Intra-variability in IB4- and IB4+ DRG neurons: a population of models study

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Abstract

The neural action potential (AP) depends on the level of expression of the different channel families, decisively influencing neuronal excitability. Since the experimental study of these phenomena has considerable limitations, the contributions of computational models are increasingly useful. In this study, a population of models of cultured dorsal root ganglion (DRG) neurons has been developed allowing the identification of parameter sets that give rise to APs congruent with the experimental ones regarding two types of DRG neurons: IB4- and IB4+. The results show a high degree of consistency with the experimental results reproducing the greater excitability of IB4- DRG neurons over IB4+ neurons. The intra-species APs have been successfully simulated by considering the intrinsic variability of the expression levels of the different families of channels. This methodology allows the determination of the correct ion channel expression levels to model the neuron AP.

1. Introduction

In-vivo and *in-vitro* assays benefit from complementary *in-silico* experiments when studying neuron excitability and underlying complex phenomena. This approach allows computationally simulating the physiological behavior of excitable cells, describing their activity through a system of mathematical equations [1]. Moreover, *in-silico* experiments help to determine the cause-effect relationships between the changes in a set of specific parameters of the model and the resulting effects.

Building a representative population of models (PoM) of excitable cells enables to simulate and analyze the variability of experimental measurements. Therefore, the high physiological variability exhibited in internal processes at every level can be represented. In the biological context, this uncertainty is manifested in changes in the values of representative biomarkers within certain ranges. Even with homogeneously cultured cells, these exhibit a significant variation between the different cells of the population, generally due to an intrinsic level of randomness.

The study of the variability in dorsal root ganglion (DRG) neurons enables to better understand the mechanisms involved in the normal and pathological behavior of the nervous system. This type of neuron plays an important role in the onset and maintenance of neuropathic pain [2, 3]. As a result of the high incidence of neurological injuries [4] and the current limited strategies to treat them [5], tissue regeneration is becoming an innovative field dedicated to discovering new ways to restore functionality [6]. Improving neuronal excitability is crucial to this point since the greater the probability of restoring the loss of functionality depends on the excitability of cells.

In this work, a PoM, commonly used in cardiac *in-silico* studies [7, 8], and its corresponding sensitivity analysis have been developed. The PoM reproduces the intrinsic variability of DRG neurons and allows the assessment of the effect of different levels of ion channel expression on the action potential (AP) features. Additionally, the developed PoM allows the identification of two DRG neuron subsets: IB4- and IB4+. These two types differ in excitability and AP features [9, 10, 11]. Using the models, a statistical analysis has been carried out on the influence of the type of neuron and the expression level of ion channels on neuronal excitability and AP characteristics.

2. Methods

2.1. Experimental dataset

Our dataset consisted of microelectrode recordings of isolated DRG preparations. The electrophysiology of these cultured neurons is defined by the varying expression of four families of Na_V channels ($Na_V1.6$, $Na_V1.7$, $Na_V1.8$, $Na_V1.9$) and four families of K_V channels (K_V1, K_V2, K_V3, K_V4). Whole-cell recordings were performed through current clamp protocol at room temperature. The extracellular solution contained (in mM): 140 NaCl, 4 KCl, 2 CaCl2, 2 MgCl2, 10 HEPES, 5 Glucose, 20 Mannitol adjusted to pH 7.4. The internal solution contained (in mM): 144 KCl, 2 MgCl2, 10 HEPES, 5 EGTA adjusted to pH 7.2. The experimental recordings yield physiological ranges of AP biomarkers for each type of neuron (IB4- and IB4+).

We used eight biomarkers to quantify the features of the DRG neuron APs: overshoot, amplitude, Resting Membrane Potential (RMP), time-to-peak, After-Hyper Polarization amplitude (AHP), time to reach half recovery to rest, width (measured as AP duration at half amplitude) and membrane potential at which depolarization speed is ≥

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10 V/s (V_{th}). Also, the rheobase current was obtained for each neuron.

2.2. DRG neuron model

For the formulation of ionic currents, the existing model of murine DRG neuron AP from Zheng et al. 2019 [12] was modified. The model was adapted to fit the conditions of our laboratory experiments. The model was implemented in MATLAB for a 0-dimensional neuron patch and solved using the explicit Euler method.

2.3. Population of models

To account for experimental variability, a population of 1000 models was constructed by randomly varying the maximum conductances of Na_V and K_V channels within a pre-established range of ± 60% from the control value, except for $Na_V1.6$ that was varied from 0 to 6 mS/cm^2.

As for the calibration, several filters were implemented during the construction of the PoM to distinguish whether the model behaves in an electrophysiological realistic way. Specifically, models that exhibited spontaneous activity, or were not able to trigger consecutive APs when periodically stimulated, were not included in the final population.

A model was considered to be "IB4-/IB4+ congruent" depending on whether its biomarkers fall within the physiological ranges of the corresponding IB4-/IB4+ cultured DRG neurons. As a result, three population sets were obtained: DRG neuron models IB4- congruent, IB4+ congruent, and non-congruent models. APs belonging to this last category were discarded since they did not faithfully represent the experimental APs for any group.

Two different stimulation protocols were implemented to reproduce our experimental recordings. A "long" stimulation protocol consisted of a single stimulus lasting 1000 ms and was used to determine the rheobase current. On the other hand, a "short" stimulation protocol was defined to obtain the remaining biomarkers by applying five consecutive stimuli 10 ms in duration separated by a stimulation period of 250 ms.

2.4. Statistical Methods

To study how the variability in the conductances of Na_V and K_V channels distinctly affect IB4- and IB4+ neurons, the partial correlation coefficients (PPC) were calculated. This method was chosen since for each biomarker the effects of the rest of the inputs on the biomarker are adjusted, thereby enabling to quantify the correlation between the varied conductances and the described biomarkers.

The PCC between x and y is defined as the correlation coefficient between the residuals as follows:

$$PPC(x, y, z_i) = \frac{Cov(r_x, r_y)}{Var(r_x) \cdot Var(r_y)} \qquad (1)$$

Biomarker-related differences between species (IB4- and IB4+) were assessed by one-way ANOVA considering $p < 0.05$ as statistically significant.

3. Results and discussion

3.1. Modelling IB4- and IB4+ DRG neurons

Both species (IB4- and IB4+) were successfully simulated

and AP morphologies were validated according to [12] and were consistent with experimental recordings.

As previously stated, the PoM is composed of 1000 models of which 10 were found to be congruent with IB4- neurons and 162 with IB4+ neurons, while 15 additional models were found to be congruent with both types of neurons. Therefore, the following sets were obtained from the PoM (Fig. 1-A): 25 congruent with DRG IB4- neurons, 177 congruent with DRG IB4+ neurons and the remaining 813 models were not consistent with any of the previous sets.

In addition, modulation of excitability as a function of stimulus strength was correctly reproduced. For long simulations (1000 ms), the increase in the firing frequency was correctly predicted, so that it increases with the increase in stimulation current, as shown in Fig. 1-B for control values of channel conductances. For stimulus currents of −26.5, −30, and −40 µA/cm2, the firing frequency is 18.20, 24.40, and 37.04 Hz, respectively, showing that the greater the stimulus strength, the higher the frequency (as experimentally observed).

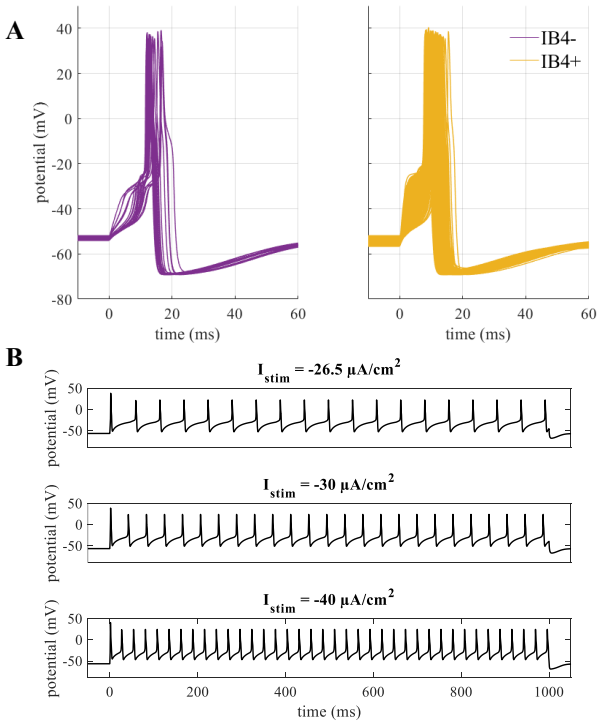

Figure 1. *A) APs of DRG IB4- (n=25) and IB4+ (n=177) neuron models obtained from the PoM. B) APs trains for the long stimulation protocol simulated under control values for the channel conductances.*

3.2. Study of the conductance variability between IB4- and iB4+ DRG neurons

The distribution of the variation of conductances is displayed in Fig. 2. Significant differences in conductance variation between IB4- and IB4+ are observed. Thus, electrophysiological differences between the two groups

can be modelled by varying the conductances of the channel families.

From both species, high values of G_{Kv1} conductance are not suitable for the building of congruent models, being more significant in the IB4- case. Therefore, the random range could be upwardly limited to the control value, since no consistent values were obtained above it.

In the IB4- case, low values of $G_{Nav1.7}$ and G_{Kv2} did not fit well to obtain congruent models. On the other hand, the conductances of the IB4+ congruent models show distributions that cover the entire range of random values except for G_{Kv1} and $G_{Nav1.8}$. Thus, the ranges from the other conductances could be extended.

The PoM has made it possible to identify sets of parameters (maximum conductances of ionic currents) consistent with the experimental results obtained in both IB4- and IB4+ DRG neurons. In addition, the intra-species APs have been successfully simulated by considering the intrinsic variability of the expression levels of the different populations of channels.

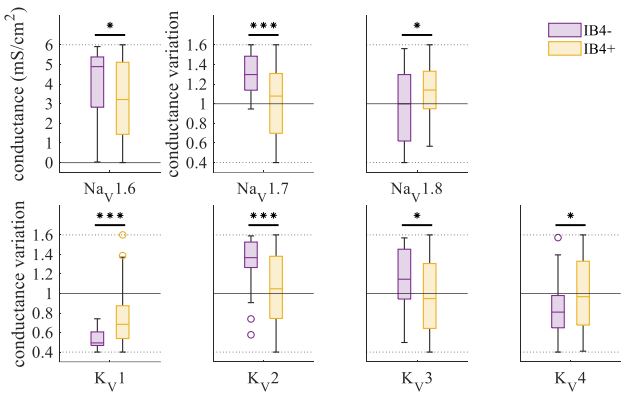

Figure 2. *Distribution of the conductances for the sets of simulated DRG neurons for IB4- (purple) and IB4+ (yellow). The dashed lines delimit the chosen ranges for the variability of the conductances and the solid black line marks the control value for these. Current conductances that show statistically significant differences are indicated as Mann-Whitney U test: *p < 0.05; **p < 0.01; ***p < 0.001.*

Therefore, the electrophysiological differences between the two types of neurons can be computationally modelled from the variation of parameters that determine their bioelectric activity since this variability influences the biomarkers measured for each specie.

3.3. Study of differences in biomarkers

As described in the literature [9, 10, 11], APs of DRG neurons show differences related to the species IB4- and IB4+. Thus, an ANOVA analysis was carried out to study if a significant difference between IB4- and IB4+ DRG neuron models regarding the AP duration (width), the rheobase current and the RMP exists. These biomarkers were chosen since the main differences between the mentioned species consist of IB4+ neurons having longer AP durations, a higher threshold for AP firing (rheobase current) and lower activity (lower RMP). A comparison of the distribution of these biomarkers between species is shown in Fig. 3.

The ANOVA analysis shows a p-value = 3.68e-08 for RMP, p-value = 0.113 for width and p-value = 1.92e-05 for rheobase current. Therefore, IB4- and IB4+ DRG neuron models can be considered significantly different based on rheobase current and RMP, but not AP width. Even so, the distribution of AP width for IB4+ models covers a wider range for higher durations being consistent with both literature [9, 10, 11] and our experimental data. Further simulations would be needed to faithfully represent the AP width observed in experimental recordings and re-examine if there is a significant difference regarding this biomarker.

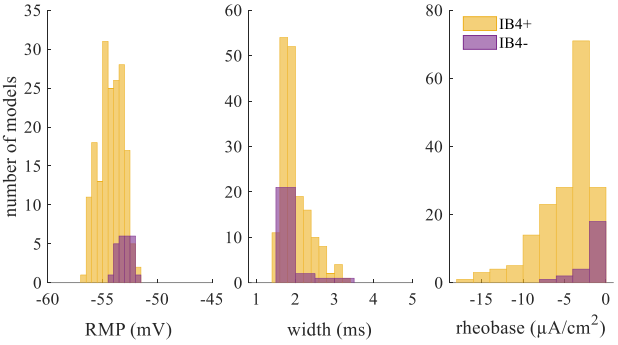

Figure 3. *Distribution of main biomarkers (RMP, width, rheobase) for the simulated DRG neuron sets of IB4- (purple) and IB4+ (yellow) species.*

Regarding neuronal excitability, the difference between IB4- and IB4+ neurons has been correctly estimated, with the rheobase being lower in the IB4- models. This result is consistent with the experimental results.

3.4. Sensitivity analysis on conductance level and biomarkers

Sensitivity analysis results are shown in Fig. 4, where the significant partial correlation coefficients for each variable-biomarker pair ($p < 0.05$) are shown. Most of the biomarkers showed a significant partial correlation with different inputs.

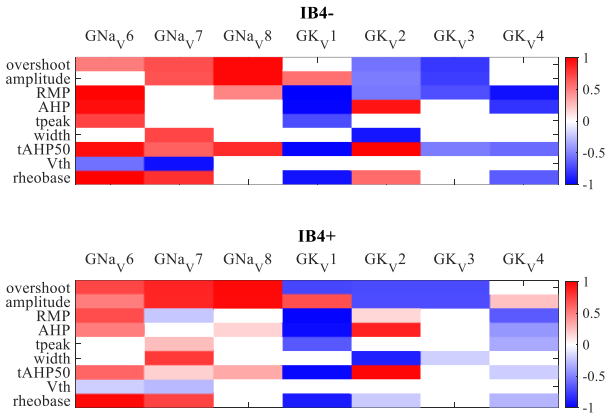

Figure 4. *Representation of the partial correlation coefficients for each variable-biomarker pair. The value of the coefficient is shown by a colour scale showing the positive correlations in red and the negative ones in blue. Pairs without significant correlation (p-value > 0.05) are shown in white.*

For both IB4- and IB4+ cases, a high positive correlation was found between the overshoot and amplitude

biomarkers and the conductances $G_{Nav1.7}$ and $G_{Nav1.8}$. This is consistent since these channel families are related to depolarization. On the other hand, $G_{Nav1.6}$ also correlated positively for both species with the threshold current for the long stimulation protocol (rheobase). This indicates that, at higher conductances of this family, the threshold current necessary for firing APs becomes less negative, thus increasing cell excitability.

For the K_V channel families, the G_{Kv1} conductance showed a negative correlation for several biomarkers including RMP, AHP, tpeak and tAHP50.

In addition, G_{Kv2} was negatively correlated to AP width for both cases, so at higher levels of this conductance, the duration of AP will be shorter. Due to its significant correlation, a small increase in conductance could drastically decrease the duration of the AP. This may explain the excessively short AP durations obtained in the simulated models compared to the recordings in cultured DRG neurons.

Therefore, it is shown that the variability in the conductances can generate models consistent with the experimental data. The variability influences the studied biomarkers to a lesser or greater extent. In any case, the ranges of the variables continue to be decisive in obtaining simulated models of DRG neurons, since they determine the electrical properties of the model. Thanks to the PoM, it has been possible both to model the electrical properties of cultured IB4- and IB4+ DRG neurons and to simulate the characteristic variability of experimental records.

3.5. Limitations and future perspectives

This study accounts the response of a 0D neuron patch. Extending the model to a 3D morphology of DRG neurons and implementing networks of synaptic-connected neurons would enable to further examine the characteristic excitability and conduction properties of these type of neurons.

Simulations have underestimated the duration of the AP, so the inclusion of the $Na_V1.9$ channel would contribute to obtaining longer durations AP and longer depolarization times since it has slower kinetics than the rest of the Na_V channels [10].

4. Conclusions

In this study, a mathematical model has been developed for the computational simulation of the electrophysiological behaviour of cultured DRG neurons. The PoM approach allows to reproduce the intrinsic variability of the expression levels of the different ionic channels, to study its influence on neuronal excitability and to build two different populations of models that faithfully represent both types of neurons, being able to reproduce the variability present in experiments with cultured neurons in the laboratory. We prove that differences in electrophysiological features and excitability between IB4- and IB4+ neurons result from the specific variation of channel conductances. As the experimental recordings, IB4- DRG models show greater excitability than IB4+ neurons. Further computational research would be needed to help design and improve strategies oriented to heal neuronal excitability-related pathologies giving a complementary approach to experimental studies.

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