), low-frequency (LF), and high-frequency (HF) bands, and total power. | Power spectra (all frequencies), mean band power (delta, theta, alpha, beta, low-gamma), individual alpha frequency (IAF), alpha asymmetry (all pairs), EEG coherence (between all non-neighbor pairs) |
| **Nonlinear** | Poincaré (SD1, SD2, SD1/SD2 ratio), fuzzy entropy, multiscale fuzzy entropy, phase-rectified signal averaging (PRSA) | Fuzzy entropy, multiscale fuzzy entropy (all scale factors, mean, SD, peak scale factor, and area under the curve) |

When features are computed, a plot displays the power spectral density (PSD) and multiscale fuzzy entropy (MFE) estimated on the NN series (**Figure 2.2., left**), and on the EEG data (the average across all electrodes is used for illustration; **Figure 2.2., Right**). For EEG MFE, each scale factor is band-pass filtered by default, following guidelines to reduce spectral bias in fine time scales (presumed to indicate fast dynamics) by broadband spectral power (dominated by low-frequency contributions, see Kosciessa et al. 2020 and Grandy et al. 2016 for more detail). As a result, the frequency bounds of each scale factors are provided. **Note**: EEG entropy measures are very computation-heavy. Thus, 20 scale factors are set by default, and when EEG signals are longer than 5,000 samples, they are resampled (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 low-pass filter). Furthermore, parallel and/or GPU computing to further accelerate the process. Original code for entropy was developed by Azami and Escudero (2016). Next, the EEG scalp topographies are displayed for each frequency band (**Figure 2.3. top and middle rows**), as well as for the individual alpha frequency (IAF; **Figure 2.3. bottom left**) and fuzzy entropy (**Figure 2.3. bottom right**).

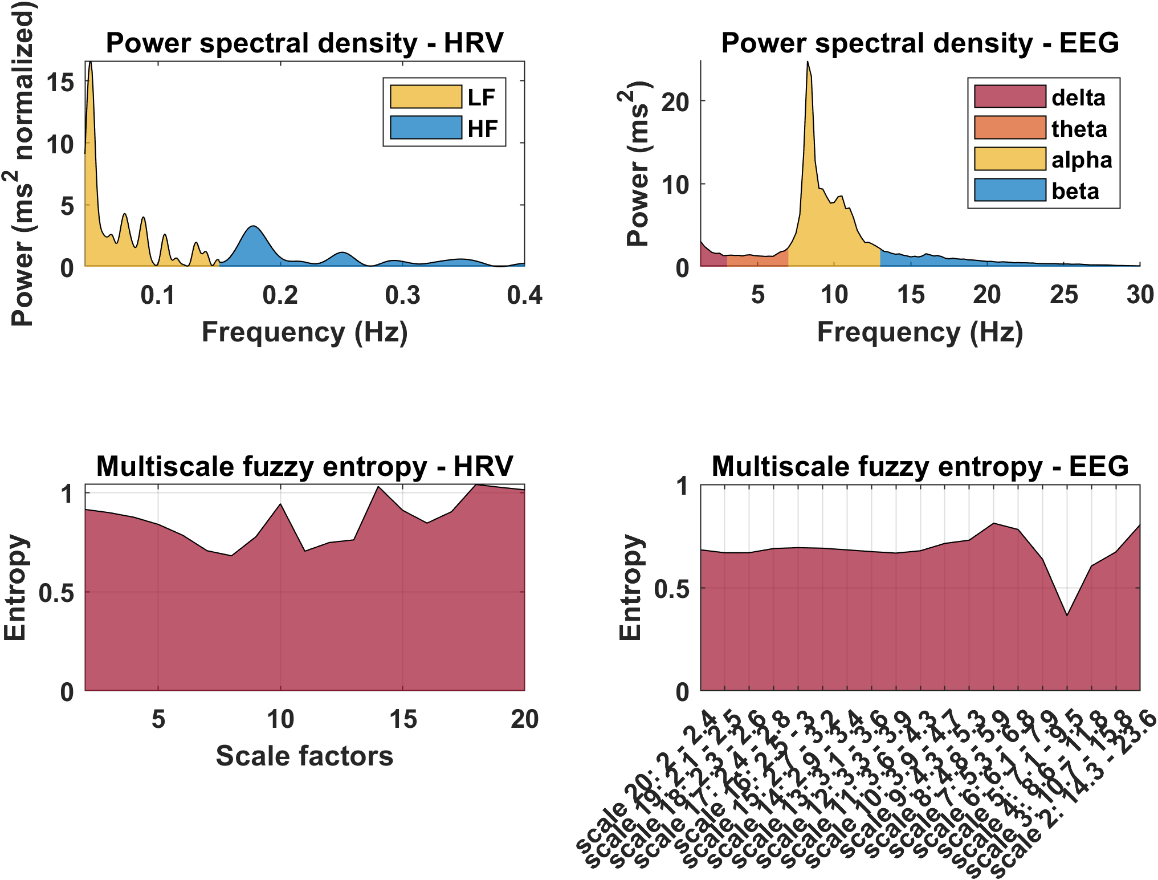
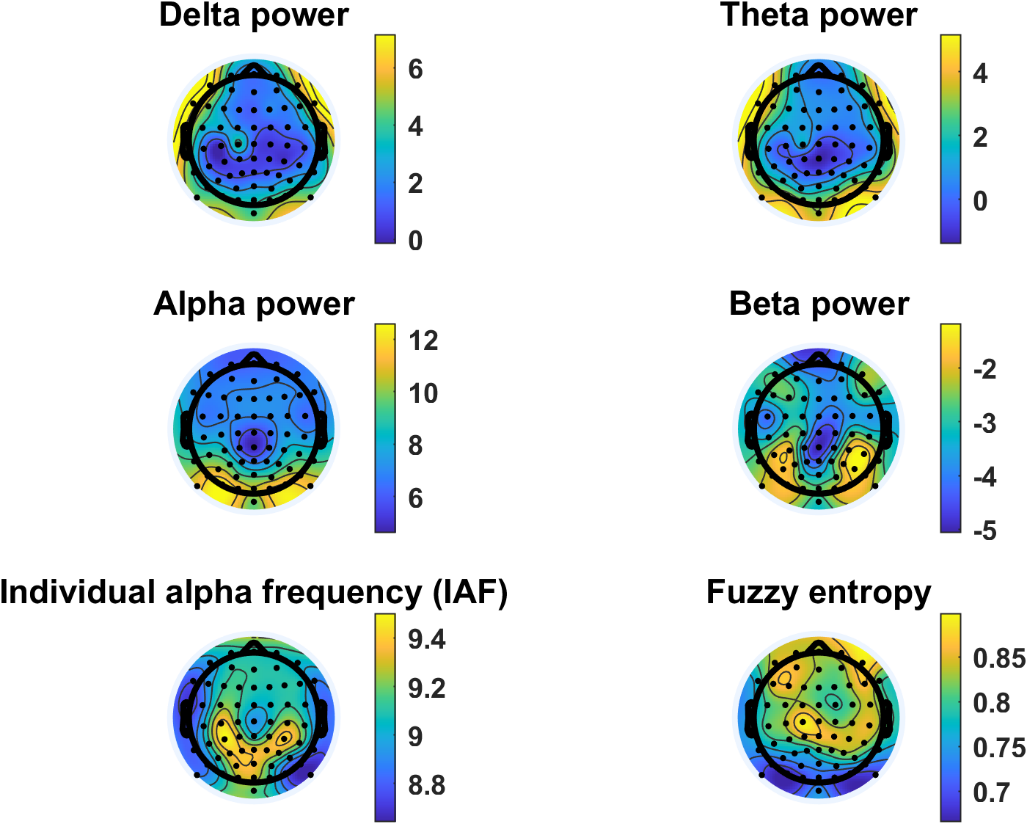


Figure 2.2. Power spectral density (PSD; top panel) and multiscale fuzzy entropy (MFE’ bottom panels) features estimated from NN intervals (left panel) and EEG data (right panel). Note: calculated on the *sample\_data1.set*, corresponding to 5 min of mindwandering eyes closed.



**Figure 2.3.** EEG scalp topographies showing the distribution of mean power spectral density (PSD) for the delta (top left), theta (top right), alpha (middle left), and beta (middle right) frequency bands. The individual alpha frequency (IAF) is plotted in the bottom left corner, and the fuzzy entropy distribution in the bottom right corner. Note: from *sample\_data1.set*, corresponding of 5 min of mindwandering eyes closed.

**METHOD 3**

BrainBeats runs Infomax ICA (or Picard algorithm if installed) using PCA dimension-reduction to account for effective data rank ([Ref](https://www.frontiersin.org/articles/10.3389/frsip.2023.1064138/full?&utm_source=Email_to_authors_&utm_medium=Email&utm_content=T1_11.5e1_author&utm_campaign=Email_publication&field=&journalName=Frontiers_in_Signal_Processing&id=1064138)). Next, the heart component(s) is automatically classified and removed with 95.3% confidence, using the ICLabel plugin. The ECG channel(s) is included in this process to increase ICA’s source separation performance and chances to separate heart components from the EEG signals. Users can click on the component to inspect its properties in more detail (see **Figure 3.1. left**). The final output is displayed, showing the heart components removed from the EEG signals in red (**Figure 3.1. right**). Notes: The ECG signal is included here only for visualization purposes, but is otherwise removed from the output dataset. When no heart components are detected, users are informed in MATLAB’s command window, and the program ends.

Graphical user interface, application

Description automatically generated A picture containing text, font, handwriting, line

Description automatically generated

**Figure 3.1.** **Left**: Heart independent component classified by ICLabel and its properties. **Right**: EEG signals after removing the heart artifacts (in red). The ECG signal is included here for visualization purposes, but is otherwise removed from the final output dataset.

**DISCUSSION:** *(3-6 paragraphs)*

Focus on protocol and not the representative results. This section should discuss the following with citations:

Critical steps in the protocol

Critical steps include installation of the EEGLAB software, BrainBeats plugin, and collection of a dataset that includes both EEG and cardiovascular (ECG or PPG) signals. Users must know the name of the cardiovascular channel to select it in the parameters. For novices, loading the dataset into EEGLAB and selecting parameters in the main GUI are crucial steps.

Modifications and troubleshooting of the method

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

Limitations of the method

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 (Costa et al., 2005). However, they are very computationally heavy and can take a very long time to compute on EEG signals. While improvements were made (parallel computing, GPU computing, signal downsampling/decimation), further accelerations are required, if possible. While statistics are provided for the Method 1 and 3 (at the subject and group levels using the EEGLAB STUDY mode), no statistical analysis is currently provided for Method 2, to assess interactions between HRV and EEG features. This is because a lot of flexibility must be implemented to account for the various analyses users may want to perform (e.g., statistics, machine learning). However, users may use the Method 2 to extract features and easily perform statistics with any standard statistical software.

* The significance of the method with respect to existing/alternative methods

The third method allows quick and easy removal of heart components from the EEG signals, in an automated manner. While this was possible before, it required various steps, such as highpass filtering the signals, running ICA, running ICLabel, tuning parameters, subtracting the heart components from the EEG signals, and removing the ECG channels.

* Future applications or directions of the method

Future development (in process) will provide statistical analysis of features as follows:

1. Dimension reduction for HRV and EEG features, separately) to remove features that are highly correlated and redundant, and therefore reduce multiple comparisons. This will be done using PCA.
2. Correlations (skipped Pearson or Spearman depending on data distribution and variance) will be performed across HRV and EEG features, with FDR-correction for multiple comparisons to control for type 1 error. Results will be plotted in an intuitive correlation matrix (available via command line).
3. Next, since correlation coefficients are non-normally distributed, they will be transformed using the Fisher r-to-z method to meet the normal distribution assumption of t-tests. Nonparametric tests (e.g., Wilcoxon signed-rank test and Wilcoxon rank-sum test) test for differences in central tendency and are not well-suited for differences in correlation coefficients, unlike t-tests. However, they will be used if N < 30.
4. If distributions are still significantly skewed, Yuen t-tests will be applied (using 20% trimmed means) to address the issue. If conditions/groups have different variance, a Welch test will be used.

Finally, other Methods will be developed to assess interactions between EEg and cardiovascular signals in a different way, such as the “time-resolved directional brain/heart interplay measurement” (Catrambone et al. 2019), or classification of HEP data using machine learning. Deep learning methods may also be added to classify patterns using the raw EEG and cardiovascular times series in the long term.

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Bedford, C.D., Harris, R.N., Howd, R.A., Goff, D.A., Koolpe, G.A. Quaternary salts of 2-[(hydroxyimino)methyl]imidazole. *Journal of Medicinal Chemistry*. **32** (2), 493-503 (1998).

Kioh, L.G. *et al.* *Physical Treatment in Psychiatry*. Blackwell Scientific Pubs. Boston (1988).