CAPPA: Consistent Analysis of Action Potential Parameters for Various Types of Cardiomyocytes

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Abstract

In recent years, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have become an emerging tool in cardiovascular research. However, due to the spontaneous electrical activity and diverse morphologies of hiPSC-CM action potentials (APs), manual extraction of AP parameters is time-consuming and depends on the operator's subjective judgment, which hampers reproducible, quantitative comparisons between various types of CMs. Here, we introduce a consistent, fast, reproducible and operator-independent algorithm, which extracts quantitative AP parameters from recordings of various CM types. Markers for the start of an AP, maximum upstroke velocity, peak potential and maximum diastolic potential (MDP) are derived automatically. The algorithm was tested on 501 AP recordings from hiPSC-CMs, 873 AP recordings from stimulated isolated native CMs, 133 AP recordings from HL-1 cells and 100 AP recordings from computational electrophysiological simulations. The markers placed by the algorithm were validated against manual expert inspection and were considered correct in over 99% of cases. This open source algorithm enables standardized, reproducible and operator-independent high-throughput analyses of diverse AP recordings, facilitating quantitative comparisons of electrophysiological properties in various types of CMs.

1. Introduction

Analyzing cardiac AP properties has been a crucial tool in arrhythmia and drug development research for decades. In recent years, hiPSC-CMs have emerged as a promising *in vitro* model for translational cardiac research. In contrast to most isolated native CMs, hiPSC-CMs show spontaneous electrical activity. This allows to simultaneously investigate parameters from both individual APs and the collective readouts from multiple APs such as beating frequency or arrhythmogenicity *in vitro*. However, due to the diverse morphologies of hiPSC-CM APs, the quantification of standard AP parameters can be challenging compared to APs obtained from stimulated isolated native CMs. Moreover, manual analyses are time-consuming and depend on the operator's subjective judgment, which hampers reproducible, quantitative comparisons between various types of CMs.

There are multiple tools available to extract standard AP parameters from AP recordings. However, all of them have specific limitations and are not capable of analyzing AP recordings from various types of CMs in a standardized way, thus a tool allowing for direct comparisons is lacking.

ParamAP focuses on the quantification of AP parameters of sinoatrial node (SAN) CMs and comes with a graphical user interface but was designed for analyzing SAN cells only [1]. Moreover, this tool is dependent on user-customization of e.g. the window size of Savitzky-Golay filtering to not mistake noise artifacts for peak potentials, which hampers inter-operator comparisons.

BAPTA is a tool for analyzing spontaneous and triggered cardiac APs from CMs of multiple species and origin. It is written in R language and was designed to overcome operator-dependent bias and improve high throughput analyses [2]. However, the peak detection relies on a user-dependent threshold, which may impede high throughput analyses of datasets showing variable AP recordings with different peak potentials and also affects the inter-operator comparability of analyzed parameters. Additionally, analyzed AP parameters are derived non-uniformly for spontaneous and triggered APs not allowing for direct comparisons.

ActionPytential is another software solution with a graphical user interface, which is based on segmenting continuous AP recordings to individual APs [3]. It enables analyses of AP parameters from multiple tissue types (e.g. ventricular or atrial trabecules, isolated CMs or Purkinje fibers) and acquisition techniques (e.g. optical mapping, single cell current clamp techniques or *in silico* modeling).

While allowing for AP analyses of both slow-response and regular APs, this software has not been tested on the great variety of hiPSC-CM APs. Moreover, this software tool offers multiple manual customization options. On the one hand, it might be beneficial to adapt the analyses to the user's needs. On the other hand, manually selecting a cut-off frequency for filtering or defining a time frame for the starting and ending of an AP hinders inter-operator comparisons. Similarly to BAPTA, there are multiple options to calculate the identical AP parameter, which does not allow for standardized comparisons between multiple operators and research teams.

In summary, there are numerous software solutions automating the extraction of standard AP parameters from cardiac electrophysiological recordings of various tissue types and acquisition techniques. However, to the best of our knowledge, none of them follows a standardized approach for extracting quantitative AP parameter regardless of the type of CMs and without manually adapting analysis parameters. Here, we introduce CAPPA, a consistent, fast and reproducible open source algorithm focusing on operator-independent results to directly compare quantitative AP parameters from various types of CMs.

2. Methods

Given the variability and noise in hiPSC-CM AP recordings, classical mathematical tools or thresholding cannot be directly applied. In contrast to most other software solutions, the foundation of our algorithm is not built on the detection of peaks or minima. Instead, APs are identified as sequences characterized by a potential rise exceeding 20 mV within a time frame of less than 25 ms. This allows reliable and robust detection of depolarization phases of spontaneously beating hiPSC-CMs of multiple maturities with a great variety in upstroke velocities and peak potentials, as well as depolarization phases of stimulated isolated CMs, SAN CMs, HL-1 cells and computational electrophysiological simulations.

The raw AP recording is filtered with a 2nd order Butterworth low-pass filter at a cutoff frequency of 1 kHz. Markers for the start of an AP, maximum upstroke velocity, peak potential and maximum diastolic potential (MDP) are derived automatically. For each index at the beginning of a sequence identified as an upstroke, the maximum upstroke velocity in a time interval of 100 ms is detected, as illustrated in Figure 1. Any subsequent markers occurring within this time interval are excluded from further analyses. This time interval was determined assuming a maximum beating frequency of 10 Hz. The peak potential is defined by the greatest potential within a time interval of 100 ms after the maximum upstroke velocity. The MDP is defined as the lowest potential between two consecutive peaks or before (after) the first (last) peak, respectively.

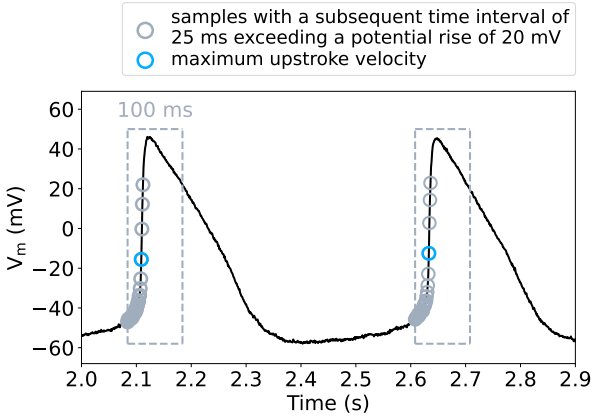


Figure 1: Grey markers denote the beginnings of sequences characterized by a potential rise exceeding 20 mV within a time frame of less than 25 ms. For each marker, the maximum upstroke velocity is determined in a time interval of 100 ms.

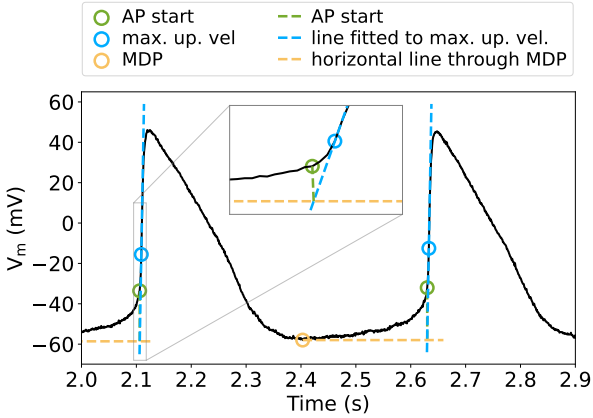


Figure 2: The start of an AP is identified by calculating the point on the time axis where a line fitted to the maximum upstroke velocity intersects the MDP prior to the depolarization phase.

The start of an AP is marked by the intersection of a line fitted to the maximum upstroke velocity and a horizontal line at the MDP before the upstroke, as shown in Figure 2. The first and last AP are investigated further to assure that both APs are entirely recorded.

Based on these markers, standard AP parameters are extracted, as illustrated in Figure 3. The AP amplitude is defined by the difference between the peak potential and the subsequent MDP. The time to peak and repolarization time are calculated as the time intervals between AP start and the peak potential, and the peak potential and the subsequent MDP, respectively. The APD is defined by the time interval between the start of an AP and the first point in time after a peak at which the potential is lower than

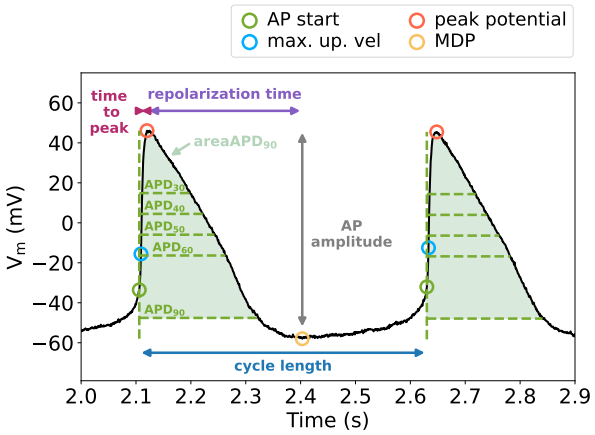


Figure 3: Analyzed AP parameters

the respective amount of repolarization, e.g. 90 % (considering the AP amplitude). To account for varying beating frequencies and to facilitate quantitative comparisons between spontaneously beating hiPSC-derived and stimulated CMs, the raw APD values are supplemented with cAPDs corrected according to Bazett's formula. To obtain further information on the shape of an AP, the area of the AP enclosed by the potential at 90% repolarization is calculated using Simpson's rule. The cycle length is derived by the time interval between two subsequent AP starts and the frequency by the inverse of the cycle length.

Analyses can be performed either on single AP recordings or on an entire directory containing multiple AP recordings by iterating through all sub-directories for high-throughput analyses. The input files can be of any type separated by a user-defined character (e.g. .csv, .txt, .dat). Axon binary files (.abf) files can be used as well. If information on the stimuli are given, stimulus artifacts are excluded from the AP analysis.

As output files, images of the AP recordings featuring the automatically derived AP markers are provided alongside a .csv table containing the mean and standard deviation of all AP parameters. These values are obtained by considering APs within a time interval of user defined length, which is applied to the sequence exhibiting the lowest variance in cycle length, thus representing the most stable sequence.

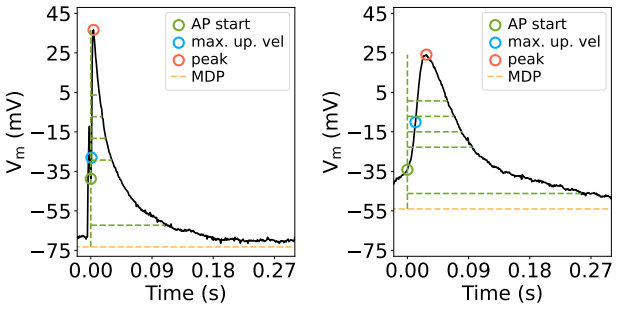
The open source code written in Python language is available under the Apache 2.0 license (gitlab.kit.edu/kit/ibt-public/cappa [4]) for use and further development.

3. Results

The algorithm was evaluated using 501 AP recordings from hiPSC-CMs, 873 AP recordings from stimulated isolated native CMs, 133 AP recordings from HL-1 cells and 100 AP recordings from various exemplary computational models, including those by Courtemanche et al., Grandi et al., Ohara et al. and ten Tusscher et al. In the absence of a ground truth, our algorithm was validated against thorough manual expert inspection. The markers placed by the algorithm were considered correct in over 99% of cases, computed in 0.4 s for a 60 s AP recording encompassing 20 APs sampled at 10 kHz.

The tested AP recordings exhibited a wide range of morphologies, including maximum upstroke velocities ranging from 2 mV/ms to 120 mV/ms, MDPs ranging from –82 mV to –40 mV, peak potentials ranging from 20 mV to 50 mV, APD_{90} ranging from 90 ms to 700 ms, and beating frequencies ranging from 0.5 Hz to 4 Hz.

Exemplarily, we compared AP parameters in 12 isolated native atrial control CMs and 16 atrial-like control hiPSC-CMs. The spontaneously beating hiPSC-CMs showed a more depolarized MDP (–49.17 mV vs. –68.86 mV, all values are means unless specified otherwise) and a lower maximum upstroke velocity (9.78 mV/ms vs. 56.73 mV/ms) and AP amplitude (75.79 mV vs. 111.16 mV) compared to isolated native CMs. However, the APD_{90} and area of APD_{90} were greater in hiPSC-CMs compared to isolated native CMs (276.77 ms vs. 89.76 ms and 7.50 mVms vs. 2.08 mVms). Two representative examples of APs from stimulated isolated native CMs and hiPSC-CMs are shown in Figure 4. Both APs show notable discrepancies. The AP from the stimulated isolated native CMs exhibits a fast upstroke, fast early repolarization phase and an overall concave shape. In contrast, the upstroke and early repolarization phase of the hiPSC-CM AP is slower compared to the stimulated isolated native CM AP. Moreover, the shape of the hiPSC-CM AP is more triangular in the first repolarization phase and exhibits a decreased curvature compared to stimulated isolated native CM APs.



(a) Stimulated isolated native CM (b) hiPSC-CM

Figure 4: Comparison between two representative examples of stimulated isolated native atrial CMs (left) and atrial-like hiPSC-CMs (right).

4. Discussion

The algorithm showed a high accuracy in placing fundamental AP markers and extracting quantitative AP parameters. This was achieved despite a large variety of the analyzed AP recordings while not manually adapting any options. Such accessible and standardized tooling has proven beneficial for example in the field of ECG analysis [5].

In our analyses, hiPSC-CMs showed a less negative MDP and a slower maximum upstroke velocity than isolated native CMs, which is in line with comparisons between ventricular-like hiPSC-CMs and native ventricular CMs [6]. This may be explained by the immaturity of hiPSC-CMs [7]. To account for this limitation, long-term culture could be utilized, albeit this is time-consuming and allows hiPSC-CMs to only develop up to a certain maturity [8]. The less negative MDP can be explained by considerably less functional expression of I_{K1} and expression of I_f, which in combination also contribute to the spontaneous electrical activity of hiPSC-CMs [9].

The APD_{90} and area of APD_{90} was larger in hiPSC-CMs than in isolated native CMs. Given the decreased amplitude observed in hiPSC-CM APs, the increased area of APD_{90} compared to isolated native CMs cannot only be explained by the longer APD_{90} but rather by the shape of the AP. While main potassium current components I_{to}, I_{Kur} I_{Kr} and I_{Ks} present in native adult CMs were also found in hiPSC-CM, I_{to} and I_{Kur} show a lower functional expression in hiPSC-CMs compared to native CMs explaining the differences in the repolarization phase [9, 10].

As described, there are notable differences between hiPSC-CM APs and native adult CM APs, which need to be considered when performing direct comparisons. Moreover, our results are in line with comparisons between hiPSC-CMs and native adult CMs found in literature, further confirming the correct automatic extraction of quantitative AP parameters.

In conclusion, the open source algorithm CAPPA enables standardized, reproducible and operator-independent high-throughput analyses of diverse AP recordings, facilitating quantitative comparisons of electrophysiological properties in various types of CMs.

Author Statement

This work was supported by research grants from the German Cardiac Society (Research Clinician-Scientist program to F.W.); German Heart Foundation / German Foundation of Heart Research (Atrial fibrillation research funding to F.W. and C.S., F/03/19 to C.S., Kaltenbach and Otto-Hess scholarship to A.P.); German Center for Cardiovascular Research (DZHK; TRP starter grant, Shared expertise, Innovation cluster) to C.S. and C.G.; German Ministry of Education and Research (BMBF, ACRIBIS to C.S. and M.P.); Else-Kröner Fresenius Foundation (EKFS Clinician-Scientist professorship to C.S.). C.S., F.W., M.K., C.G. and A.P. are members of the CRC1425 and CRC1550, funded by the German Research Foundation (#422681845 and #464424253).

The study involving human tissue samples was approved by the responsible Ethics Committee of the Medical Faculty of Heidelberg University (Germany; S-017/ 2013) and was conducted in accordance with the 1964 Declaration of Helsinki. hiPSC-CMs were derived from dermal fibroblasts of a healthy male donor and experimental protocols were approved by the ethics committee of the University Medical Center Göttingen (10/9/15).

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