A Computational Tool for Evaluation of Heart Rate Variability in Rats

Mariana da Palma Valério[1], Silvia Becker[2], Higor Barreto Campos[1], Pietra Aguiar dos Reis[1], Vlasta Bari[3,4], Luan de Almeida Moura[1], Axel Loewe[2], Alessandro Pereira da Silva[1]

[1]University of Mogi das Cruzes, Mogi das Cruzes, Brazil
[2]Karlsruhe Institute of Technology, Karlsruhe, Germany
[3]Department of Biomedical Sciences for Health, University of Milan, Milan, Italy
[4]Department of Cardiothoracic, Vascular Anesthesia and Intensive Care, IRCCS Policlinico San Donato, San Donato Milanese, Milan, Italy

Abstract

This study presents ECGRats, a software with a graphical user interface (GUI) that calculates heart rate variability features in rats, such as SDNN, rMSSD, NN20, and pNN20. The algorithm was developed in Python using high-pass, low-pass, and notch filters, along with a Haar wavelet transform to identify R-peaks. ECG data from Wistar rats, provided by the University of Parma, Italy, were used. 100 ECG recordings were grouped into sets of ten per rat. Each signal underwent baseline correction to remove DC offset before HRV parameters were extracted. HRV analysis included both time-domain and frequency-domain features, providing insights into autonomic nervous system activity and cardiac arrhythmia. Data dispersion was significant in some groups with variations from 300 to 460 bpm, whereas other groups had lower variability, indicating a more homogeneous distribution. rMSSD was generally higher than SDNN, indicating greater short-term variability. Additionally, NN20 values were higher than pNN20, as expected. The tool's accuracy was validated by comparison with manual heart rate variability analysis methods, showing correlation. The results suggest that the tool may be useful for assessing heart rate variability in animal studies.

1. Introduction

In the human body, the cardiovascular system is responsible for transporting fluids, driven by cardiac cycles of contraction and relaxation initiated by depolarizations of cardiac cells [1][2]. These cells, at rest, are polarized and, upon depolarization, generate a charge inversion that propagates in a cascading effect between cells until the entire heart is depolarized [3], giving rise to the electrical activity that sustains cardiac function [4].

Capturing this electrical activity is essential to understanding heart function. Furthermore, the use of automated tools to process this information is becoming increasingly necessary [5]. One example is the electrocardiogram (ECG), a low-cost, easy-to-use device that is widely used to monitor the heart's electrical activity [6][7]. The use of software that performs automatic ECG analysis allows many recordings to be evaluated quickly, without the need for manual annotation by physicians or researchers [5]. This is useful not only in hospitals but also in animal research, such as with rats, which are widely used in disease studies and drug testing [8].

In studies involving rats, automated heart rate variability (HRV) analysis helps to understand how the heart responds to different conditions, such as medication use or the presence of disease [9][10]. In addition, it allows for comparison of data over time, assisting researchers in identifying significant patterns and effects [9][10]. The analysis begins with the identification of key components of the ECG waveform, such as the P waves, QRS complexes, and T waves [7][11]. Then, the R peaks are marked, enabling the calculation of HRV, which reflects how the heart adapts to physiological changes and is commonly used in rodent studies [12].

In this work, we present a graphical user interface (GUI) developed in Python, called ECGRats, which integrates algorithms for ECG preprocessing and calculates HRV features such as heart rate (HR), Standard Deviation of NN intervals (SDNN), Root Mean Square of Successive Differences (rMSSD), NN20, and pNN20, specifically designed for use in rodent research. The tool is freely available on GitHub and Zenodo as an open-source resource.

The main goal of this work is not to propose new methods for wave delineation, as the algorithms used have already been applied in various studies. Instead, we aim to provide complementary documentation for this tool, making it easier to understand and use by other researchers. In this paper, we briefly describe the core concepts behind the implemented algorithms and compare the obtained results with reference values found in the literature.

2. Methods

The tool was developed using Python, and the open-source Kivy framework was used to create the graphical interface. This framework allows the development of interactive and responsive applications compatible with multiple platforms, including desktop and web environments. The tool was made available as open source at https://doi.org/10.5281/zenodo.14559400 [13].

2.1. Raw ECG signal processing

The raw ECG signal processing was performed with the aim of reducing noise and distortions that compromise the accuracy in detecting the R wave. Initially, a baseline removal technique [14] was applied to eliminate slow variations caused by movement or breathing.

The signal then passed through a Butterworth band-pass filter to detect the QRS complex. A filter with a band width of 5 Hz to 100 Hz was used to attenuate the P and T waves and highlight the high frequency component characteristic of the QRS. Additionally, a notch filter was used to suppress power line interference at 50 Hz. Finally, an electrical isoline correction was applied to remove residual displacements after filtering, ensuring that wave amplitude and duration measurements were not affected by offset artifacts.

Next, a stationary wavelet transform (SWT) using the Haar wavelet was applied to highlight the rapid transitions present in the QRS complex. The detail coefficients were calculated at the level corresponding to the 45 Hz frequency band, which represents the main spectral band of the QRS.

To locate R peaks, the algorithm identifies QRS intervals based on an adaptive threshold adjusted according to signal statistics within moving windows. In each identified interval, the R peak is determined as the point of greatest amplitude in the time domain.

2.2. Requirements engineering

Initially, software requirements engineering was performed, analysing the needs of users in this area. Based on this analysis, the requirements were documented, including their origin, priority, and potential dependencies. Finally, validation was conducted through screen prototyping. The first screen is designed to be the tool's home screen, while the second screen is for system configuration. The third screen generates data graphs and heart rate variability calculations. Finally, the fourth screen presents the statistical analysis.

2.3. Tool validation

To validate the tool, ECG signals from Wistar rats provided by the University of Parma, Italy, were used [15]. In this study, cardiac electrical activity was recorded using ECGs captured by platform receivers (RPC-1, Data Sciences International) positioned under the animals' cages and acquired with the ART-Gold 4.2 system (Data Sciences International) at a sampling rate of 1000 Hz. Electrocardiographic recordings were performed for 1 hour during the dark (active) phase of the light-dark cycle, between 10:00 and 11:00 am, on different days.

From the complete data set, the ECG signals were segmented into smaller parts, totalling 100 recordings lasting approximately 6 minutes each, organized into groups of ten recordings per animal. All signals were processed using the tool developed in this work.

The results obtained were then compared with reference values described in the literature [16][17][18], considering classic parameters of HRV in rodents. The metrics evaluated included: HR, mean NN intervals, SDNN, rMSSD, as well as NN20 and NN6 counts, and their respective pNN20 and pNN6 ratios. This comparison allowed us to verify the consistency of the results produced by the tool in relation to the physiological standards expected for rats.

3. Results

The tool was developed in Python, with a graphical interface built using the Kivy framework. The screen structure was designed to facilitate the loading of signals, the execution of algorithms, and the interactive visualization of results.

The first screen, Figure 1A, presents the tool named ECGRats. The second screen, Figure 1B, corresponds to the system configuration, allowing the user to define parameters such as the sampling rate, high-pass and low-pass filters, and the notch filter. Additionally, there is a button to select an Excel file containing the pre-collected rat ECG data.

The third screen, Figure 1C, displays the data graph, allowing visualization of the raw signals, the filtered signals, and the R peak markings (Figure 2). Below the graph, cardiac variability calculations are displayed, including HR, mean NN intervals, SDNN, rMSSD, NN20, NN6, pNN20, and pNN6. Finally, the fourth screen, Figure 1D, presents the statistical analysis using two graphs: a Poincaré diagram and a histogram, providing a more detailed view of cardiac variability patterns.

Regarding HRV, rMSSD was generally higher than SDNN, indicating a predominance of short-term variability in the analyzed recordings, a characteristic consistent with the autonomic pattern of rodents. Furthermore, absolute NN20 values were higher than pNN20 ratios, as expected, and variations in NN6 and pNN6 indices also contributed to the distinction between the groups. Overall, some groups demonstrated higher HRV, while others presented

a more stable profile with less fluctuation in NN intervals.

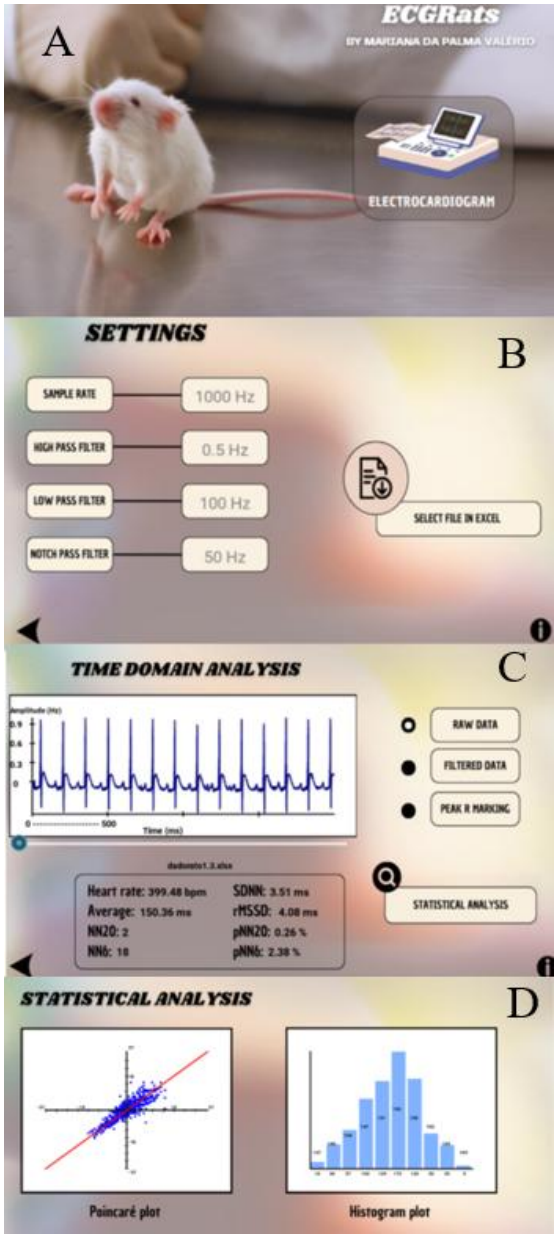

Figure 1. ECGRats computerized tool interface. A. Home screen. B. Second screen for selecting files and setting parameters. C. Results screen. D. Last statistical analysis screen.

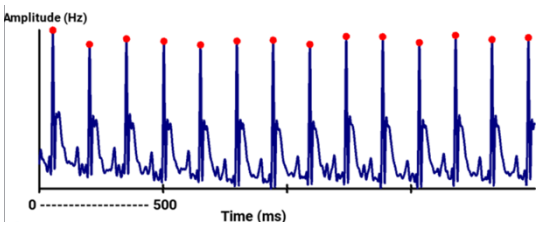

Figure 2. Marking of R peaks in the ECG signal.

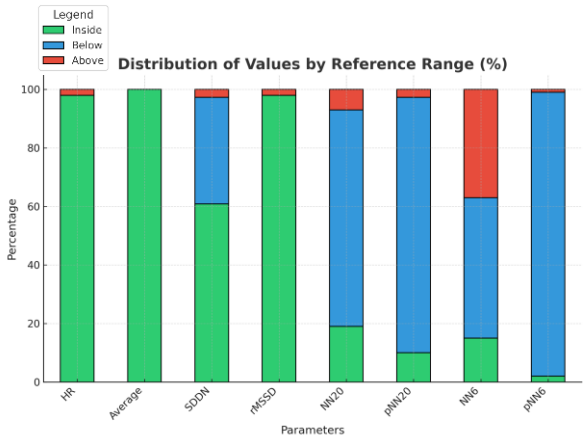

Figure 3. Result of the distribution of values by reference range (%).

After analyzing the parameters' compliance with the reference ranges established in the literature, the results showed that HR presented 97.3% of values within the expected range, while the average of the NN intervals (Average) presented 100% compliance. The SDNN parameter presented 61.8% of values within the reference range, and the rMSSD demonstrated high compliance, with 99.1%. In contrast, the NN20, pNN20, NN6, and pNN6 parameters presented low compliance, with less than 20% of values within the reference limits. These results are illustrated in Figure 3.

4. Discussion

The ECGRats tool demonstrated satisfactory performance in the automated analysis of rat ECGs, with results that, for the most part, approximate the physiological reference values described in the literature. The tool's accuracy was assessed by comparing it with manual methods of HRV analysis, demonstrating good agreement and reliability of the implemented automated approach.

HR values were 97.3% within the expected range, indicating effective detection of R peaks and consistent calculation of RR intervals. The average showed 100% compliance, demonstrating accuracy in calculating the average heart rate.

Among the HRV parameters, SDNN represents the standard deviation of all NN intervals, considered a global indicator of heart rate variability as it reflects the combined action of the sympathetic and parasympathetic nervous systems [16]. Higher SDNN values generally indicate better autonomic balance and a healthier physiological state [19]. In this study, SDNN values were within the reference range for 61.8% of the values, representing an overall positive trend, but one that may have been impacted by factors such as signal noise, sample size, and individual

biological variability [20].

The rMSSD parameter demonstrated high compliance, with 99.1% of values within the reference range. The rMSSD is used as a marker of vagal function, as it calculates the square root of the mean of the squares of the successive differences between adjacent NN intervals [17]. Because it is sensitive to rapid heart rate fluctuations, this index primarily reflects short-duration parasympathetic activity. Its adherence to the expected range reinforces the tool's sensitivity to vagal modulation in rats [17].

In contrast, the parameters NN20, pNN20, NN6, and pNN6, which quantify the relative amount of variation above certain thresholds between successive NN intervals, showed poor adherence to the reference values, with less than 20% of the data within the established limits. NN20, for example, measures the number of pairs of NN intervals whose absolute difference exceeds 20 milliseconds and, like rMSSD, is related to parasympathetic activity [19]. However, this parameter is less robust in short recordings and may be more susceptible to the presence of noise or rapid non-physiological variations [20].

It is important to highlight that the literature presents few well-established reference values for parameters such as NN20 and NN6 in rodents. This limitation hinders direct comparison and reinforces the need for additional studies that explore the physiology of these indicators in greater depth in animal models. Furthermore, because these are more recent metrics in experimental application with rodent ECGs, there is still room for standardization and validation of these indices.

Overall, the findings indicate that ECGRats is reliable for research applications with rodent ECGs, especially when the focus is on global and short-term HRV metrics. However, the discrepancies observed in the counting parameters reinforce the need for continued improvement of the tool, as well as for expanding the reference database for animal studies.

Acknowledgments

This project received funding from the German Academic Exchange Service (DAAD) under grant number 57710871, from the Coordination for the Improvement of Higher Education Personnel (CAPES) under grant number 88887.712116/2022-00 and from São Paulo State Research Support Foundation (FAPESP) under grant #2017/16292-1. The authors also thank Dr. Andrea Sgoifo and Dr. Luca Carnevali, both from the University of Parma, Italy.

References

[1] J. D. Humphrey, A. D. McCulloch, “The cardiovascular system anatomy, physiology and cell biology”, *Biomec. Soft Tissue Cardio. Syst.*, vol. 441, pp.1-14, 2003.

[2] M. S. Thaler, “ECG essencial”, *Artmed Editora*, 2023.

[3] F. A. Antonio, “Eletrocardiograma em 7 aulas: temas avançados e outros métodos”, *2. ed. Barueri, Manole*, 2016.

[4] L. S. Fernandes, M. C. Lira, V. V. França, “Conhecimento teórico-prático de enfermeiras sobre eletrocardiograma”, *Revista Baiana de Enfermagem*, vol. 29, no. 2, pp.98-105, 2015.

[5] L. Schwarz, “Artigo de Revisão: eletrocardiograma”, *Rev. Ilha Dig*, vol. 1, pp.3-19, 2009.

[6] P. Kanani, M. Padole, “Recognizing real time ecg anomalies using arduino, ad8232 and java”, *Communications in Computer and Information Science*, pp.54–64, 2018.

[7] S. I. Fox, “Fisiologia humana”, *7. ed. Baurueri, SP: Manole*, 2007.

[8] J. B. Olvera, R. A. Rios, R. R. Camacho, “Blood pressure measurement system based on oscillometric method”, *Celaya*, vol. 39, no. 128, pp.248-265, 2018.

[9] A. K. Farraj, M. S. Hazari, W. E. Cascio, “The utility of the small rodent electrocardiogram in toxicology”, *Toxic. Scien.*, vol.121, no.1, pp.11-30, 2011.

[10] B. Egner, “High definition oscillometry: non-invasive blood pressure measurement and pulse wave analysis”, *P. Safety Pharm.*, pp.243-264, 2015.

[11] P. M. S. Cannavan, R. N. Aoki, R. D. Gomes, “The teaching of electrocardiogram in higher education in nursing: integrative review”, *R. Socie. Devel.*, vol.12, no.1, pp.e5012139411, 2023.

[12] A. Dasí, C. Nagel, A. Loewe, J. Camps, A. Bueno-Orovio, B. Rodriguez, “Electrogram analysis reveals ionic current dysregulation relevant for atrial fibrillation”, *CinC*, pp.1-4, 2022.

[13] M. Valério, S. Becker, M. Houillon, A. Loewe, A. Silva, “ECG rat”, *Zenodo*, 2024.

[14] N. Pilia, C. Nagel, G. Lenis, S. Becker, O. Dössel, A. Loewe, “ECGdeli - an open-source ecg delineation toolbox for matlab”, *SoftwareX*, vol.13, pp.100639, 2021.

[15] B. De Maria, et al., “Concomitant evaluation of heart period and qt interval variability spectral markers to typify cardiac control in humans and rats”, *Front Physio.*, vol.29, no.10, pp.1478, 2019.

[16] R.M Frasier et al., “Sex differences in heart rate variability measures that predict alcohol drinking in rats”, *Add. Biol.*, vol. 29, no. 3, 2024.

[17] A. Dharamvir et al., “Morin attenuates inflammasome-dependent myocardial injury in diabetic rats: modulation of the signaling pathway”, *J. Hyper.*, vol. 42, pp.e250, 2024.

[18] T. Wilasrusmee et al., “Epigallocatechin gallate enhances sympathetic heart rate variability and decreases blood pressure in obese subjects: a randomized control trial”, *Sci. Rep.*, vol. 14, pp.21628, 2024.

[19] L. Ang et al., “Inflammatory markers and measures of cardiovascular autonomic neuropathy in type 1 diabetes”, *J. Am. Heart Assoc.*, vol. 14, no. 1, pp. e036787, 2025.

[20] E. Salaz-Martínez et al., “Heart rate variability: obtaining the stress score from sdnn values”, *Isok. Exer. Sci.*, vol.32, no. 4, pp.301-307, 2024.

Address for correspondence:

My Name. Mariana da Palma Valério
My Full postal address. Av. Dr. Cândido X. de Almeida e Souza, 200 - Centro Cívico, Mogi das Cruzes – SP
My E-mail address. marianavalerio566@gmail.com