Sinusoidal voltage protocols for rapid characterization of ion channel kinetics: Supplementary Material

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March 12, 2017

A Additional Methods

This section contains further description of the methods that were used, with a particular focus on details of the Bayesian Inference scheme in Section A2. These sections do not feature in the Online Methods purely due to space constraints.

A1 Protocol schematics

Figures A1–A3 show plots of the voltage clamps that are used in the repeated activation step and activation kinetics protocols (Pr0–Pr2). The voltages and times of the steps are given in the Online Methods 4.2.
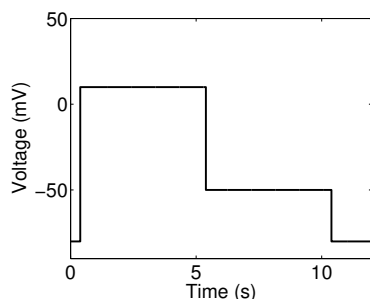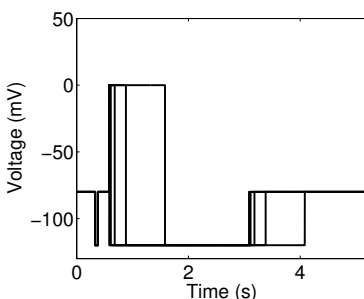

Figure A1: Repeated Activation Step Protocol (Pr0).
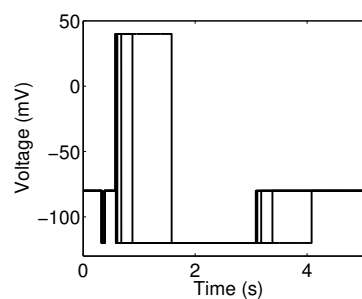
Figure A2: Activation Kinetics 1 Protocol (Pr1).

Figure A3: Activation Kinetics 2 Protocol (Pr2).

A2 Bayesian Inference Scheme

A2.1 Conductance estimation to inform the prior

Preliminary work revealed that using sine wave protocols alone often allowed kinetic parameters in the hERG model to be recovered, but there was potential for identifiability problems (or at least we encountered difficulties in finding a global optimum due to a rugged likelihood surface) when simultaneously fitting the conductance parameter and transition rate parameters P_1 to P_8 (although

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previous work suggests all parameters are theoretically identifiable[42]). To add extra information on conductance, we incorporated a voltage-step to $+40\,\text{mV}$ followed by a step down to $-120\,\text{mV}$, as described in the definition of the sine wave protocol in Section 4.2. The aim being to provoke a large current. We then fitted a single exponential through the slow time constant of the tail current exhibited during the $-120\,\text{mV}$ step (fitting was performed in the Clampfit software, using the Levenberg-Marquardt algorithm with a tolerance of 10^{-6}). We then extrapolated back to the point at which the voltage step to $-120\,\text{mV}$ was made, and used the extrapolated current value at this point to estimate a conductance at this time point (this extrapolation method is described in Vandenberg *et al.*[2]). The conductance we estimated was used as a lower bound for the prior distribution of the conductance, as we describe below.

A2.2 Prior

In this section we describe our prior assumptions on the values that each model parameter can take. The prior for the conductance G_{Kr} is assumed to be independent of the kinetic parameters, and to take a uniform distribution. As discussed above, the lower bound is formed by estimating a lower bound on the conductance value 'directly' from the experimental data; the upper bound is assumed to be 10 times the value of the lower bound.

The other model parameters are within transition rates of the form

$$k = A\exp(BV), \tag{A.1}$$

where V is voltage and A and B are model parameters (P_1 to P_8 for k_1 to k_4, as shown in Figure 3). For parameters of the form A we assumed that the prior distribution is uniform between 10^{-7} and $1000\,\text{ms}^{-1}$, again to cover (and extend beyond) the full physiological range expected with hERG channel gating.

We assume that the prior distributions for B parameters are uniform between 10^{-7} and 0.4 mV^{-1}. The lower bound for this parameter was selected as the voltage-dependence becomes practically redundant when B becomes small: when $B = 10^{-7}$ the value of $\exp(BV)$ will change by less than 0.0015% across the voltages we reach in this study. The upper value is beyond the physiologically expected range.

We also impose a prior on the maximum rate of transition k between any states (maximum across the full voltage range in the protocol (that is from -120 to 58.25 mV)). If the maximum rate k is greater than $1000\,\text{ms}^{-1}$, or less than $1.67 \times 10^{-5}\,\text{ms}^{-1}$, the pair of parameter values that give rise to this are assigned prior probability zero (strictly, this is equivalent to defining 2D prior on A and B, but is easier to describe here, and code, as an additional constraint): the lower bound is based on the assumption that a transition is not physiologically realistic if it occurs over a time scale slower than one minute; the upper bound was decided based on the prior for the individual parameters A and B in the transition rate expression and to prevent the transitions occurring over a time scale much faster than would be physiologically expected.

Note that our analysis is relatively insensitive to the precise form of the prior that is used as there are around 80,000 data points (8 s of 10 kHz samples) in the likelihood product calculation of Equation (13), which is then also in a product with the prior in Equation (12). So, effectively, each of the 10,000 data points has the same impact as the prior does on the posterior. Given our likelihood is extremely peaked around its maximum (Figure 3C), we have observed no notable influence of the shape of the prior, as long as the maximum posterior density point is well away from the limits described above — which it has been in all cases. Note that the same concept means that, in our case, the "maximum likelihood estimate" (MLE — parameter set that maximizes

Equation (13)) would be practically indistinguishable from the "maximum a posteriori estimator" (MAP — parameter set that maximizes Equation (12)) even if we had a non-uniform prior.

A2.3 Global minimization

The Covariance Matrix Adaptation — Evolution Strategy (CMA-ES) algorithm was used to perform an initial exploration of the surface of the posterior density, and to identify parameter sets which allow the model to fit the experimental data well. The tolerance used is 10^{-4} and all other settings are the defaults in MatLab implementation of CMA-ES v3.61, downloaded from `https://www.lri.fr/~hansen/cmaes.m`. We imposed bounds based on the prior as we describe above in Section A2.2.

We run the CMA-ES algorithm from different starting points and continue to do so until we identify the same region of parameter space for optimal parameter sets for each experimental data trace when starting from many different starting points. In this way, we can be confident that we identify the same region of high likelihood consistently (not simply the first local minimum that is found), and we have more confidence that this corresponds to the globally optimal likelihood.

These initial starting points for the CMA-ES algorithm are sampled from within the prior defined for each parameter, described in section A2.2. To sample from the prior we simply select the voltage-dependent transition rate parameters (of the form B described above) uniformly from the defined range. The same approach is used to sample the conductance parameter.

For the parameters of the form A above we sample starting points in a logarithmic fashion across the range of the uniform prior. This approach helps to restrict the initial guesses of parameters to the region of measurable time scales we imposed by defining the maximum and minimum ranges on the overall transition rate, as described above. We also run a small selection of starting points with both A and B parameter values sampled uniformly from $[10^{-7}, 0.1]$ (the range in which most existing model parameters lie), again to ensure we identify the global optimal solution to the optimization problem. We log-transform all parameters within CMA-ES to aid the optimization process by making all values similar orders of magnitude.

A2.4 Markov Chain Monte Carlo parameter inference

We use *Markov Chain Monte Carlo* (MCMC) methods to explore the posterior probability distribution. The approach we use is the *Metropolis-Hastings* algorithm. In this algorithm, candidate parameter sets are proposed from a *proposal distribution* $q(\theta_{\mathbf{cand}}|\theta_{\mathbf{i}})$ which depends only on the previously accepted parameter set $\theta_{\mathbf{i}}$. We use a multivariate normal distribution as our proposal distribution. Any candidate parameter set θ_{cand} is compared to the current parameter set θ_i by calculating the ratio of the likelihood of the two parameter sets. The value of the ratio determines whether or not the proposed parameter set is accepted as part of the MCMC chain. If the candidate parameter set has a greater posterior density value than the existing parameter set then it will be added to the Markov chain, that is $\theta_{\mathbf{i+1}} = \theta_{\mathbf{cand}}$. Otherwise, the parameter set may still be accepted with a probability equal to the ratio of likelihood/posterior density values. That is, a proposed parameter set generated from a multivariate normal distribution is accepted with probability

$$\alpha = \min\left\{\frac{L(\theta_{\mathbf{cand}}|\mathbf{y})}{L(\theta_{\mathbf{i}}|\mathbf{y})}, 1\right\}. \tag{A.2}$$

Also note that if the proposed parameter set contains any parameters outside the range of the prior, or violates any of the conditions on the parameters that we have imposed, the parameter set is assigned an acceptance probability of 0 and immediately rejected and the previously accepted parameter set is again added to the Markov chain — that is, $\theta_{i+1} = \theta_i$.

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In practice, we use a covariance matrix adaptive version of the Metropolis-Hastings Algorithm which helps identify the directions in parameter space which have the highest likelihood values, the algorithm is described in Haario *et al.*[43]. At each iteration of the algorithm, the covariance matrix of the multivariate normal distribution is updated and a scalar value is also updated to define the width of the distribution. We run our MCMC chains for 250,000 samples and discard the first 50,000 samples as 'burn in' (for an introduction to MCMC see Gilks *et al.*[44]).

B   Details of Published hERG Channel Models

Figure 1A of the main text features simulations from 29 literature hERG or I_{Kr} models. In Table B1 we list these models, give references, and show the seven different structures that they feature in Figure B4.

There are two models in this table that are not in Figure 1A: the Kiehn *et al.*[45] model as it is defined only at certain voltages; and the Piper *et al.*[46] model as it does not easily fit into the Hodgkin-Huxley/Markov model framework we used in our simulation code.
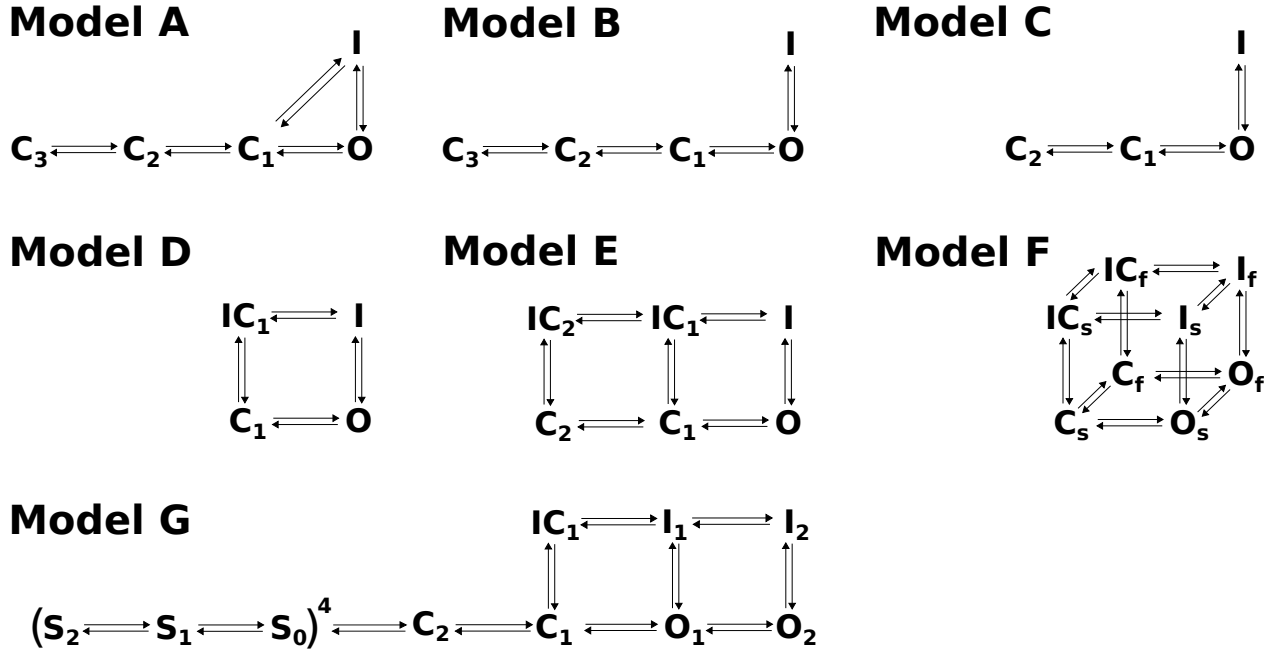

Figure B4: Different mathematical model structures for the literature models listed in Table B1. The model we use in the main text takes structure D as shown in Figure 3B.

Table B1: Table summarizing details of each published I_{Kr} model formulation, in chronological order. '*# Params*' indicates the total number of free parameters (the number given includes a G_{Kr} parameter for the conductance). The *Model Type* is 'HH' for Hodgkin–Huxley models and 'MM' for Markov Models, or a hybrid of the two (MM/HH, which generally means a MM with some symmetry in transition rates).

Model	Model Type	# Params	Experimental Cell Type	Temperature	Structure in Figure B4
Zeng *et al.*[30]	HH	11	Guinea pig ventricular myocytes	Physiological	D
Lindblad *et al.*[47]	HH	11	Rabbit SA, AV, atrial myocytes and rabbit and guinea pig ventricular myocytes	Physiological	D
Liu *et al.*[48]	MM	11	Ferret atrial myocytes	Room	C
Wang *et al.*[25]	MM	15	*Xenopus* oocytes	Room	B
Courtemanche *et al.*[49]	HH	10	Human atrial myocytes	Physiological	D
Nygren *et al.*[50]	HH	9	Human atrial/rabbit atrial myocytes and *Xenopus* oocytes	Physiological	D
Priebe & Beuckelmann[51]	HH	9	Human ventricular myocytes	Physiological	D
Kiehn *et al.*[45]	MM	9*	*Xenopus* oocytes	Room	A
Winslow *et al.*[52]	HH	7	Guinea pig ventricular myocytes	Physiological	D
Ramirez *et al.*[53]	HH	13	Canine atrial myocytes	Physiological	D
Zhang *et al.*[54]	HH	15	Rabbit sino-atrial node cells	Physiological	F
Clancy & Rudy[55]	MM	14	Guinea pig ventricular	Physiological	A
Lu *et al.*[56]	MM	17	Chinese Hamster Ovary (CHO)	Physiological	A
Mazhari *et al.*[31]	MM	17	Human Embryonic Kidney (HEK) 293	Physiological	A
Fox *et al.*[57]	HH	10	Canine ventricular myocytes	Physiological	D
Kurata *et al.*[58]	HH	18	Rabbit sino-atrial node cells	Physiological	F
Oehmen *et al.*[59]	MM	11	Rabbit sino-atrial cells	Physiological	C
Matsuoka *et al.*[60]	HH	23	Rabbit pacemaker and guinea pig ventricular myocytes	Physiological	F
Piper *et al.*[46]	MM/HH	43	*Xenopus* oocytes	Room	G
Seemann *et al.*[61]	HH	7	Human ventricular myocytes	Physiological	D
Hund & Rudy[62]	HH	11	Canine ventricular myocytes	Physiological	D
Shannon *et al.*[63]	HH	11	Rabbit ventricular myocytes	Physiological	D
Ten Tusscher *et al.*[32]	HH	13	HEK 293/CHO/*Xenopus* oocytes	Physiological	D
Fink *et al.*[64]	MM	15	Human Embryonic Kidney (HEK) 293	Physiological	B
Aslanidi *et al.*[65]	HH	8	Canine Purkinje cells	Physiological	D
Inada *et al.*[66]	HH	20	Rabbit atrio-ventricular node cells	Physiological	F
Grandi *et al.*[67]	HH	12	Human ventricular myocytes	Physiological	D
O'Hara *et al.*[68]	HH	19	Human ventricular myocytes	Physiological	F
Severi *et al.*[69]	HH	17	Rabbit sino-atrial node cells	Physiological	F
Di Veroli *et al.*[33]	MM/HH	13	Chinese Hamster Ovary (CHO)	Room	E
Di Veroli *et al.*[33]	HH	17	Human Embryonic Kidney (HEK293) expressing canine ERG	Physiological	D

* The transition rates of the Kiehn *et al.*[45] model are defined at specific voltages, so for this model there are 8 parameters (and 1 conductance parameter) for each voltage at which the model is defined.

C Synthetic Data Study to Assess Protocol Information Content

In order to verify that there was sufficient information within the sinusoidal voltage protocol to parameterize our model we performed a synthetic data study. The aim in such a study is to ascertain whether we can recover the parameters used in the simulation from a simulated data trace (with added noise in this case).

C1 Producing synthetic data

In order to produce synthetic data we simulated the mathematical model with the parameter values obtained when fitting to the experimental data trace. We scale the simulated trace by multiplying by this factor, so it becomes approximately the same magnitude (in nA) as the experimental trace. We estimated the typical level of noise from the experimental trace by calculating the standard deviation σ of the experimental current during the first $200\,\text{ms}$ (where the current is around zero at the initial holding potential of $-80\,\text{mV}$). We then generate a synthetic data trace by adding normally distributed noise with a mean of zero and the standard deviation equal to the noise estimated from the experimental trace ($\sim N(0,\sigma^2)$) to the conductance-scaled simulated trace.

The example we present here uses the experimental reference trace from cell 5, featured in much of the manuscript.

C2 Inferring parameters from synthetic data

We then attempt to infer parameters from this 'synthetic data' trace, using the CMA-ES algorithm followed by MCMC as described in Section 4.7. In Figure C5 we present probability density distributions obtained when using both synthetic and experimental traces. We are able to recover the original parameters underlying the synthetic trace with high accuracy.
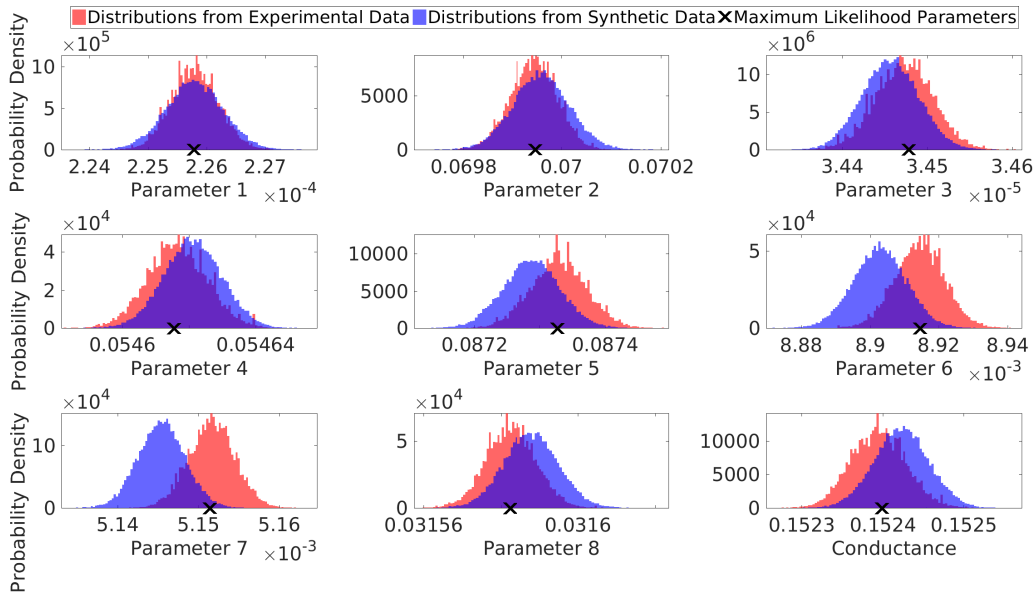

Figure C5: Probability density distributions for each parameter estimates from fitting to both experimental data (red) and simulated data (blue). Crosses indicate the parameter set with the maximum posterior density.

The synthetic data study provides us with confidence in the suitability of our protocol for accurately identifying parameters of the model presented in Figure 3B in the main text, and also that the parameter inference protocol(s) we are using are suitable for the task. We believe such an approach should always be used to test whether there is sufficient information in the experimental data being proposed for calibration of a mathematical model. The test should be performed twice: before conducting the experiment (with the pre-existing best guess at the parameters); and also after conducting the experiment (with the new maximum posterior density estimate of the parameters — as we illustrate in Figure C5).

D Cell-Specific versus Literature Model Predictions

In Tables D2–D10 we compare the predictions given by each cell-specific model with a range of literature model predictions. We compare their ability to predict the full current traces for the validation protocols Pr3–6 discussed in the main text. Each table provides the mean (over each time point) square difference between an experimental current recording in one particular cell and its cell-specific model prediction under each of the validation protocols, and compares this with current predictions from a range of literature models. Equation (F.3) gives the formula that was used to calculate the error entries.

Note that we have to choose a conductance value, G_{Kr}, for the literature models. G_{Kr} is selected differently for each cell by minimizing the error metric for the predicted current trace under the action potential protocol (Pr6) for each model (a best-case scenario for each literature model). Our new cell-specific models' conductances were fitted to the sine wave protocol (Pr7), along with the rest of their parameters. N.B. the literature model predictions are worse if we scale them to fit the sine wave; we considered this perhaps unjustified since they were developed never having seen such a protocol.

Despite literature models having their conductance scaled to minimize error in the Pr6 (action potential clamp) current prediction; only the Wang *et al.*[25] model for Cells #1, #3 and #4, and the Di Veroli *et al.*[33] model for Cell #9 perform better than our cell-specific models. The sine-wave fitted model outperforms all other literature models for all other cells.

Additionally, the Wang *et al.*[25] model gives better predictions for the deactivation protocol current for some cells; and for the inactivation protocol for Cell #9. The Di Veroli *et al.*[33] model gives better predictions for the inactivation protocol for Cells #5 and #9; and the deactivation protocol for Cell #6.

Table D2: Table quantifying square root of mean square difference (units nA) between experimental current traces and simulation predictions for the validation protocols shown in Figures 4 and 5 for Cell #1. Here the color scale is set so that ⬜ represents zero error and 🟥 represents the highest error for each protocol/column.

Model	Sine Wave (Pr7)	AP (Pr6)	Steady Act. (Pr3)	Deact. (Pr4)	Inact. (Pr5)
New model for Cell #1	0.0268	0.0757	0.0745	0.2115	0.1312
Wang *et al.*[25]	0.1196	0.0746	0.2079	0.2574	0.1543
Di Veroli *et al.*[33]	0.1406	0.0910	0.2097	0.3005	0.1706
Mazhari *et al.*[31]	0.1213	0.0885	0.1962	0.3171	0.1619
Ten Tusscher *et al.*[32]	0.1827	0.1079	0.2563	0.3335	0.2265
Zeng *et al.*[30]	0.1928	0.1381	0.2961	0.3617	0.2275

Table D3: Table quantifying square root of mean square difference (units nA) between experimental current traces and simulation predictions for the validation protocols shown in Figures 4 and 5 for Cell #2. Here the color scale is set so that ▮ represents zero error and ▮ represents the highest error for each protocol/column.

Model	Sine Wave (Pr7)	AP (Pr6)	Steady Act. (Pr3)	Deact. (Pr4)	Inact. (Pr5)
New model for Cell #2	0.0262	0.0549	0.0481	0.1421	0.0478
Wang et al.[25]	0.0923	0.0732	0.1081	0.1075	0.0818
Di Veroli et al.[33]	0.0687	0.0564	0.0859	0.1427	0.0655
Mazhari et al.[31]	0.0618	0.0664	0.0882	0.1793	0.0629
Ten Tusscher et al.[32]	0.1280	0.1159	0.1460	0.1902	0.1518
Zeng et al.[30]	0.1356	0.1395	0.1835	0.2190	0.1497

Table D4: Table quantifying square root of mean square difference (units nA) between experimental current traces and simulation predictions for the validation protocols shown in Figures 4 and 5 for Cell #3. Here the color scale is set so that ▮ represents zero error and ▮ represents the highest error for each protocol/column.

Model	Sine Wave (Pr7)	AP (Pr6)	Steady Act. (Pr3)	Deact. (Pr4)	Inact. (Pr5)
New model for Cell #3	0.0348	0.0997	0.0917	0.1320	0.1106
Wang et al.[25]	0.1015	0.0900	0.1071	0.1030	0.1178
Di Veroli et al.[33]	0.1365	0.1298	0.1639	0.1660	0.1502
Mazhari et al.[31]	0.1167	0.1263	0.1404	0.1885	0.1599
Ten Tusscher et al.[32]	0.1833	0.1337	0.1603	0.1929	0.1953
Zeng et al.[30]	0.2024	0.1826	0.2123	0.2272	0.1971

Table D5: Table quantifying square root of mean square difference (units nA) between experimental current traces and simulation predictions for the validation protocols shown in Figures 4 and 5 for Cell #4. Here the color scale is set so that ▮ represents zero error and ▮ represents the highest error for each protocol/column.

Model	Sine Wave (Pr7)	AP (Pr6)	Steady Act. (Pr3)	Deact. (Pr4)	Inact. (Pr5)
New model for Cell #4	0.0346	0.0649	0.0931	0.1337	0.0864
Wang et al.[25]	0.0962	0.0624	0.0804	0.0736	0.0928
Di Veroli et al.[33]	0.0713	0.0871	0.1381	0.1462	0.1028
Mazhari et al.[31]	0.0744	0.0992	0.1098	0.1828	0.1249
Ten Tusscher et al.[32]	0.1374	0.1350	0.1268	0.1771	0.1663
Zeng et al.[30]	0.1545	0.1867	0.1939	0.2194	0.1713

Table D6: Table quantifying square root of mean square difference (units nA) between experimental current traces and simulation predictions for the validation protocols shown in Figures 4 and 5 for Cell #5. Here the color scale is set so that ▮ represents zero error and ▮ represents the highest error for each protocol/column.

Model	Sine Wave (Pr7)	AP (Pr6)	Steady Act. (Pr3)	Deact. (Pr4)	Inact. (Pr5)
New model for Cell #5	0.0338	0.1003	0.0964	0.2788	0.5713
Wang et al.[25]	0.1409	0.1185	0.2236	0.2856	0.5715
Di Veroli et al.[33]	0.1498	0.1648	0.2086	0.3864	0.5659
Mazhari et al.[31]	0.1400	0.1760	0.1982	0.4443	0.5726
Ten Tusscher et al.[32]	0.2643	0.2453	0.3169	0.4653	0.6482
Zeng et al.[30]	0.2845	0.3116	0.4001	0.5245	0.6262

Table D7: Table quantifying square root of mean square difference (units nA) between experimental current traces and simulation predictions for the validation protocols shown in Figures 4 and 5 for Cell #6. Here the color scale is set so that ▮ represents zero error and ▮ represents the highest error for each protocol/column.

Model	Sine Wave (Pr7)	AP (Pr6)	Steady Act. (Pr3)	Deact. (Pr4)	Inact. (Pr5)
New model for Cell #6	0.0149	0.0419	0.0482	0.0883	0.0443
Wang et al.[25]	0.0396	0.0503	0.0537	0.0412	0.0528
Di Veroli et al.[33]	0.0362	0.0569	0.0596	0.0794	0.0458
Mazhari et al.[31]	0.0511	0.0621	0.0603	0.1048	0.0560
Ten Tusscher et al.[32]	0.0788	0.0844	0.0776	0.1041	0.1014
Zeng et al.[30]	0.0867	0.1101	0.1051	0.1255	0.0988

Table D8: Table quantifying square root of mean square difference (units nA) between experimental current traces and simulation predictions for the validation protocols shown in Figures 4 and 5 for Cell #7. Here the color scale is set so that ▮ represents zero error and ▮ represents the highest error for each protocol/column.

Model	Sine Wave (Pr7)	AP (Pr6)	Steady Act. (Pr3)	Deact. (Pr4)	Inact. (Pr5)
New model for Cell #7	0.0825	0.1343	0.2060	0.3167	0.1239
Wang et al.[25]	0.1914	0.2176	0.2506	0.2358	0.2726
Di Veroli et al.[33]	0.1994	0.2654	0.2827	0.4101	0.2605
Mazhari et al.[31]	0.2361	0.2987	0.2597	0.5102	0.2637
Ten Tusscher et al.[32]	0.3966	0.4124	0.4205	0.5287	0.5326
Zeng et al.[30]	0.4147	0.5013	0.5597	0.6246	0.5357

Table D9: Table quantifying square root of mean square difference (units nA) between experimental current traces and simulation predictions for the validation protocols shown in Figures 4 and 5 for Cell #8. Here the color scale is set so that ▮ represents zero error and ▮ represents the highest error for each protocol/column.

Model	Sine Wave (Pr7)	AP (Pr6)	Steady Act. (Pr3)	Deact. (Pr4)	Inact. (Pr5)
New model for Cell #8	0.0426	0.1331	0.1302	0.1796	0.2222
Wang et al.[25]	0.1043	0.1367	0.2036	0.2100	0.2491
Di Veroli et al.[33]	0.1053	0.1545	0.1828	0.2656	0.2538
Mazhari et al.[31]	0.1071	0.1621	0.1860	0.3058	0.2528
Ten Tusscher et al.[32]	0.1839	0.1957	0.2624	0.3248	0.3334
Zeng et al.[30]	0.1902	0.2305	0.3122	0.3641	0.3347

Table D10: Table quantifying square root of mean square difference (units nA) between experimental current traces and simulation predictions for the validation protocols shown in Figures 4 and 5 for Cell #9. Here the color scale is set so that ▮ represents zero error and ▮ represents the highest error for each protocol/column.

Model	Sine Wave (Pr7)	AP (Pr6)	Steady Act. (Pr3)	Deact. (Pr4)	Inact. (Pr5)
New model for Cell #9	0.0243	0.1478	0.1109	0.1806	0.2183
Wang et al.[25]	0.0507	0.1493	0.1277	0.1755	0.2181
Di Veroli et al.[33]	0.0474	0.1474	0.1153	0.1830	0.2164
Mazhari et al.[31]	0.0408	0.1482	0.1176	0.1917	0.2163
Ten Tusscher et al.[32]	0.0773	0.1547	0.1443	0.1961	0.2246
Zeng et al.[30]	0.0820	0.1606	0.1589	0.2060	0.2253

E   Additional Peak Current-Voltage Relationship Predictions

Here we show the remainder of the predictions of the current-voltage relationships for the validation
data of cell 5 that were not included in the main text (the results of Pr1 and Pr2). Figure E6 shows
the summary curves for Pr1 (voltage clamp shown in Figure A2) and Pr2 (voltage clamp shown in
Figure A3).

   Traditionally these peak current curves would be plotted by normalizing to the peak current
recorded in each activation kinetics protocol. However, as we have used a shorter version of the
activation kinetics protocol, we do not expect that the channel would be fully open at the longest
duration test step in Pr1 and Pr2. We have therefore instead normalized the curves using the peak
current during the initial deactivation step in the sine wave protocol (around 1.6 seconds) where we
expect the channel to be maximally open.

Figure E6: Predictions of peak current-voltage relationship derived from experiment and model
predictions in response to; A) Activation Kinetics Pr1, B) Activation Kinetics Pr2, with compari-
son of our model prediction with predictions from existing literature models. Currents have been
normalized to the peak current in the initial deactivation step in the sine wave protocol (around 1.6
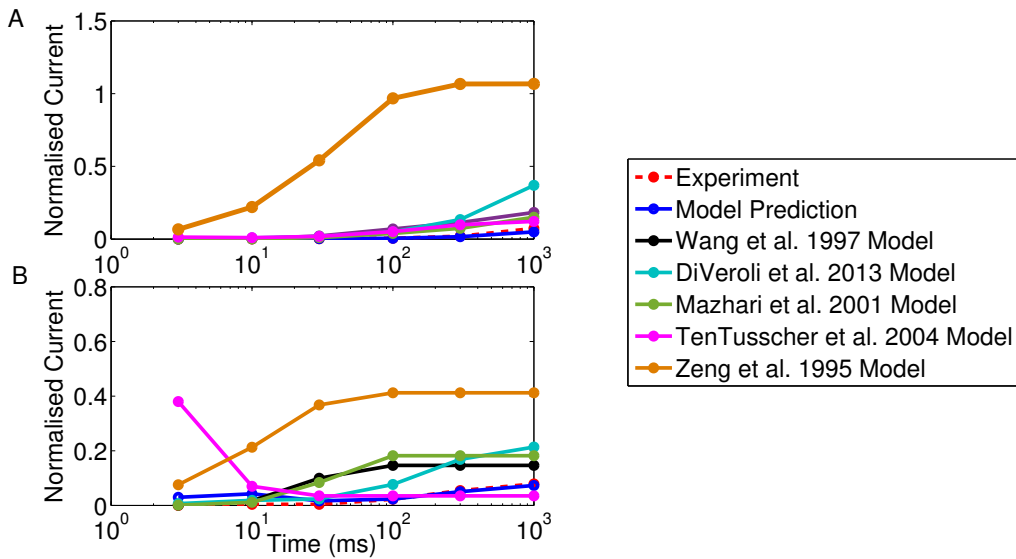seconds) as we do not expect the channel to be fully open at the longest T_{step} in these activation
kinetics protocols.

F Comparing Cell-Specific with Average Model

In addition to creating cell specific models as described so far we also created an averaged model by first normalizing each experimental trace to one reference trace (so that each trace was given equal weight in the averaging regardless of the conductance of the channel) and then summing and averaging the current value at each time point along the protocol.

The parameter values obtained when calibrating each cell-specific and averaged model are shown in Table F11. These values correspond to the parameter sets with maximum posterior density identified in the MCMC chain. The full posterior density distributions for each parameter for each of the 9 cells are shown in Figure F7.

Table F11: Table of parameter values at the maximum posterior density for each cell-specific model, and the model fitted to averaged data. Here the model parameter numberings correspond to those detailed in Figure 3B, and G_{Kr} represents the conductance value fitted for each model. *Note that the conductance fitted for the 'Averaged' model reflects mainly the conductance for the reference experimental trace (used for scaling all other traces before averaging), and should not be considered the 'average' conductance, hence its omission from Figure 6A.

	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	G_{Kr}
Cell #1	1.9742×10^{-4}	0.0594	7.1664×10^{-5}	0.0493	0.1048	0.0139	0.0038	0.0360	0.1350
Cell #2	3.2387×10^{-4}	0.0653	7.8183×10^{-5}	0.0497	0.0805	0.0025	0.0049	0.0324	0.0902
Cell #3	4.7883×10^{-4}	0.0661	5.1621×10^{-5}	0.0523	0.1375	0.0094	0.0039	0.0375	0.1011
Cell #4	6.7417×10^{-4}	0.0563	5.8605×10^{-5}	0.0516	0.0893	0.0057	0.0059	0.0324	0.0743
Cell #5	2.2578×10^{-4}	0.0699	3.4477×10^{-5}	0.0546	0.0873	0.0089	0.0052	0.0316	0.1524
Cell #6	6.1015×10^{-4}	0.0662	1.2729×10^{-4}	0.0380	0.0810	0.0165	0.0092	0.0253	0.0218
Cell #7	5.5188×10^{-4}	0.0477	6.6263×10^{-5}	0.0457	0.0628	0.0087	0.0054	0.0317	0.1555
Cell #8	3.1062×10^{-4}	0.0485	5.0455×10^{-5}	0.0491	0.0722	0.0063	0.0060	0.0328	0.0983
Cell #9	5.5916×10^{-4}	0.0435	1.2377×10^{-4}	0.0444	0.0658	0.0028	0.0036	0.0343	0.0514
Averaged	4.0177×10^{-4}	0.0578	6.5137×10^{-5}	0.0487	0.0807	0.0068	0.0052	0.0334	0.0673*

To quantitatively compare the average model predictions and the cell-specific model predictions shown in Figure 6B of the main text we calculated the mean square difference at each point between the average model and the cell-specific models for each cell when predicting the full current trace in response to the steady-state activation protocol. We also repeated this for the deactivation and inactivation protocols and the action potential protocol shown in Figures 4 and 5. The differences for each cell are shown in Table F12 with a comparison between the experimental result and the average model predictions with the cell-specific predictions.

We note that we have ordered the cells in this table (as in Figure 6) according to the percentage change in leak resistance between performing the vehicle and dofetilide repeats of the sine wave voltage protocol used to construct the model. This ordering acts as an estimated ranking for the quality of each recording. The benefit of a cell-specific approach occurs when using the highest quality data for both model construction and validation. We should note that even though in cells #4 and #6 the average model provides the better prediction of the steady-state activation peak current-voltage relationship than the cell-specific model, the cell-specific models are still providing very good predictions in these cases, it is just that the experimental behavior is more like the average model behavior for these cells. We also note that for six out of the nine cells, the cell-specific model provides a better prediction of the current response to the action potential protocol than the average model, however, in the cases where the cell-specific model is worse the difference is only a small amount.
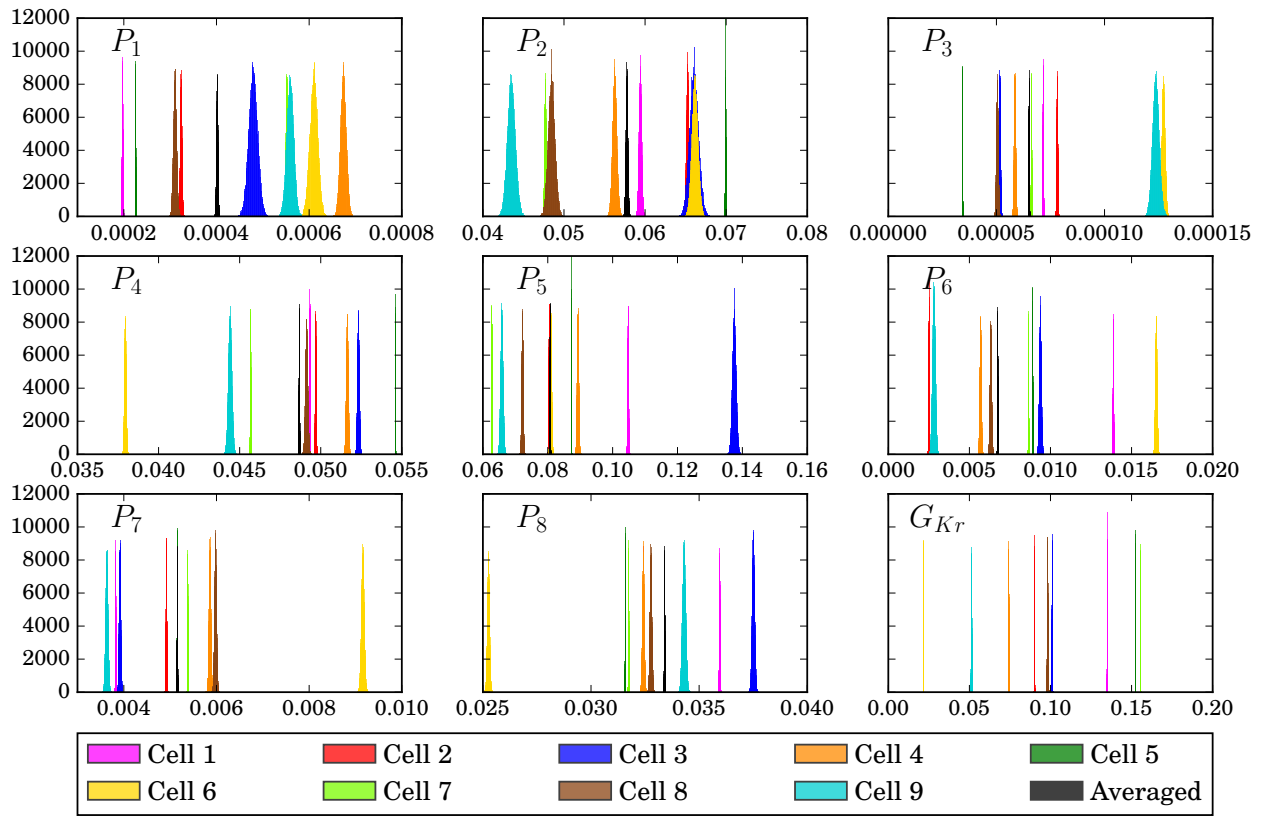
Figure F7: Distributions for each parameter for each of the 9 cell-specific models and the averaged data model. To aid comparison these are all histograms with 100 bars (plotting probability distributions here leads to very different maxima, obscuring the spread information), and so the y-axis is in arbitrary units related to the number of samples. We see that the parameter values tend to be given distinct distributions and so we would consider most of them to be 'significantly different'.

We use a measure of

$$\text{error} = \left(\frac{1}{T} \sum_{t=1}^{T} \left(\text{simulated current at time step } t - \text{experimental current at time step } t \right)^2 \right)^{\frac{1}{2}},$$

(F.3)

to evaluate the error in model predictions for individual cells (using the whole current traces, apart from removing regions in the sine wave protocol with capacitive spikes as explained in Online Methods 4.7.1).

Table F12: Table showing the error measure defined by equation F.3 between cell-specific or average models and the experimental current recording for fit (sine wave Pr7) and predictions with validation protocols (all other columns). Cells are ordered in ascending order according to the percentage change in leak resistance R_{leak}. Here the color scale is set so that within each pair of columns ▢ represents lowest error and ▮ represents the highest error for each protocol/pair of columns. Note that the cells with larger currents will show larger errors, but the left column cell-specific predictions tend to perform better than the average model, particularly for cells where the average model gives a relatively large error.

Cell #	ΔR_{leak} (%)	Sine Wave (Pr 7)		APs (Pr 6)		Steady Act. (Pr 3)		Deact. (Pr 4)		Inact. (Pr 5)	
		Specific	Average	Spec.	Aver.	Spec.	Aver.	Spec.	Aver.	Spec.	Aver.
1	0.0	0.0268	0.0650	0.0757	0.1195	0.0745	0.1369	0.2115	0.2334	0.1312	0.1250
2	7.7	0.0262	0.0401	0.0549	0.0516	0.0481	0.0490	0.1421	0.1249	0.0478	0.0489
3	12.5	0.0348	0.0609	0.0997	0.1403	0.0917	0.1489	0.1320	0.1317	0.1106	0.1600
4	16.7	0.0346	0.0497	0.0649	0.0690	0.0931	0.0797	0.1337	0.1301	0.0864	0.0959
5	20.0	0.0338	0.0374	0.1003	0.1149	0.0964	0.1274	0.2788	0.3358	0.5713	0.5668
6	28.6	0.0149	0.0335	0.0419	0.0401	0.0482	0.0372	0.0883	0.0739	0.0443	0.0419
7	32.5	0.0825	0.1073	0.1343	0.1635	0.2060	0.1772	0.3167	0.3595	0.1239	0.1398
8	42.9	0.0426	0.0514	0.1331	0.1356	0.1302	0.1494	0.1796	0.2345	0.2222	0.2233
9	58.3	0.0243	0.0266	0.1478	0.1472	0.1109	0.1068	0.1806	0.1766	0.2183	0.2174

For predictions of the action potential protocol currents, Table F12 demonstrates that the cell-specific modeling approach yields predictions that are very close to or better than the average model. Additionally, for the predictions of the steady-state activation protocol the cell-specific approach generally yields very good and more accurate (for 4/5) predictions of validation data when the highest quality data is used (cells #1–5). This benefit is absent when lower quality experimental data is used where the average model provides very similar, but slightly better, predictions (cells #6–9).

We also compare cell-specific and average predictions for each of the 9 cells for the deactivation, recovery from inactivation and instantaneous inactivation time constants as were shown for one cell in Figure 4. We show this comparison for each cell in Figure F8 and F9 for 8/9 cells and in Figure F10 for all cells. Cell #6 was omitted in the first two plots because this cell had a particularly low current and it was difficult to accurately fit exponential curves to the experimental data for this cell. We also note that we have not plotted the time constant values for $-90\,\text{mV}$ in Figures F8 & F9 for the same reason; we could not confidently fit an exponential decay curve to determine an accurate time constant value for this voltage step.

We see in Figures F8–F10 that the same observations that were made for the results shown in Figure 6 generally hold: for lower cell numbers #1–5, we see enhanced predictions of the experimental time constants from the cell-specific model rather than the averaged model. i.e predictions
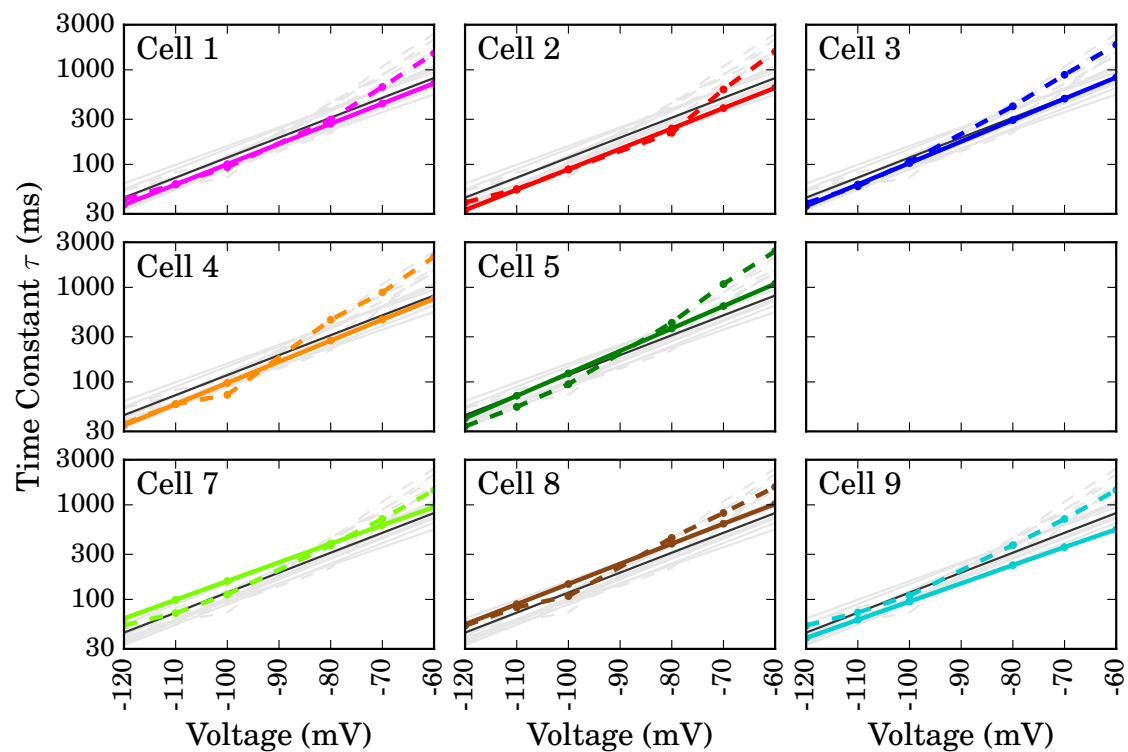
Figure F8: Cell-specific model predictions of time constant/voltage relationships for deactivation (Pr5). Each plot represents a different cell, with cell-specific model prediction depicted by the bold line, and the dashed line showing the cell's experimental data. Black lines on each plot represents the average model prediction. Cells are ordered as in Table F12.
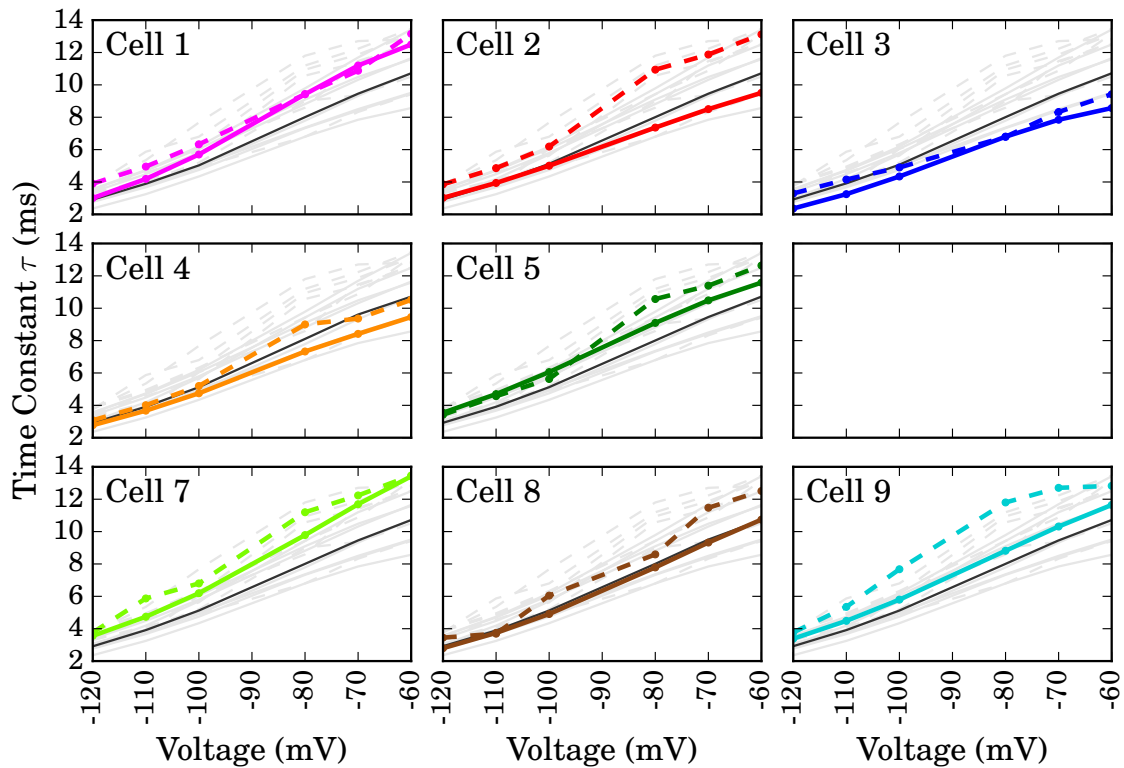
Figure F9: Cell-specific model predictions of time constant/voltage relationships for recovery from inactivation in Pr5. Each plot represents a different cell, with cell-specific model prediction depicted by the bold line, and the dashed line showing the cell's experimental data. Black lines on each plot represents the average model prediction. Cells are ordered as in Table F12.
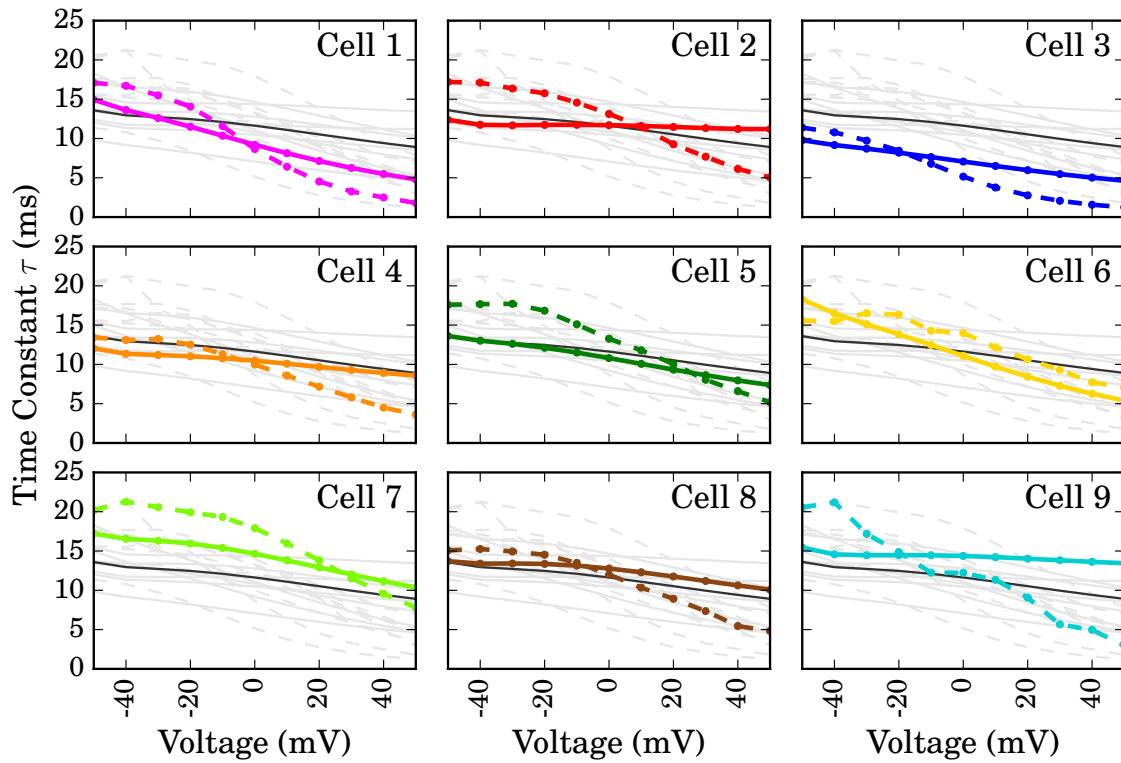
Figure F10: Cell-specific model predictions of time constant/voltage relationships for instantaneous inactivation (Pr4). Each plot represents a different cell, with cell-specific model prediction depicted by the bold line, and the dashed line showing the cell's experimental data. Black lines on each plot represents the average model prediction. Cells are ordered as in Table F12.

are better in the cells with lower percentage changes in leak current resistance, which correspond to better quality data.

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