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The UNICA sleep HRV analysis tool: an integrated open-source tool for heart rate variability analysis during sleep

Parisa Sattar^{1,2,*} , Giulia Baldazzi^{2,3} , Monica Puligheddu^{3,4} and Danilo Pani^{2,3} ¹ Neuroscience PhD program, Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy² MeDSP Lab, Department of Electrical and Electronic Engineering, University of Cagliari, Cagliari, Italy³ Interdepartmental Sleep Disorder Research Center, University of Cagliari, Cagliari, Italy⁴ Azienda Ospedaliero-Universitaria di Cagliari, Cagliari, Italy

* Author to whom any correspondence should be addressed.

E-mail: parisasattar92@hotmail.com, giulia.baldazzi@unica.it, monicamf.puligheddu@unica.it and danilo.pani@unica.it**Keywords:** heart rate variability, hypnogram, sleep phases, polysomnography, toolSupplementary material for this article is available [online](#)

Abstract

Heart rate variability (HRV) analysis during sleep plays a key role for understanding autonomic nervous system function and assessing cardiovascular health. The UNICA Sleep HRV analysis (UNICA-HRV) tool is a novel, open-source MATLAB tool designed to fill the gap in current HRV analysis tools. In particular, the integration of ECG and HRV data with hypnogram information, which illustrates the progression through the different sleep stages, eases the computation of HRV metrics in polysomnographic recordings. This integration is crucial for accurate phase-specific analysis, as autonomic regulation changes markedly across different sleep stages. The tool supports single- and multiple-subject analyses and is tailored to enhance usability and accessibility for researchers and clinicians without requiring extensive technical expertise. It implements and supports a variety of data inputs and configurations, allowing for flexible, detailed HRV analyses across sleep stages, employing classical and advanced metrics, such as time-domain, frequency-domain, non-linear, complexity, and Poincaré plot indexes. Validation of the tool against established tools like Kubios and PhysioZoo indicates its robustness and precision in generating reliable HRV metrics, that are essential not only for sleep research, but also for clinical diagnostics. The introduction of UNICA-HRV represents a significant simplification for sleep studies, and its open-source nature (licensed under a Creative Commons Attribution 4.0 International License) allows to easily extend the functionality to other needs.

1. Introduction

Heart rate variability (HRV) provides hints on the autonomic nervous system (ANS) status. HRV analysis dates back to the early 1960s, when it was initially used to study fetal distress during labor (Hon 1958). The technique gained more attention in the 1970s as a method to assess cardiac mortality (Akselrod *et al* 1981, Acharya *et al* 2006), when Akselrod (Akselrod *et al* 1981) further demonstrated its value in understanding autonomic regulation through spectral analysis. Then, HRV analysis has become an important tool for evaluating cardiac health and detecting autonomic dysfunction (Acharya *et al* 2006, Shaffer and Ginsberg 2017). It serves as a valuable indicator of physiological resilience and adaptability (Lehrer and Eddie 2013). HRV has diverse applications across various fields, including medical diagnosis (Stauss *et al* 2005), sports science (Plews *et al* 2013), and mental health (Kemp *et al* 2012). Clinical studies have proved its utility for the diagnosis of myocardial infarction (Malik and Camm 1994), the detection of atrial fibrillation (Stauss *et al* 2005), the scoring of sepsis severity (Ahmad *et al* 2009), the diagnosis of obstructive sleep apnea (Jahrami *et al* 2023), and the measurement of mental stress levels (Lee *et al* 2022), just to cite a few application areas.

HRV analysis is highly sensitive to accurate R-peak detection (Kim *et al* 2009, 2012, Molina-Picó *et al* 2013). However, detecting R-peaks accurately can be challenging due to noise, ectopic beats (Nabil and Reguig 2015), and the intrinsic variability of ECG waveforms. Noise can stem from various sources, such as muscle contractions, electrode movement, respiration, or external electrical interference, hampering the correct R-peak annotation (Sim *et al* 2015). Ectopic beats complicate the detection process by introducing anomalous waveforms, compared to normal heart cycles, that do not follow the other cycles' timing. Additionally, the morphology of ECG signals exhibits not only a strong inter-individual variability, but also an intra-individual variability due to different physiological and pathological conditions, further complicating the R-peak detection process (Hu *et al* 2023). Therefore, the availability of graphical user interfaces (GUIs) that facilitate accurate visualization/correction of R-peak annotations by automatic software routines is of paramount importance. Moreover, significant advancements in HRV research have been fostered by the development and release of tools for the automatic computation of the different indexes. HRV analysis software such as Kubios (Tarvainen *et al* 2014), PhysioZoo (Behar *et al* 2018), HRVAS (Ramshur Jr. 2010), gHRV (Rodríguez-Liñares *et al* 2014) and HRnV-Calc (Niu *et al* 2023), although well-established and reliable, suffer some limitations when used on polysomnographic data analysis. In fact, none of them can allow the full-night HRV analysis on a sleep-stage-basis, i.e. by performing the analysis in the different sleep stages. This is because currently available tools do not incorporate hypnogram data, so the automatic segmentation of the traces according to this signal is not supported. However, the analysis of HRV evolution during the different sleep stages is fundamental to understand possible ANS dysregulations impacting the overall sleep structure (van der Woerd *et al* 2024).

Based on the significant use of HRV analysis in sleep research, and considered the limits of current solutions, we present an open-source tool for sleep-related HRV analysis based on a MATLAB GUI not requiring any programming skill. This tool simplifies HRV analysis in sleep studies by integrating hypnogram data in order to enable the analysis separately in the different sleep stages, including several presets for automatic analysis. Developed with a strong interaction with the neurologists specialized in sleep studies, it was expressly conceived to support both clinical and non-clinical researchers, regardless of their technical or programming expertise on the use of similar tools, by putting in a single GUI all the controls and analysis results they need. UNICA-HRV, version 1.0, is licensed under a Creative Commons Attribution 4.0 International License and can be downloaded from the GitHub repository.

2. Material and methods

This section provides an overview of the UNICA Sleep HRV Analysis tool by explaining its user interface and back-end computational methods. Additionally, it describes the methodology adopted for the validation of the proposed software, which includes the comparison of the HRV features extracted by the UNICA tool with the HRV parameters obtained by other available HRV analysis tools, as well as a statistical analysis to assess the consistency and reliability of the results.

2.1 The UNICA sleep HRV analysis tool front-end

Figure 1 displays the front-end layout of the UNICA Sleep HRV Analysis tool (UNICA-HRV). Different panels group the features required for the analysis to facilitate their use. On the top left side, the Data Input & Configuration area allows users to import single or multiple-subject ECG signals from ECG/PSG files for (batch) analyses. This area also enables users to specify the sampling rate of the data and supports resampling and plot features. Moreover, users can select the specific sleep phases and relative segments, as well as the time window to be displayed, expressed in different units. Just below it, the ECG Display Panel shows the imported ECG trace with R-peak annotations marked by the automatic QRS-detection algorithm featured by the back-end of the tool, enabling manual correction of misplaced peaks. This feature is particularly important when annotating ECGs of patients with cardiac rhythm abnormalities, as it displays the detected R-peaks on an interactive display window, allowing users to manually adjust any inaccurate annotation. Next to the Data Input & Configuration area, the Analysis Configuration area enables users to specify various analysis methods and perform the QRS detection. The HRV Analysis Panel allows starting the computation of the HRV metrics; it also shows, in different tabs, the results of the performed analysis in a simple and interactive way. On the top right, the Output File Generation area allows users to save obtained HRV metrics, the ECG signal, and the associated R-peak annotations. All these panels are better detailed in the following sections.

Hereafter, the settings and features supporting single individual and batch analyses of multiple subjects are presented, whereas the detail of the signal processing methods adopted to achieve them is reported in the next section.

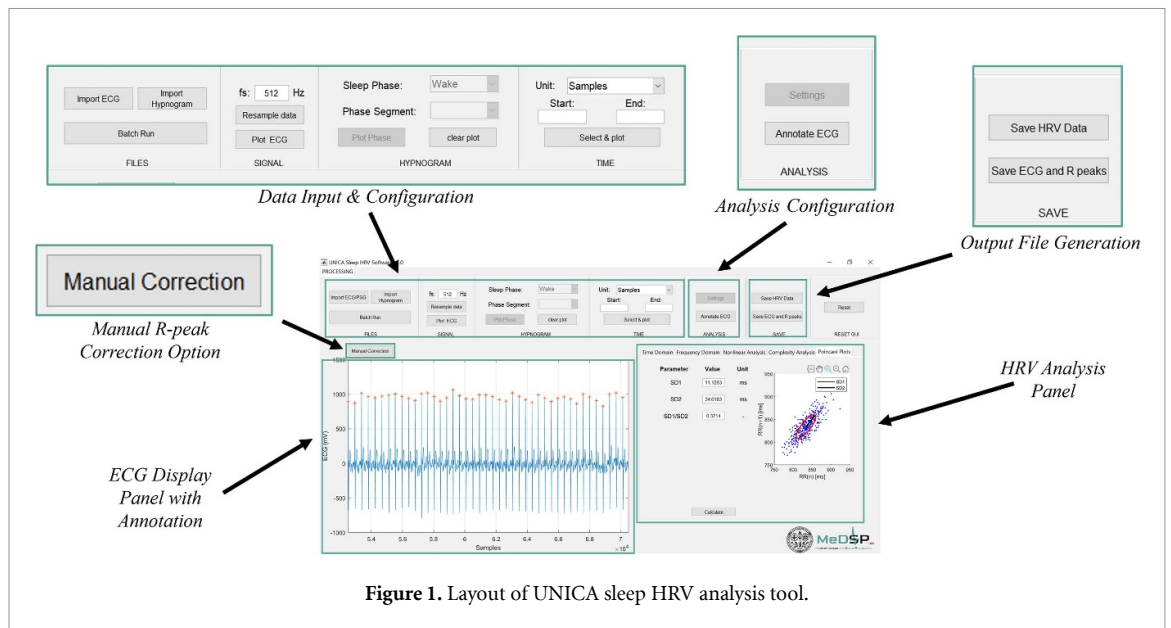


Figure 1. Layout of UNICA sleep HRV analysis tool.

2.1.1 Single-subject assessment

Data input and configuration section: In this section, both ECG and hypnogram files are managed. ECG signals acquired from either polysomnography (PSG) data or just ECG recordings can be uploaded in the software using the ‘Import ECG/PSG’ button, which opens the operating system window for the file selection. The current implementation of the software computes everything from the raw ECG signal, so it is not designed to accept tachograms (i.e. the time series of the RR intervals). The accepted file formats for raw ECG signals, both single- and multi-channel, are: .mat, .edf, and .txt. As the software is conceived to analyze a single ECG lead, in case of multi-channel ECG data, the GUI requires the indication of the lead to be used for HRV analysis. Similarly, if PSG data is provided in input, provided that PSG is a multichannel recording by nature, the software asks the user to specify the channel number containing the ECG signal.

After that, if HRV analysis during sleep is pursued, users can select the ‘import hypnogram’ button to load a file containing the hypnogram in .xlsx, .csv, or .txt formats. Remarkably, this step is not mandatory, as the UNICA-HRV enables HRV analysis also independently from sleep phases. If hypnogram data has been imported, the GUI presents a guided procedure to enable reading of hypnograms described in different formats, assuming that the information is stored column-wise. To this aim, users are prompted to select the criteria used to mark the sleep phases (i.e. duration, end time, or 30 s epochs). Then, the user needs to select the unit of measure and specify the column numbers for the relevant data, such as the start time column, end time/duration column, and phase label column. Then, a label-mapping interface (figure 2) is prompted to associate the names adopted for the different sleep phases in the file with the unique ones used by the tool. At the moment, the software is designed to use the sleep stages described by the American Academy of sleep medicine (AASM) in their scoring criteria.

After successful data loading, the user must specify, in the ‘Signal’ subsection, the sampling rate of the ECG to proceed in the analysis. In order to increase time resolution or reduce the number of samples of a long signal, the user can resample the ECG by pressing ‘Resample data’ and entering the desired sampling rate.

In the ‘Hypnogram’ subsection, users can select the specific sleep phase to be analyzed, and the specific segment of that phase, by selecting its number (segments are phase-wise progressively numbered, from the first to the last one). Once a segment of a given phase has been selected, users can choose to plot it by pressing the ‘Plot Phase’ button. For improved visual inspection, the ‘Time’ subsection allows users to extract a portion of the selected phase and segment, by specifying the start and end points, before pressing the ‘Select & plot’ button.

In the same panel, the ‘Batch run’ button allows for the multiple subjects’ assessment, as described in Section 2.2.1.

Analysis configuration section: This section encloses the settings and the modality for HRV analysis during sleep.

To perform the HRV analysis only on the selected phase and segment (either on the entire segment or on the specified timeframe of that segment), the user must click the ‘Annotate ECG’ button. Then, the back-end performs the QRS detection at first, and the R-peak locations are marked with red mark on the display panel

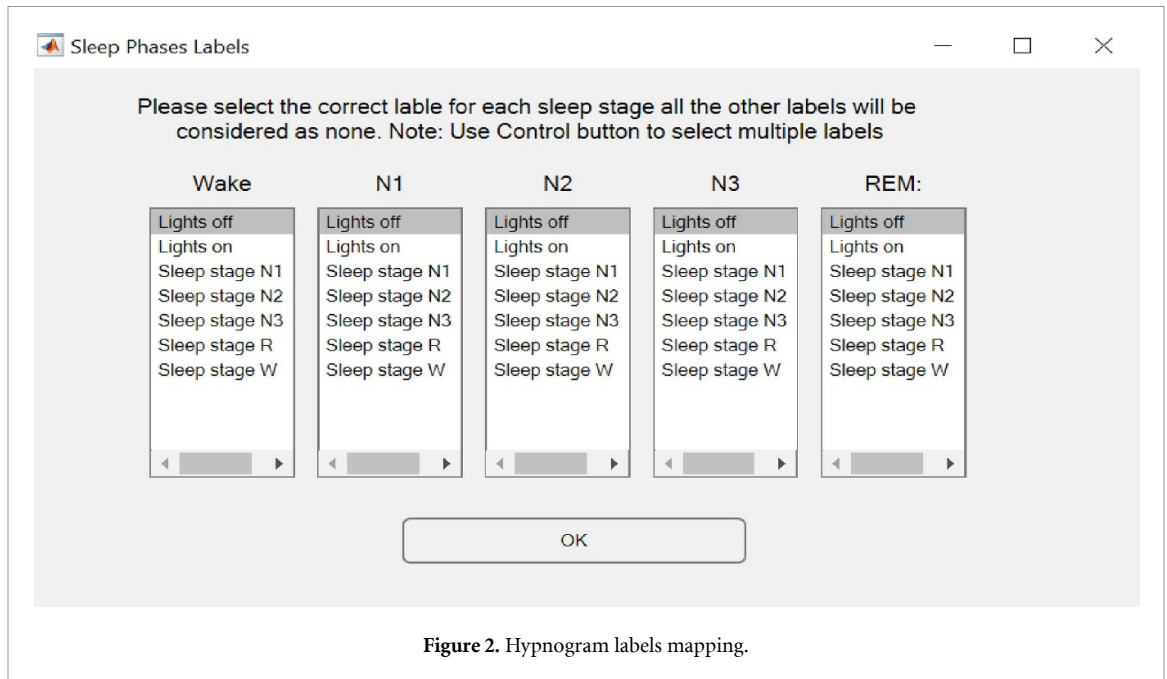


Figure 2. Hypnogram labels mapping.

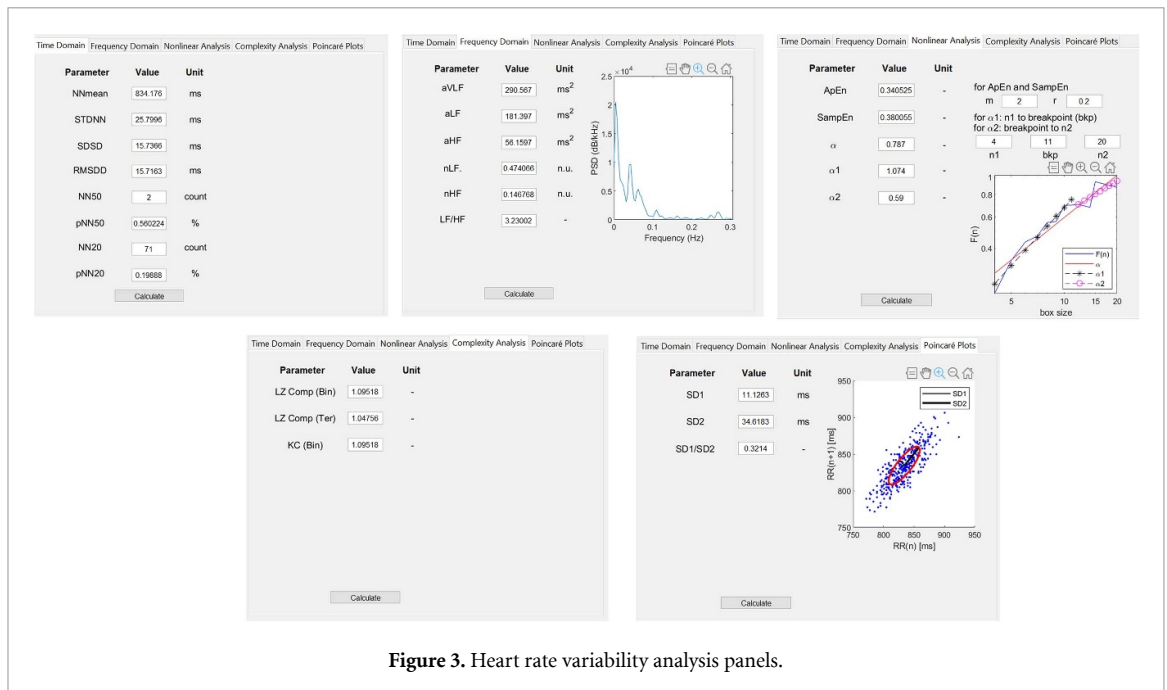


Figure 3. Heart rate variability analysis panels.

of the GUI. Once the annotation is over, the ‘Calculate’ buttons are enabled, allowing users to compute a large number of HRV metrics, including time domain, frequency domain, nonlinear, complexity-based, and Poincaré plot-based. An exemplary visual output of this computation is reported in figure 3.

Alternatively, HRV analysis can be automatically performed on the entire ECG, for all or specific sleep phases, via the ‘Settings’ button in the ‘Analysis’ subsection. Upon clicking the ‘Settings’ button, a pop-up window with several options for analysis appears, as shown in figure 4. In this window, the desired sleep phase(s) for analysis can be chosen; for each of them, three options for the segments are proposed:

- all available ECG epochs of sleep phase/s regardless of their duration (Method AAE) are considered;
- all ECG epochs of sleep phase/s greater or equal to five minutes with 50% overlap (Method AEW0) are considered;
- the first five-minute ECG epoch of sleep phase/s only (Method FEO) is/are considered;

These options are offered because different HRV metrics are influenced by the duration of the analyzed segment. Notably, complex HRV metrics such as detrended fluctuation analysis (DFA) require long segments

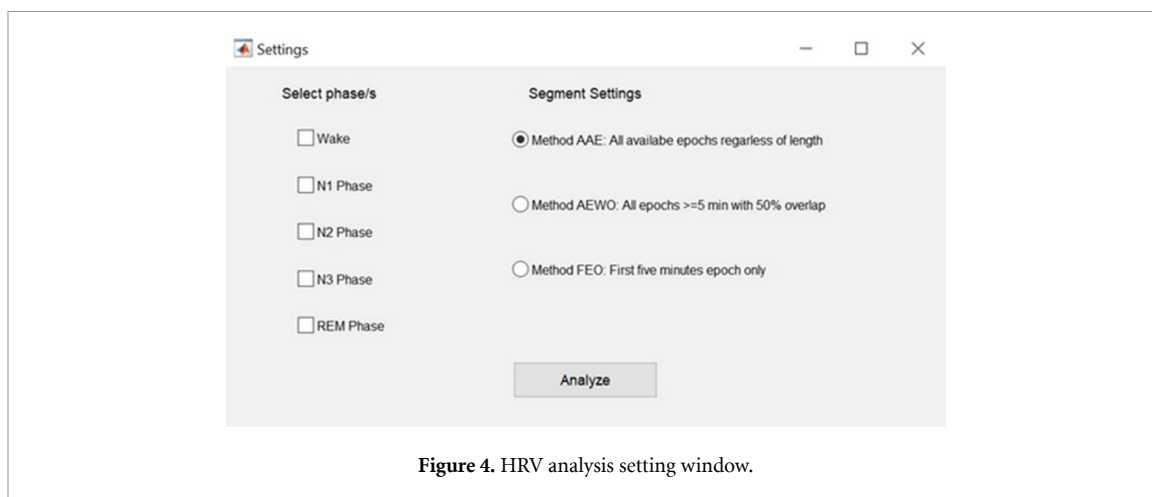


Figure 4. HRV analysis setting window.

to accurately assess long-range correlations and chaotic behaviors. The three segment settings provided by the tool can be extended according to the researchers' needs. An exemplary analysis using these methods is presented in the supplementary data. Once the users selected the appropriate method and sleep phase(s) for the analysis, they simply need to click the 'Analyze' button to start the analysis. The software will then begin the analysis, displaying the current sleep phase and segment being analyzed along with the progress towards the end. Upon completion, the GUI will automatically generate a .mat file and an .xlsx file for each analyzed sleep phase. More details on the generated output files are provided in the following section.

Output file generation section: The GUI generates two distinct output files: a .mat file and an .xlsx file for the selected sleep phase(s). The former consists of a two-column table variable: the first column contains the segmented ECG signals of a given phase, according to the user settings, while the second column contains the locations of the R peaks within that ECG segment. This file enables the creation of a dataset for further comparative analyses, review, or can be employed for further HRV metrics and feature extraction.

The second output is an .xlsx file structured to facilitate statistical analyses. It begins with two columns indicating the start and end times of the ECG segments, respectively, followed by the segment number/information, and 25 additional columns, each one representing a different HRV metric. These include time domain, frequency domain, nonlinear, complexity-based, and Poincaré plot-based indexes. The .xlsx file also includes a report on issues encountered during the analysis, which includes data quality concerns or annotation errors. Examples:

- If the analysis was targeting ECG segment in N1 phase of 5 min or longer, and no segment with such characteristics can be extracted, the Excel file will report that no N1 phase of the required length is available in the 'segment information' column.
- If the data is corrupted by noise, it will be reported that QRS detection could not be trustable due to poor signal quality. In this case, users can manually review the specific segment in the .mat file using the provided start and end time information.

2.1.2 Multiple subjects' assessment (batch analysis)

Multiple subjects' analyses are allowed by the 'Batch Run' button in the 'Files' subsection.

In case of HRV analysis during sleep, to run a multiple subjects' analysis, the user must provide the software with the hypnogram files and their corresponding ECG data files with identical names (regardless of the file extension). This naming consistency is crucial for the back-end to analyze matched files, considering that the procedure is completely automatized. In case ECG and hypnograms are both in .txt format, the naming consistency requires them to be stored in different folders, as the simultaneous existence of the files with the same name and extension is impossible. Upon all hypnogram data files loaded, the GUI asks if the hypnograms have a uniform file structure among them or not. If not, the GUI will present the guided procedure to enable reading of hypnograms described in different formats, including the phase-labeling mapping (figure 2) for each file, otherwise only once.

From this point on, the procedure is similar to that described in the single-subject case when uploading PSG or ECG files: the back-end checks whether all ECG files are uniformly structured and share the same sampling frequency. If so, the GUI prompts the PSG or ECG data setup window only once, otherwise the GUI will provide the handling and configuration options separately for each file, requiring the selection of a uniform sampling rate for all the recordings.

This approach ensures the correct handling and analysis of both homogeneous and heterogeneous datasets, avoiding incoherent parameterizations and preventing errors. Once the hypnogram and ECG data files have been correctly loaded, the GUI will prompt the HRV Analysis Setting Window (figure 4), as in the single-subject case, to proceed with the same analysis approach on the whole dataset. During the analysis, the GUI provides real-time updates on the processing, indicating the specific subject, sleep phase, and segment currently being processed. Upon completion, the GUI will display a notification indicating the directory where the generated files have been saved, following the format previously described. These output files retain the names of the originally uploaded files but include a prefix corresponding to the selected sleep phase (e.g. N2-SN0012).

2.2 The UNICA sleep HRV analysis tool back-end

2.2.1 ECG data pre-processing

The ECG pre-processing consists in a single zero-phase bandpass filtering between 0.67 Hz (Kligfield *et al* 2007) and 100 Hz (Luo and Johnston 2010), like in other HRV analysis tools (pecg documentation 2024), to attenuate baseline wander, respiration artifacts and high-frequency noise. According to the chosen QRS detection algorithm downstream, there is no need for a more aggressive filtering, thus preserving the possibility to analyze and correct the R-peak annotations on a signal with adequate morphology. This preprocessing stage is obtained by a 4th-order Butterworth infinite impulse response filter applied bidirectionally to obtain a zero-phase response without delays and phase distortions (Mitra 2001, MathWorks Italia 2024).

2.2.2 QRS detection

A renowned wavelet-based QRS detector (Martínez *et al* 2004) was selected to accurately identify the R peaks. As the optimal performance of this delineator is achieved at a sampling frequency of about 250 Hz, the back-end resamples all the input signals to match this sampling frequency, resampling back to the selected sampling frequencies after the detection has been accomplished. To ensure an accurate marking of the R-peak locations, a refinement process is implemented on the output of the QRS detector: a window with a number of samples equal to the maximum between ± 10 samples and ± 20 ms around the identified R peaks is used to detect the exact maxima/minima location corresponding to the R-peaks after resampling. This refined information is used to create the tachograms, which are automatically inspected by the back-end to identify and correct potential false positives or negatives in R-peak detection by comparing all estimated RR intervals against the median RR interval: RR intervals that are less than 70% of the median RR interval are assumed to be the effect of a false positive, whereas false negatives are associated to RR intervals larger than 130% of the median RR interval. In the former case, the second R peak contributing to the suspicious RR interval is deleted; in the latter, missing beat annotations are added. The location of the missing annotations is determined as follows: the distance between two detected R peaks is measured and compared to the median RR interval; if it is twice larger, a single R peak is added. Similarly, up to three R-peak may be added, depending on the RR distance. This procedure is then followed by subsequent R-peak refinement. Remarkably, epochs with more than three missing R peaks are discarded as noise-corrupted by the back-end, ensuring signal integrity and reliability in the analysis.

2.2.3 Featured HRV metrics

The back-end implements a wide range of HRV analysis metrics, which are calculated using either fixed settings or customizable parameters, as shown in tables 1 and 2, respectively. These metrics include time-domain (Shaffer and Ginsberg 2017), frequency-domain [4], non-linear (Kaspar and Schuster 1987, Peng *et al* 1995, Ladysz 2009, Burns and Rajan 2015, 2019, Acharya *et al* 2016, Delgado-Bona and Marshak 2019, MATLAB Central 2024a, 2024b), and Poincaré plot (Hsu *et al* 2012) indexes.

2.2.4 Comparative analysis of the HRV parameters

Since the automatic HRV analysis across different sleep phases was not provided by other available software tools for HRV analysis, to provide a quantitative assessment of UNICA-HRV performance, we compared the HRV metrics obtained by our tool against other well-established HRV analysis tools, i.e. Kubios, PhysioZoo, HRVAS, and gHRV, on a 15 min ECG segment from PSG data.

For this benchmark, we used the publicly available Haaglanden Medisch Centrum sleep staging database, which includes 151 full-night PSG recordings (85 males and 66 females, with age of 54 ± 15) (Alvarez-Estevez and Rijsman 2021). The recordings, sampled at 256 Hz, involved different patients who were randomly chosen and had been referred for PSG testing due to various sleep disorders. The dataset also contains EEG, EOG, chin EMG, and ECG data, along with event annotations related to sleep-pattern scoring in the related hypnograms (Goldberger *et al* 2000, Alvarez-Estevez and Rijsman 2021, PhysioNet 2021).

Table 1. HRV metrics with fixed settings.

Metrics	Unit
NNmean: mean normal-to-normal (NN) RR interval, where 'normal' is referred to a physiological beat	ms
STDNN: standard deviation of all NN intervals	ms
SDSD: standard deviation of differences between adjacent NN intervals	ms
RMSSD: square root of the mean of the squared differences between adjacent NN intervals	ms
NN50: number of adjacent NN intervals with differences greater than 50 ms	count
pNN50: NN50 divided by total number of NN intervals	%
NN20: number of adjacent NN intervals with differences greater than 20 ms	count
pNN20: NN20 divided by total number of NN intervals	%
aVLF: absolute power in very low frequency band (VLF) [0.003 0.04]	ms ²
aLF: absolute power in very low frequency band (LF) [0.04 0.15]	ms ²
aHF: absolute power in very low frequency band (HF) [0.15 0.4]	ms ²
nLF: normalized power in LF band	n.u.
nHF: normalized power in HF band	n.u.
LF/HF: ration of the absolute power in LF and HF bands	a.u.
LZc: Lempel-Ziv complexity (Binary)	a.u.
LZc: Lempel-Ziv complexity (Ternary)	a.u.
Kc: Kolmogorov complexity (Binary)	a.u.
Poincaré plot geometric features, i.e. SD1 and SD2:	ms
SD1: geometrical dispersion (by standard deviation) in the perpendicular direction w.r.t. the $y = x$ axis (i.e. the width of the ellipse)	
SD2: geometrical dispersion (by standard deviation) in the parallel direction w.r.t. the $y = x$ axis (i.e. length of the ellipse)	
SD1/SD2: ratio of the SD1 and SD2 features	a.u.

Table 2. HRV metrics with customizable parameters.

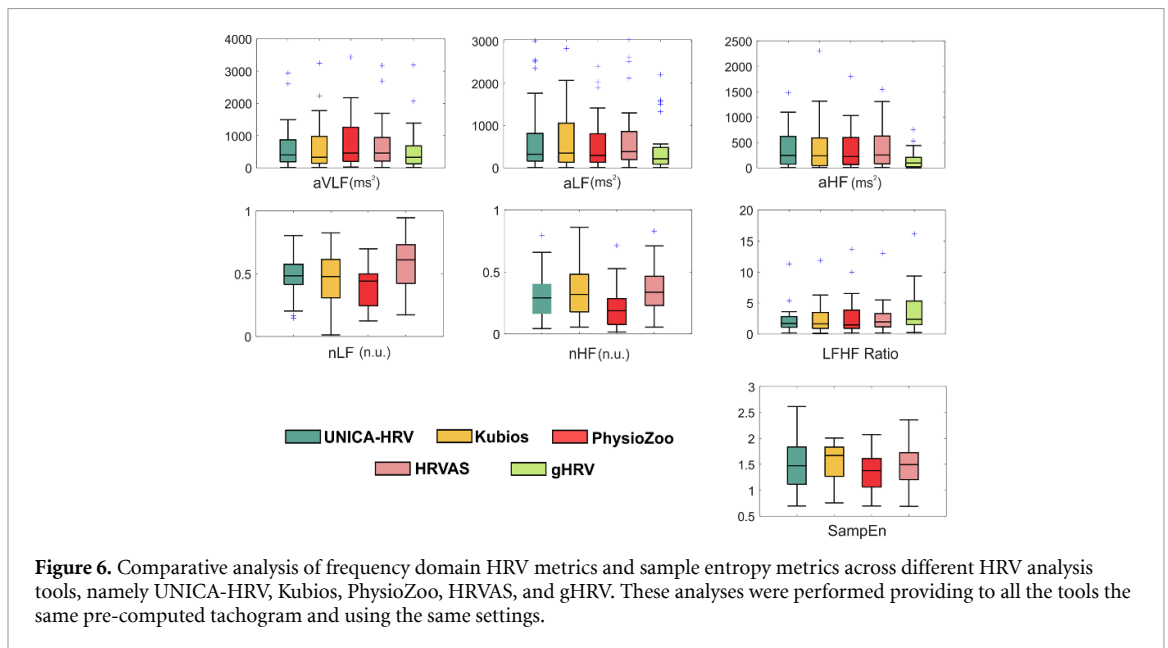
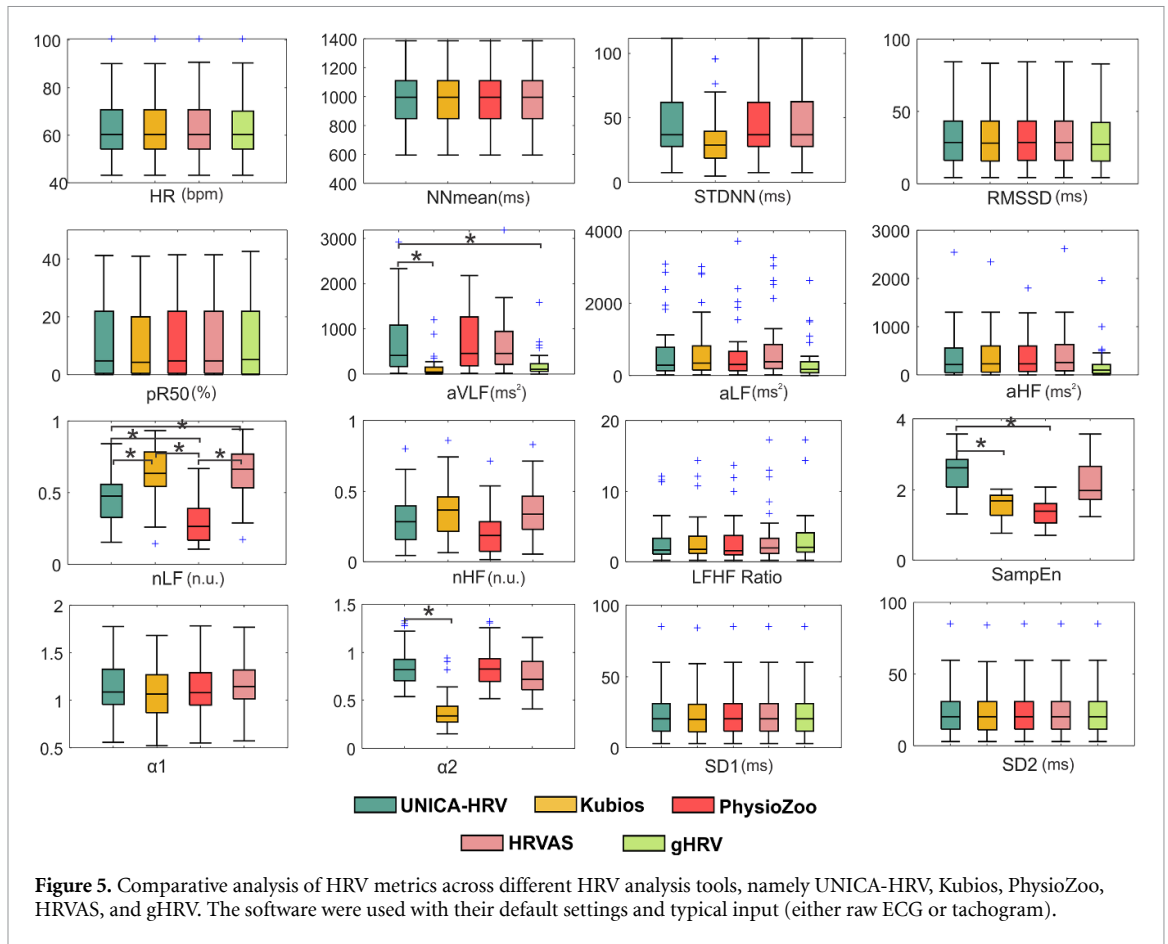
Metrics	Unit	Customizable parameter	Default Settings
ApEn: approximate entropy	a.u.	m and r	$m = 2$ and $r = 0.2 * \text{STDNN}$
SampEn: sample entropy	a.u.	m and r	$m = 2$ and $r = 0.2 * \text{STDNN}$
DFA: detrended fluctuation analysis	a.u.	for $\alpha 1$, $n1$ and bkp for $\alpha 2$, b2 bkp and $n2$	for $\alpha 1$, $n1 = 4$ and $\text{bkp} = 11$ for $\alpha 2$, $\text{bkp} = 11$ and $n2 = 20$

After the HRV metric computation by the different software tools, a statistical comparison was carried out by the non-parametric Kruskal–Wallis test, eventually followed by a pairwise cWilcoxon signed-rank test. After Bonferroni's correction, to control type I error, we set $p < 0.05$ for statistical significance.

3. Results

3.1 Assessment of the UNICA sleep HRV analysis tool

Figure 5 presents the results of the comparative analysis. In the time domain, a good consistency across the compared software tools can be appreciated. Indeed, HR, NNmean, RMSSD, pR50, SD1, and SD2 show almost equal values across all software tools, but the same does not hold for all the HRV metrics analyzed. In the time domain, STDNN presents some minor differences for the Kubios tool, but there is no statistically significant difference to highlight. Conversely, for frequency domain indexes, the variability among the different tools is higher. Statistically significant differences were observed for aVLF, considering UNICA-HRV vs Kubios ($p < 0.01$) and UNICA-HRV vs gHRV ($p < 0.01$), and for nLF, among all the tools ($p < 0.01$). This finding could be attributed to the different methodologies employed in calculating nLF (whose value is influenced by aVLF, that also revealed significant differences) or to the various pre-processing techniques used. Conversely, the other frequency-domain metrics, although not identical for the different tools, did not lead to statistically significant differences, which supports the reliability of the tool also in frequency domain. However, in figure 6, the comparison of aVLF and nLF demonstrated no significant differences across the various HRV analysis software when the metrics are computed by the different software tools from the same tachogram (externally provided) and identical parameter settings (except for the number of FFT points, specifically for Kubios and PhysioZoo software, as its value was not editable).



In nonlinear HRV analysis, except for α_1 , which presents a very high consistency among the different tools, both SampEn and α_2 parameter revealed statistically significant differences on a subset of the tools (respectively, UNICA-HRV vs Kubios ($p < 0.01$) and UNICA vs PhysioZoo ($p < 0.01$) for SampEn, and UNICA-HRV vs Kubios ($p < 0.01$) for α_2). This issue can be due to the different parameterizations adopted in the different tools. In particular, the handling of α_2 requires careful consideration, as it reflects the long-term correlations in HRV data. Any change in the range settings for its computation can alter the interpretation of underlying physiological conditions, particularly those related to autonomic function and intrinsic HR modulation dynamics.

Table 3. Feature comparison for the HRV analysis tools used for the UNICA-HRV comparative assessment.

Software	UNICA-HRV	Kubios free version	PhysioZoo	HRVAS	gHRV
Open source	Yes	No	Yes	Yes	Yes
Programming skills required	No	No	Yes	Yes	Yes
Hypnogram integration	Yes	No	No	No	No
Batch analysis	Yes	No	No	Yes	No
User defined setting	Yes	No	Limited	Yes	Limited
QRS detection and annotation	Yes	No	Yes	No	No
Manual correction of R-peaks	Yes	No	No	No	No
Automatic tachogram correction	Yes	Yes	Yes	Yes	Yes
Time domain indexes	Yes	Yes	Yes	Yes	Limited
Frequency domain indexes	Yes	Yes	Yes	Yes	Limited
Nonlinear indexes	Yes	Limited	Limited	Limited	No
Poincaré plot indexes	Yes	Yes	Yes	Yes	Yes

Nonetheless, this assumption was investigated more deeply by standardizing the adopted parameters in the different tools and by using a pre-computed tachogram for the metric computation, thus bypassing the software-specific differences in the identification of the RR intervals: indeed, also in this case, no significant difference was obtained in SampEn (see figure 6). Lastly, the Poincaré plot metrics (SD1 and SD2) reveal consistent distributions across the examined tools.

Compared to the other tools used for this assessment, UNICA-HRV also overcomes some of their limitations. The absence of hypnogram integration is the most significant one for sleep studies, as the autonomic regulation varies considerably between rapid eye movement (REM) and non-REM (NREM) sleep stages (van der Woerd *et al* 2024).. Remarkably, the possibility of adapting the code is impossible for tools that are not released under an open-source license, such as Kubios, which however is a popular tool for HRV analysis. The other tools used in this assessment are all open-source, but their adoption by users without programming skills is not straightforward, thus hampering their use by people with a clinical background. Moreover, while traditional HRV metrics are well-covered, the absence of nonlinear metrics in some tools limits the analysis that can be performed. Table 3 synthetically presents the feature comparison among the tools considered in this section.

4. Discussion

The development and validation of the UNICA Sleep HRV Analysis Tool represents a significant milestone in the field of HRV research during sleep. A major innovation of the UNICA-HRV tool is its integration of hypnogram data, enabling HRV analysis to be automatically segmented by sleep stages. This feature addresses a critical gap in existing HRV analysis tools, which often lack the ability to account for the distinct autonomic regulation patterns observed across REM and NREM sleep stages. Interestingly, the ANS exhibits parasympathetic dominance during NREM sleep and increased sympathetic activity during REM sleep. By facilitating stage-specific analyses, UNICA-HRV enhances research ability to investigate the physiological underpinnings of sleep and autonomic function (Boudreau *et al* 2013, Herzig *et al* 2018). This feature is particularly valuable for exploring alteration in expected HRV patterns from individuals with sleep disorders, such as OSA, where autonomic dysregulation is a hallmark (Stein and Pu 2012, Bradicich *et al* 2020).

Previous research has demonstrated notable differences in HRV analysis between patients with sleep disorders and healthy individuals. For instance, patients with conditions such as OSA, insomnia, restless legs syndrome, and narcolepsy often exhibit lower HRV during sleep, reflecting altered autonomic balance and increased sympathetic activity (Stein and Pu 2012, Dodds *et al* 2017, Sforza *et al* 2019, Bradicich *et al* 2020). Studies have shown that patients with sleep disorders may display significantly reduced HRV parameters, RMSSD and total power, indicating parasympathetic withdrawal and higher cardiovascular risk. Additionally, sleep fragmentation commonly observed in disorders like OSA, periodic limb movement disorder, and REM sleep behavior disorder is linked to pronounced changes in nocturnal HRV, highlighting sympatho-vagal imbalance characterized by sympathetic dominance (Stein and Pu 2012, Boudreau *et al* 2013, Dodds *et al* 2017, Sforza *et al* 2019, Bradicich *et al* 2020). These findings emphasize the application of HRV analysis in diagnosing and managing sleep-related conditions, particularly for understanding possible autonomic dysfunction. By enabling sleep-stage-specific HRV analysis, UNICA-HRV software is uniquely contributing to these investigations across a variety of sleep disorders.

Remarkably, the proposed software tool is also able to deal with possible pathological conditions commonly encountered in sleep studies. For example, patients with OSA experience recurrent apneic events

that cause abrupt autonomic shifts, reflected in altered HRV patterns. Similarly, cardiac arrhythmias, such as PVCs, are prevalent in individuals with sleep disorders and can significantly affect HRV metrics (Quintal 2023, Statello *et al* 2023). UNICA-HRV accounts for irregular beats, making it possible to adjust the RR interval automatically. Moreover, it also includes interactive features for manual correction of R-peak annotations, thus ensuring accurate data processing even in the most challenging scenarios. Following these corrections, the tool automatically adjusts the RR intervals again to ensure precise data processing. Expanding these capabilities with additional, clinically validated automated detection algorithms could further enhance the software effectiveness. Thanks to the open-source nature of the proposed software, these aspects could be also integrated by the scientific community itself, according to specific needs.

A comparative analysis with well-established HRV tools, including Kubios, PhysioZoo, gHRV and HRVAS, demonstrated the reliability and precision of UNICA-HRV in calculating time-domain, frequency-domain, and nonlinear metrics. While minor differences in specific frequency-domain and nonlinear metrics were observed, it is important to highlight that such incoherencies were caused by differences in the pre-processing methods and parameters setting (when default values are used). When these differences have been eliminated, the robustness of the proposed tool clearly emerged, ensuring its outputs are consistent with existing well-established software, while offering additional functionalities, such as sleep-stage-specific segmentation. On the other hand, despite the UNICA-HRV tool providing significant advancements, some limitations must be mentioned. Firstly, the current software version only supports AASM-based hypnograms. In the future, it could be expanded to incorporate hypnogram files compliant with the Rechtschaffen and Kales (R&K) method. Secondly, the choice of MATLAB for the development of the interface limits the adoption of the tool by research facilities. However, the widespread adoption of MATLAB in the bioengineering community is the key for the continuous development and improvement of the tool, and for its exploitation in teaching activities.

5. Conclusion

The UNICA Sleep HRV Analysis Tool represents a significant advancement in HRV research, providing automated, sleep-stage-specific analysis that addresses critical gaps in existing tools. By integrating hypnogram data, the tool enables precise HRV analysis within the context of specific sleep stages. Supporting a wide range of HRV metrics, including time-domain, frequency-domain, and nonlinear parameters, the tool serves as a comprehensive solution for sleep-related HRV analysis. Its user-friendly interface, designed for researchers without programming expertise, simplifies complex tasks, reduces the potential for errors, and streamlines workflows in research settings. By automating and enhancing HRV analysis, the UNICA-HRV tool empowers clinicians to investigate sleep-related autonomic changes with greater precision, advancing our understanding of sleep disorders and potential biomarkers for early disease detection and risk stratification, especially in the context of sleep-related disorders.

Data availability statement

No new data were created or analysed in this study.

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ORCID iDs

Parisa Sattar  <https://orcid.org/0000-0002-9461-0568>

Giulia Baldazzi  <https://orcid.org/0000-0003-1275-4961>

Monica Puligheddu  <https://orcid.org/0000-0002-6837-6608>

Danilo Pani  <https://orcid.org/0000-0003-1924-0875>

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