Normalisation of Action Potential Data Recorded with Sharp Electrodes Maximises Its Utility for Model Development

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Abstract

In silico models of cardiomyocyte electrophysiology describe the various ionic currents and fluxes that lead to the formation of action potentials (APs). Experimental data used to create such models can be recorded in adult human cardiac trabeculae using sharp electrodes. During these experiments, the stability of the electrode's position can not always be maintained, leading to spontaneous changes in the recorded voltage and to partial loss of data for model development. In this study, we explored the normalisation of APs recorded with sharp electrodes to reduce the impact of electrode movement on data quality. We show that APs normalised with peak voltage and resting membrane potential as reference points were identical before and after electrode movement, and can still be used for model development. Using a synthetic (simulated) dataset and the Tusscher & Panfilov 2006 model we show that normalising experimental AP traces does not significantly impact predictions of the model. We conclude that normalisation of APs increases the effective size of sharp-electrode datasets without compromising the identifiability and accuracy of inferred model parameters. In addition, our findings suggest that the electrophysiological activity of the recorded cardiac cells was not affected by the electrode's movement, and that changes in electrode offsets can explain the variations observed in the non-normalised recordings.

1. Introduction

Models of the cardiac action potential (AP) are used to study the process by which several ionic currents and intracellular fluxes interact, leading to the formation of an AP, a calcium transient, and ultimately the coordinated contraction of the heart. To calibrate these models, their parameters are adjusted (manually or in an automated process) until model outputs match experimental data [1]. In addition to data collected under baseline conditions, data collected after the application of channel-blocking drugs can be extremely useful for model development and/or validation.

Although human adult ventricular AP data is rare, it can sometimes be obtained from ventricular trabeculae extracted from donor hearts not suitable for transplantation. This data is commonly recorded with a sharp electrode impaled into isolated cardiac muscle fibers [2]. It can be difficult to maintain a stable electrode position over time [3], especially in contracting heart tissue over longer periods. This electrode movement can generate spontaneous changes in the resting membrane potential, thus compromising data quality and reducing the amount of data suitable for model development.

However, action potential duration at 90% repolarisation (APD_{90}) remains relatively stable even when resting membrane potential (RMP) fluctuates (data not shown) suggesting that some features of the data (those that are not sensitive to voltage offset and rescaling) are preserved. Therefore, in this study we explore normalisation of the AP recordings as a way to mitigate instability of the electrode's position and increase the size of the usable data set.

Finally, we investigate how the inference of conductance and missing charge [4] parameters is influenced by the normalisation of AP data. For this study, synthetic data was generated with the AP model by Ten Tusscher & Panfilov 2006 (TTP06, [5]) selected for its low computational cost, under similar conditions to those for AP data recordings.

2. Methods

2.1. Sharp electrode experiments

Experimental AP data was produced by the AnaBios Corporation following the protocol described by Page et al. [2]. Trabeculae were extracted from the inner endocardial wall of ventricles of human hearts. Tested tissues were exposed to drugs that inhibit the rapid repolarisation K^+ (I_{Kr}) and/or the L-type Ca^{2+} (I_{CaL}) current(s) to observe the response of APs to perturbations. In total, five experimental conditions were investigated in each trabecula: control, three perturbed conditions, and positive control for APD_{90} prolongation.

At each drug concentration, the trabecula was stimulated at 1 Hz for an initial 25 min. 1 Hz stimulation was then continued until a stable continuous 2 min segment had been obtained. The trabecula was then stimulated at 2 Hz for 3 min and then stimulated again at 1 Hz for 3 min. The stability of APs was assessed qualitatively by the experimenter, based on measurements of RMP, AP amplitude (APA) and APD_{90}.

Data was acquired in `.adicht` format, and read using the `adi` Python package (`https://github.com/JimHokanson/adinstruments_sdk_python`). 60 Hz noise was filtered out, using the `numpy.fft` package.

Upstroke time was measured for each AP as the time point such that $V_{\text{upstroke}} < -40\,\text{mV}$ and after which $V > -40\,\text{mV}$ was sustained for more than 50 ms. The RMP was measured as the mean voltage between 890 ms and 990 ms after upstroke with 1 Hz stimulation. The peak voltage was very vulnerable to noise and varied between otherwise similar paces, so instead we used a more robust measurement to rescale our data, V_{95}, measured as the upper 95$^{\text{th}}$ percentile of the voltage recorded over one AP [6]. To normalise the AP data, V_{95} and RMP were used as reference points for 1 and 0, respectively, and the normalised voltage V_{norm} was computed from the raw voltage V_{raw} as:

$$V_{\text{norm}} = \frac{V_{\text{raw}} - RMP}{V_{95} - RMP}. \quad (1)$$

APA was computed as the difference between RMP and V_{95}. APD_{90} was computed as the time between the upstroke and the moment the normalised voltage returned below 0.1.

The continuity of RMP was used as a surrogate marker for the stability of the electrode's position. Discontinuities of RMP were identified as: continuous drift of the moving average (over 50 paces) of RMP < -0.002 mV/pace or > 0.005 mV/pace, or sudden change in RMP of more than 6 mV in absolute value. Non-normalised AP data after discontinuities was deemed unusable for model development.

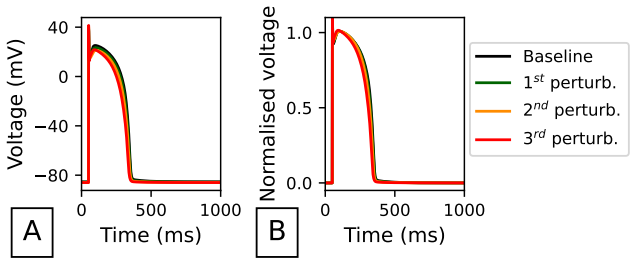

Figure 1. Non-normalised, i.e., control (A) and normalised (B) synthetic datasets generated with the TTP06 model, from which the posterior distribution of parameters was inferred.

2.2. Simulation & inference

Synthetic AP data was generated with the TTP06 model, constituting the known ground truth, to investigate how AP normalisation affects model training. The TTP06 model was downloaded from the Physiome CellML repository and imported and solved with Myokit [7]. APs were simulated with four I_{Kr}/I_{CaL} perturbations (0%/0%, 25%/20%, 50%/40%, and 75%/60% inhibition) similar to perturbations applied experimentally to the real tissues. The synthetic dataset is shown in Figure 1.

Conductance parameters for 8 major ionic currents of the TTP06 model ($\hat{g}_{Kr}$, $\hat{g}_{CaL}$, $\hat{g}_{Na}$, $\hat{g}_{NaCa}$, $\hat{g}_{NaK}$, $\hat{g}_{K1}$, $\hat{g}_{Ks}$, $\hat{g}_{to}$) and missing charge Γ_0 [4] were selected as parameters to infer. To enable direct comparison between errors in inferred conductances, the parameters were considered as rescaling factors (in log-scale) from the "true" value used to generate the synthetic data.

Parameters were inferred from the normalised and non-normalised synthetic data, then compared with the true parameter values (0). An algorithm (Haario-Bardenet Adaptive Covariance Markov Chain-Monte-Carlo (MCMC) [8] implemented in the PINTS Python package [9]) relying on Bayesian inference was used to sample the parameter space in the region with the highest likelihood. Five independent chains were used, initialised at the "true" parameters values, and run for 10,000 iterations. Parameters values taken by the Markov chains at the equilibrium after the 7000$^{\text{th}}$ iteration followed the posterior distribution which was used to estimate the uncertainty on inferred parameters.

The significance of differences between posteriors inferred from normalised and non-normalised data was investigated by comparing the APD_{90} predicted from them. 50 random parameter values were sampled from the posterior distributions, and APD_{90} predictions were computed for the 4 I_{Kr}/I_{CaL} inhibition combinations used to generate the synthetic data.

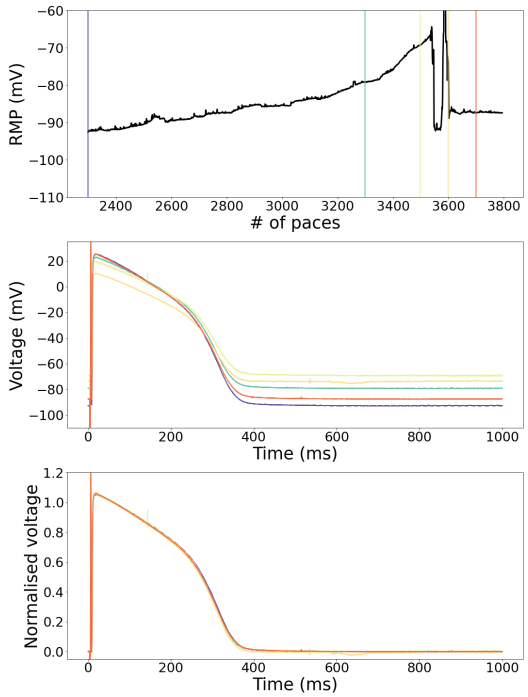

Figure 2. Evolution of the normalised AP is unaltered even when the RMP is unstable. **Top:** Evolution of the RMP. **Middle:** Original APs. **Bottom:** Normalised APs. The time points are indicated in the top panel by a line of the same color as the AP.

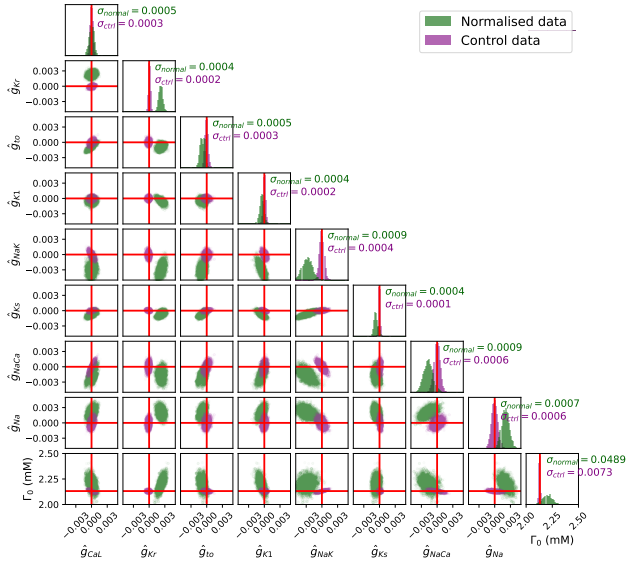

Figure 3. Posterior distributions of parameters inferred from the synthetic dataset with and without normalisation. The "true" parameter values used to generate the synthetic data are indicated by red lines. The posterior distribution inferred from non-normalised AP data (purple) is closer to the true values than the distribution inferred after the normalisation of the AP data (green).

3. Results

3.1. The normalised AP data is not affected by electrode movement

In the representative example plotted in Figure 2, a drift in the recorded voltage, associated with electrode movement, happened between the 2200$^{\text{th}}$ and the 3500$^{\text{th}}$ paces after the beginning of the experiment, which was corrected by the experimenter after the 3600$^{\text{th}}$ pace. The normalised and non-normalised APs were observed over the course of the instability and after the correction.

Non-normalised APs were considerably impacted by the instability, with RMP varying by 25 mV. The data after the 3500$^{\text{th}}$ pace did not meet the quality control criteria, so that it would have to be discarded.

Changes in the cell's RMP by 25 mV are associated with major changes in intracellular balance of ions, which should lead to visible changes in the shape of the AP and AP features [4]. However, the normalised APs were affected neither by the electrode movement nor by the correction at the 3600$^{\text{th}}$ pace. Therefore the spontaneous changes in recorded voltage were likely recording artefacts due to electrode movement.

Like in this example, we found that spontaneous changes in recorded voltage could be safely attributed to electrode movement in the majority of our measurements. As a result, the spontaneous voltage changes can be corrected using normalisation, making the APs usable for model development.

3.2. Accuracy of parameters inferred from APs is preserved under normalisation

We hypothesised that normalisation of AP data recorded under I_{Kr} and I_{CaL} inhibition conditions does not significantly impact parameters inferred from it. To test this hypothesis, the two posterior distributions of parameters inferred from normalised and non-normalised synthetic AP data (Figure 1) are compared in Figure 3.

As expected, inference on normalised AP data returned wider posteriors for the rescaling factors of conductance parameters ($4 \times 10^{-4} < \sigma_{\text{normal}} < 9 \times 10^{-4}$) than on the non-normalised data ($10^{-4} < \sigma_{\text{raw}} < 6 \times 10^{-4}$). The posterior distribution of the missing charge parameter Γ_0 was 7-fold wider when inferred from normalised APs ($\sigma_{\text{normal}} = 0.05$ vs $\sigma_{\text{raw}} = 0.007$), and its mean was shifted by 0.2 mM.

APD_{90} predictions obtained from the two posterior dis-

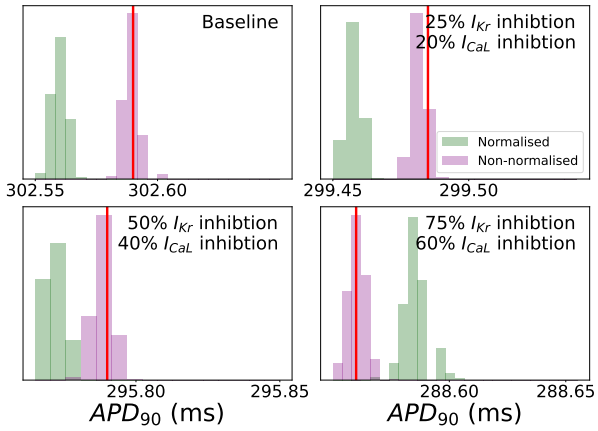

Figure 4. Distribution of APD_{90} predicted by 50 random samples taken from the posterior parameter distributions inferred from non-normalised and normalised datasets (Figure 3). APD_{90} predictions of the model inferred from the non-normalised datasets are closer to the true APD_{90} (red line), but errors in APD_{90} predictions of the model fitted to normalised AP data are small (error < 0.1 ms).

tributions are plotted in Figure 4. APD_{90} predictions were very similar and very close to simulations with the true model ($APD_{90,\,\text{error}} < 0.1$ ms). This confirms that the loss of information induced by the normalisation of the data did not impact sensibly the predictions of the model calibrated with normalised AP data.

In conclusion, the normalised APs obtained under 4 I_{Kr}/I_{CaL} inhibition combinations from the same cell contain sufficient information to calibrate maximal conductance parameters of the TTP06 model, and normalisation does not affect sensibly the model's predictive power.

4. Discussion & conclusion

An important limitation to sharp electrode experiments is that electrode movements occur which perturb the recorded voltage [3]. In most cases, normalised APs are not affected by electrode instability, making them usable for model development. For the TTP06 model, inferred posterior parameter distributions were slightly widened by the normalisation, but this did not lead to major changes in model outputs, such as APD_{90}.

In our recordings with I_{Kr} and I_{CaL} perturbation, the RMP and APA information was not necessary for parameters to be identifiable. In contrast, when studying inhibition of fast Na^+ or Na^+-K^+ pump currents, which are strongly related to the APA and RMP, normalisation is expected to reduce the accuracy of inferred parameters. However, normalising APs can still lead to larger data sets, which may have a counterbalancing effect on the accuracy of inferred parameters [10].

Parameter identifiability should be assessed for each study, on a case-by-case basis. In particular, *in silico* AP models are unavoidably misspecified [1], which leads to less accurate parameters inferred from real experimental data than may be expected from synthetic data studies [11].

Although this study focuses on AP recordings in human heart trabeculae *ex vivo*, the analysis presented in this study can be applied to the development of any model based on sharp electrode AP recordings.

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