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In-silico analysis of the dynamic regulation of cardiac electrophysiology by Kv11.1 ion-channel trafficking

Stefan Meier, Adaïa Grundland, Dobromir Dobrev, Paul GA Volders, and Jordi Heijman
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Dear Dr Heijman,

Re: JP-RP-2022-283976 "In-silico analysis of the dynamic regulation of cardiac electrophysiology by Kv11.1 ion-channel trafficking" by Stefan Meier, Adaïa Grundland, Dobromir Dobrev, Paul GA Volders, and Jordi Heijman

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-'n' clearly defined (e.g. x cells from y slices in z animals) in the Methods. Authors should be mindful of pseudoreplication.

-All relevant 'n' values must be clearly stated in the main text, figures and tables, and the Statistical Summary Document (required upon revision)

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-Statistics Summary Document completed appropriately upon revision

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EDITOR COMMENTS

Reviewing Editor:

Both reviewers find the work interesting, exciting, and potentially impactful. The presentation is balanced and model assumptions and limitations are well discussed. However, the reviewers found that clarification and justification is needed in several aspects of the model and simulations.

REFEREE COMMENTS

Referee #1:

The article entitled "In-silico analysis of the dynamic regulation of cardiac electrophysiology by KV11.1 ion-channel trafficking" by Meier and colleagues introduces an ion channel trafficking model to demonstrate complex regulation of ventricular cardiomyocyte repolization by mutations, temperature, and different medications that impact KV11.1 channel trafficking/gating across different time scales. The in-silico trafficking framework is a novel step in the modeling the physiology of ion channel function on the ventricular cell action potential and likely represents the initial step in what will be an important component of future modeling. The strengths of the article are the model is that although the model is very simple, it recapitulates many aspects of published experimental data. The authors apply the model to several applications that help to validate its utility (e.g., mutations, medications that negative or positively impact KV11.1 channel trafficking, and temperature). The authors do a good job of discussing the limitations/simplifying assumptions of the modeling. However, some concerns exist. Some of the modelization as presented is not intuitive and, in some cases, it is unclear what the authors are trying to demonstrate. Additional clarification and justification is needed.

1) Most KV11.1 missense mutations that cause long QT syndrome disrupt the trafficking of the mutant KV11.1 channels. As noted by the authors, this is thought to primarily occur in the forward trafficking step. However, relative to the model, this includes disruptions in the "production step (Psi)" of the model because this encompasses so many different steps. These data are qualitatively captured in Figure 3 but this concept seems underexplored. The inclusion of data for a unique type of KV11.1 missense mutation that does not traffic normally, A57P, are intriguing. However, as presented the data in Figure 4 and Table 1 are not easy to interpret. Table 1 only shows the parameters for the mutant simulations (why not include WT parameters for reference?). Additional description for how the four parameter sets were obtained would be helpful.

"Subsequently, the four model rates were individually scaled to match the relative difference in membrane levels between the wild-type and mutant Kv11.1 channel, while the other three rates were kept constant, providing four starting points for parameter optimization."

Can the authors clarify which rate constants were maintained for a given parameter set?

2) Embedding the trafficking model as part of the ventricular action potential simulation is exciting. However, some of the output results are not intuitive. This reviewer is surprised that only a 35% reduction in KV11.1 current was sufficient to cause such a prolongation of the AP (Figure 4D)? Or how simulated reductions in IKR sometimes caused the AP to fail to repolarize in other situations (Figure 5 and 7). Is the O'Hara-Rudy action potential used overly sensitive to small changes in the IKR? Or does this reflect the challenges of embedding the IKr model in the ventricular AP simulation? How does the duration of the "embedded O'Hara-Rude model" action potential compare to the duration of the original O'Hara-Rudy action potential model? Do analogous reductions in IKR in both models cause similar changes in action potential duration?

Given the differences in patient QTc interval data with fever and hypothermia and the lack of concordant/unusual result with the AP modeling in Figure 7- it is unclear how including the AP simulations improves the manuscript. As noted by the authors, many things change with fever that are not incorporated in the simulation- please clarify the purpose of including

these data given these limitations.

3) Figure 5D and 5E need clarification. Are these simulations done with pentamidine? Or simulating dofefilide alone? If done without pentamidine, does dofetilide alone increase the trafficking of KV11.1 channels (in the absence of pentamidine)? Please clarify.

Minor

Do the waveform used to simulate currents in Figure 6B have corresponding experimental data? Why was this voltage waveform chosen to show the model output? Can the authors use these data to compare the simulated KV11.1 to experimental data of IKV11.1 measured at high temperatures (see PMID: 18551196)?

As noted by the authors, there are many known modulators of KV11.1 channel trafficking and while several are included here, but some of the more common clinically relevant ones are not. Particularly how extracellular K+ levels impact cardiac cellular action potentials and KV11.1 channels trafficking (e.g., with hyopkalemia). There are rich experimental data set on the effects extracellular K+ on modifying KV11.1 channel trafficking (as included in the cited reference Guo et al., 2009 and PMID: 20133899).

Is it significant that Kanner et al., also reported two other mutations (N33T and R56Q), which disrupt forward trafficking like A57P but also decrease surface stability, express similar KV11.1 channel protein levels at the cell surface member. From a modeling perspective- what might this mean/suggest?

Referee #2:

Summary Comments:

• The authors present a framework for incorporating ion-channel trafficking into computational models of cardiac electrophysiology, which currently do not incorporate them. This model uses Kv11.1 (hERG), the channel for which the most experimental data are available, as a proof-of-concept. This model incorporates temperature- and drug-dependent effects on channel kinetics and trafficking to enable a more sophisticated approach to modeling channel behavior. After optimization and parameter sensitivity analyses, the authors identified key differences between the acute and chronic electrophysiology of temperature changes and drugs that modify channel kinetics and trafficking. Moreover, this provides a potential framework for future work investigating the role of channel trafficking and its modulation to arrhythmogenesis.

• Overall, the paper flowed relatively well. There were a few points of confusion:

o In the Methods section, it was not immediately clear how certain parameter modifications were explicitly integrated into the model. Additional explanatory text and updates to Figure 1 would strengthen the paper.

o The "inverse" or "opposite" effects of temperature increases and decreases are referenced before the precise effects are described in detail near the end of the Results section. Clarifying the language in these locations would be helpful.

• Overall, model predictions of experimental data were fairly robust, save for a few minor discrepancies. These may not significantly impact the results, but a discussion of their potential impact (and/or tweaking the model to verify this) would strengthen the paper. There were a few places where the figures could be modified to resolve ambiguities in presentation and clarify the methods. See specific comments below.

• Discussion and Limitations were robust and comprehensive. The model predictions with respect to temperature contradicted observed clinical impacts, but potential reasons for this discrepancy were discussed in detail. It may be beneficial to include a brief discussion on fever as a trigger for arrhythmia in those with a vulnerable substrate (e.g., Brugada Syndrome).

• This is an important contribution to the modeling literature and suggests several future directions for further research.

-

Specific Suggestions:

• 85-87

o Please clarify this sentence: "Dofetilide substantially increased Kv11.1 membrane levels at supraphysiological concentrations that produce significant channel block, but not at clinically-relevant concentrations."

o Does "supraphysiologic concentrations" refer to dofetilide or Kv11.1? Does this mean that subclinical levels of dofetilide increased Kv11.1 membrane levels AND produced significant channel block?

• 89-98

o "The opposite was true" is unclear. Does this mean that lower temperature slowed kinetics, decreased IKr acutely, had increased trafficking after 24 hours, and increased IKr chronically?

• 129

o "The opposite is true for hypothermia."

o Same comment as above. I think this would benefit from additional clarification.

• 169

o The notation is confusing. What do "M#(1)" and "S#(2)" denote? Is this a typo?

o On second look, this appears to be a formatting error for equation numbering that is carried through the document?

• 209 (Equation 5)

o Additional clarity on how the lambda parameter is explicitly embedded within the model would be helpful.

• 254-255

o Figure 2B: At 3 minutes, model appears to imply substantially less recycling than Dennis et al. (2011)? This is potentially important for acute phenomena. While authors acknowledge this, an explanation of potential impacts of this discrepancy (either here, or in the Discussion/Limitations section) would strengthen the paper. Overall, the model predictions were in line with experimental data.

• 334-341

o These sentences help clarify the confusion in lines 89-98 and 129 regarding the effects of temperature on kinetics, channel trafficking, and acute vs. chronic IKr current. The earlier sections would benefit from this level of clarity, since they precede this section.

• 406-407

o This section would benefit from an explanation of how the rapid kinetics in Kanner et al. (2018) (minutes vs. hours in other literature) could potentially impact the conclusions of this paper, in addition to clinical implications. It is clear from the methods section (288-292) that this was compensated for by taking relative differences in dynamics between the wild-type and mutant Kv11.1, but what impact would the timescale of kinetics have clinically?

• Figure 1:

o The paper would benefit from a modification to Figure 1A/1B (or an additional subfigure) to make explicit how temperature and drug modifications of model parameters affect the model. While there is text in the figure description that discusses this, a visual representation would make the methods easier to follow.

• Figure 2:

o Overall, the model fit experimental data well. One exception is the acute phase of recycling in Figure 2B, in which model underpredicts Denis et al. (2011) at 3 minutes. See discussion of lines 254-255.

• Figure 5:

o Overall, the model fit experimental data well. There is a minor underprediction of mature channel trafficking at low concentrations of dofetilide (Figure 5B). A small description of possible impacts in the Discussion could be beneficial.

o In Figure 5D, the upper and lower graphs are quite close together. In combination with the color change in the middle section (drug application), this makes the graphs somewhat confusing, on first glance. Separating the upper and lower graphs (there appears to be sufficient space above Figure 5E) would make this clearer. Alternatively/additionally, a break in the dashed lines would help the reader to distinguish the graphs from one another.

END OF COMMENTS

Confidential Review **14-Oct-2022**

Responses to Referees on the paper:

'In-silico analysis of the dynamic regulation of cardiac electrophysiology by Kv11.1 ion-channel trafficking' by Stefan Meier, Adaïa Grundland, Dobromir Dobrev, Paul G.A. Volders, and Jordi Heijman.

We are grateful for the excellent feedback from both referees. Based on their comments, we expanded the model with regulation of $K_v11.1$ trafficking by hypokalaemia. We subsequently updated the model parameters to account for these changes and the new data, which also resulted in an improved fit for the effects of dofetilide on $K_v11.1$ trafficking. As such, we re-ran all the simulations and updated the figures and tables accordingly. None of the original conclusions of our paper were affected by these changes.

Referee #1:

The article entitled "In-silico analysis of the dynamic regulation of cardiac electrophysiology by KV11.1 ion-channel trafficking" by Meier and colleagues introduces an ion channel trafficking model to demonstrate complex regulation of ventricular cardiomyocyte repolization by mutations, temperature, and different medications that impact KV11.1 channel trafficking/gating across different time scales. The in-silico trafficking framework is a novel step in the modeling the physiology of ion channel function on the ventricular cell action potential and likely represents the initial step in what will be an important component of future modeling. The strengths of the article are the model is that although the model is very simple, it recapitulates many aspects of published experimental data. The authors apply the model to several applications that help to validate its utility (e.g., mutations, medications that negative or positively impact KV11.1 channel trafficking, and temperature). The authors do a good job of discussing the limitations/simplifying assumptions of the modeling. However, some concerns exist. Some of the modelization as presented is not intuitive and, in some cases, it is unclear what the authors are trying to demonstrate. Additional clarification and justification is needed.

We would like to thank the referee for their careful assessment and important comments and suggestions that helped us to further improve our manuscript. We did our best to better clarify our model and have provided a point-by-point response to the comments below.

1) Most KV11.1 missense mutations that cause long QT syndrome disrupt the trafficking of the mutant KV11.1 channels. As noted by the authors, this is thought to primarily occur in the forward trafficking step. However, relative to the model, this includes disruptions in the "production step (Psi)" of the model because this encompasses so many different steps. These data are qualitatively captured in Figure 3 but this concept seems underexplored. The inclusion of data for a unique type of KV11.1 missense mutation that does not traffic normally, A57P, are intriguing. However, as presented the data in Figure 4 and Table 1 are not easy to interpret. Table 1 only shows the parameters for the mutant simulations (why not include WT parameters for reference?). Additional description for how the four parameter sets were obtained would be helpful.

"Subsequently, the four model rates were individually scaled to match the relative difference in membrane levels between the wild-type and mutant Kv11.1 channel, while the other three rates were kept constant, providing four starting points for parameter optimization."

Can the authors clarify which rate constants were maintained for a given parameter set?

Based on the referee's suggestion, we have added the wild-type parameters to Table 1 and Table 6:

Table 1. Calibrated ion channel trafficking parameters.

Parameter	***Value (per hour)***
α	*6.56850075*
β	*2.101663125 · (κ_b/κ_{bref})*
δ	*0.599592 · (κ_d/κ_{dref})*
ψ	*423.26175750000004 · λ · θ*

**Note, θ, λ, κ_b, and κ_d reflect the temperature, drug and extracellular [K^+] modulators from equations 4, 5, 8, and 9, respectively. Moreover, the κ_{bref} and κ_{dref} are the results from equations 8 and 9 with extracellular [K^+] set at 5.4 mmol/L.*

Table 6. *Parameter sets related to K_v11.1 p.(A57P) mutation simulations.*

	α	**β**	**δ**	**ψ**

WT parameters	6.56850075	2.101663125	0.599592	423.2617575
Parameter set 1	4.39878871	1.93333205	0.65697293	408.40110484
Parameter set 2	7.98396124	2.06770286	0.81350517	298.70774597
Parameter set 3	6.71166945	2.06613057	0.97941628	421.38040913
Parameter set 4	6.83359678	2.05937586	0.62798972	269.66811756

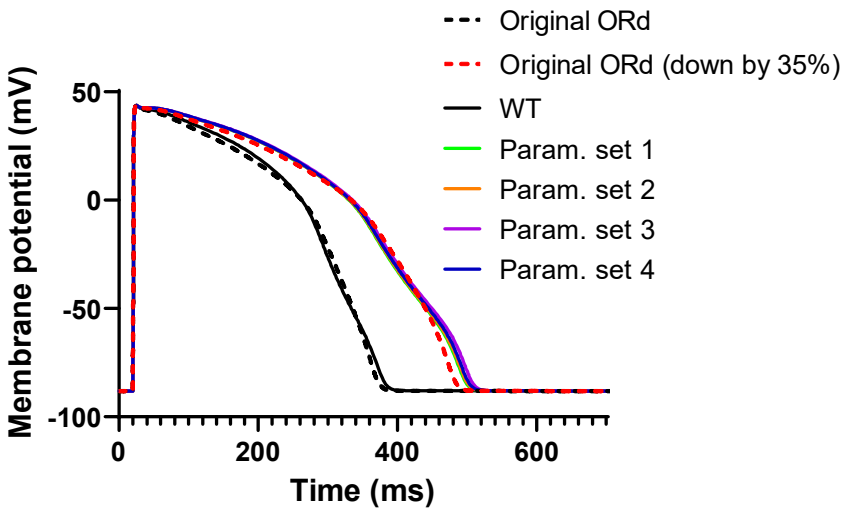
Furthermore, we have provided additional clarification on the derivation of the mutant parameter sets in the text at lines 321-327 as: *"Four starting points for parameter optimization were created by scaling each model rate individually to approximate the 35% reduction of K_v11.1 membrane channel levels, while keeping the other rates constant. For example, parameter set 1 was obtained by reducing α by approximately 35% while the other rates were kept at their WT values. Initial values for parameter sets 2-4 were obtained similarly by scaling β, δ, and ψ. Thereafter, all four parameter sets were optimized by updating all the rates from each parameter set."*

Finally, we have also added an additional sentence to the discussion at lines 432-435 that addresses the fact that channel production and first part of forward trafficking are lumped together: *"In this model structure, ψ reflects both channel production and the first part of forward trafficking (e.g., microtubule-mediated trafficking between ER and GC), so we cannot distinguish between the effects of modulators on these two components."*

2) Embedding the trafficking model as part of the ventricular action potential simulation is exciting. However, some of the output results are not intuitive. This referee is surprised that only a 35% reduction in KV11.1 current was sufficient to cause such a prolongation of the AP (Figure 4D)? Or how simulated reductions in IKR sometimes caused the AP to fail to repolarize in other situations (Figure 5 and 7). Is the O'Hara-Rudy action potential used overly sensitive to small changes in the IKR? Or does this reflect the challenges of embedding the IKr model in the ventricular AP simulation? How does the duration of the "embedded O'Hara-Rude model" action potential compare to the duration of the original O'Hara-Rudy action potential model? Do analogous reductions in IKR in both models cause similar changes in action potential duration?

To investigate the sensitivity of our embedded trafficking model, we compared the action potentials of our model with the original ORd and evaluated the effect of a 35% reduction in I_{Kr} conductance in the original ORd with the simulated mutation in our trafficking Markov model I_{Kr} formulation. During this comparison we realised that the baseline ORd I_{Kr} was smaller than the trafficking I_{Kr} model. We compensated for this difference by scaling down the conductance by 30% in the Markov model. **Referee Figure 1**, below, shows that with this update the original ORd APD is comparable to the WT trafficking model APD (approximately 400 ms at a basic cycle length of 60 s). Then, the mutant behaviour was approximated by scaling down the I_{Kr} conductance of the original ORd by 35%, which resulted in an approximately 100 ms APD prolongation. This matches the MT APD prolongations seen in our simulations with the trafficking model (**Referee Figure 1** & **Figure 4D**) and indicates that our model doesn't increase the sensitivity of the ORd model.

We have indicated this aspect in the revised manuscript at lines 334-336: "*A similar I_{Kr} reduction in the original ORd model resulted in a comparable APD prolongation of approximately 100 ms (data not shown).* "



Referee Figure 1. *Action potential duration comparison.* The original I_{Kr} formulation of the ORd model is presented as the black dotted line. The MT behaviour was mimicked by scaling down the original ORd I_{Kr} formulation (through the conductance) by 35%, which is presented as the red dotted line.

Given the differences in patient QTc interval data with fever and hypothermia and the lack of

concordant/unusual result with the AP modeling in Figure 7- it is unclear how including the AP simulations improves the manuscript. As noted by the authors, many things change with fever that are not incorporated in the simulation- please clarify the purpose of including these data given these limitations.

We agree with the referee that fever has multiple effects. The goal of the simulations in **Figure 7** was to highlight the combined effects of acute temperature-dependent changes in $K_v11.1$ channel gating and long-term regulation of $K_v11.1$ channel trafficking on repolarization. Since these are opposing effects (with higher temperatures increasing I_{Kr} through $K_v11.1$ gating modulation, but reducing the number of channels in the membrane), the combined effects are difficult to dissect experimentally and can only be predicted with a computational model. We have added a sentence to the discussion in lines 562-564 to clarify this: "*As such, the goal of **Figure 7** was not to show the macroscopic effects of fever, but rather the temporal effects of temperature on gating and trafficking, and their combined effect on APD.*"

3) Figure 5D and 5E need clarification. Are these simulations done with pentamidine? Or simulating dofefilide alone? If done without pentamidine, does dofetilide alone increase the trafficking of KV11.1 channels (in the absence of pentamidine)? Please clarify.

This has been clarified in the results at lines 345-347 as: *"We subsequently employed the model to investigate the combined effect of acute channel inhibition and long-term trafficking promotion by dofetilide in the absence of pentamidine*."

Furthermore, the legend of **Figure 5** was updated with: *"Note, these simulations were performed without pentamidine."*.

A recent study has shown an approximately 60% increase in WT $K_v11.1$ levels after 24 hours of incubation with 5 µmol/L E-4031 (Al-Moubarak *et al.*, 2020) in the absence of pentamidine or other compounds impairing $K_v11.1$ trafficking. Our simulations with 1 µmol/L dofetilide showed a comparable increase in $K_v11.1$ channels over 24 hours. In agreement, previous work has shown that E-4031 and dofetilide have qualitatively similar effects on $K_v11.1$ trafficking, with dofetilide being a more potent rescuer of trafficking-deficient $K_v11.1$ mutants (Qile *et al.*, 2020). Thus, it appears plausible that dofetilide alone can indeed increase $K_v11.1$ membrane levels, as suggested by our simulations.

Minor

Do the waveform used to simulate currents in Figure 6B have corresponding experimental data? Why was this voltage waveform chosen to show the model output? Can the authors use these data to compare the simulated KV11.1 to experimental data of IKV11.1 measured at high temperatures (see PMID: 18551196)?

We implemented the protocol from the suggested literature and re-ran the simulations for the corresponding temperatures (i.e., 23, 35, and 40 °C) (Amin *et al.*, 2008). We replaced the I_{Kr} traces in **Figure 6B** with the new results and added a comparison of the relative temperature dependence of experimental and simulated tail current amplitudes to **Figure 6C**. Although our model is slightly less sensitive to temperature changes, the tail current amplitudes are still smaller at lower temperatures and larger at higher temperatures, while being within the standard deviation of the experimental data (Amin *et al.*, 2008).

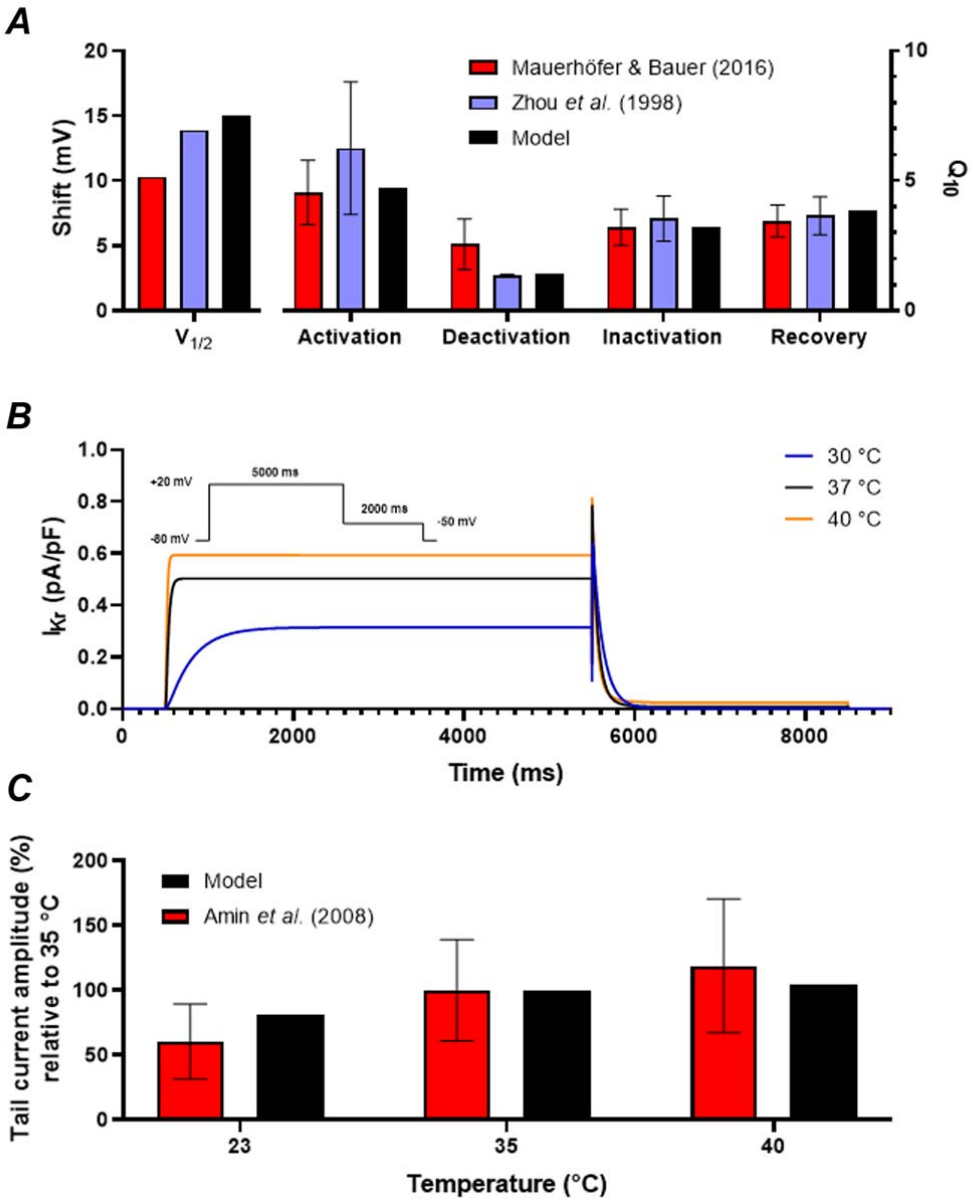


Figure 6. Temperature-dependent regulation of K_v11.1 gating. *A*, Calibration of the shift in midpoint of voltage dependence and Q_{10} values for activation, deactivation, inactivation and recovery of I_{Kr} in experimental recordings (Zhou *et al.*, 1998; Mauerhofer & Bauer, 2016) and model. *B*, Combined effects of temperature-dependent changes in midpoint and Q_{10} on I_{Kr} at 30, 37, and 40 °C. Inset shows voltage-clamp protocol for steady-state and tail I_{Kr}. *C,* Relative tail current amplitudes at 23, 35 and 40 °C normalised to 35 °C obtained with the voltage-clamp protocol from panel B in experimental recordings (Amin *et al.*, 2008) and model.

As noted by the authors, there are many known modulators of KV11.1 channel trafficking and while several are included here, but some of the more common clinically relevant ones are not. Particularly how extracellular K+ levels impact cardiac cellular action potentials and KV11.1 channels trafficking (e.g., with hyopkalemia). There are rich experimental data set on the effects extracellular K+ on modifying KV11.1 channel trafficking (as included in the cited reference Guo et al., 2009 and PMID: 20133899).

We thank the referee for this excellent suggestion and have extensively revised our model to include regulation of $K_v11.1$ trafficking by extracellular $[K^+]$, enabling simulations of the effects of hypokalaemia. The hypokalaemia equations (eq. 8 & 9) are presented in the methods at lines 227-241 and the parameters in **Table 5** as:

"*The ORd model is sensitive to changes in extracellular $[K^+]$, which, besides changing the driving force for all K^+ currents, also modulates the gating of I_{Kr} and the inward-rectifier K^+ current (I_{K1}). However, extracellular $[K^+]$ also modulates $K_v11.1$ trafficking (Guo et al., 2009; Massaeli et al., 2010), which is not part of the original ORd model. Here, we modelled the trafficking effects of hypokalaemia through changes in β and δ, because experimental studies have shown that hypokalaemia primarily affects $K_v11.1$ channel internalisation and degradation (Guo et al., 2009; Massaeli et al., 2010). In particular, the β rate was scaled by a factor κ_b (**Figure 1A**), as follows:*

$$\kappa_b = 1 + \frac{\alpha_k - 1}{1 + \left(\frac{[K^+]}{k_m}\right)^{h_k}} \quad \#(8)$$

where α_k is the magnitude of the extracellular $[K^+]$-induced internalisation, $[K^+]$ is the extracellular K^+ concentration, k_m is the affinity for extracellular $[K^+]$, and h_k is the Hill factor for $[K^+]$. Similarly, the effects of extracellular $[K^+]$ on δ were modelled as:

$$\kappa_d = 1 + \frac{s \cdot \alpha_k - 1}{1 + \left(\frac{[K^+]}{k_m}\right)^{h_k}} \quad \#(9)$$

*where s is a scalar determining the relative impact of extracellular $[K^+]$ on δ vs β. The final parameters related to extracellular $[K^+]$ can be found in **Table 5**."*

Table 5. *Calibrated parameters for the effects of extracellular [K^+] on trafficking for 'overnight' (i.e., 12 hours) and 'week' model configurations.*

Parameter	***'Overnight' value***	***'Week' value***
a_k	*7.249733*	*7.249733*
k_m	*0.278542*	*0.871920*
h_k	*2.895935*	*2.691968*
S	*0.226791*	*0.226791*

The results are presented in lines 379-403 and **Figure 8** as:

*"Extracellular [K^+] is a prominent regulator of ventricular electrophysiology, with both hyper- and hypokalaemia being associated with increased risk of cardiac arrhythmias. Previously, hypokalaemia has been shown to negatively regulate K_v11.1 channel gating and membrane stability in a concentration-dependent manner through increased internalisation and degradation (Guo et al., 2009; Massaeli et al., 2010). The model's extracellular [K^+] dependence was calibrated to experimental data from Guo et al. (2009), which revealed a distinct half-maximal concentration after overnight (i.e., 12 hours) incubation compared to incubation for a week (**Figure 8A, left vs. right panel**). The rate of decrease in K_v11.1 membrane levels in the presence of low (0.1 mmol/L) extracellular [K^+] and the rate of recovery of K_v11.1 membrane expression after switching back to 5.0 mmol/L extracellular [K^+] following overnight incubation at 0.1 mmol/L were also calibrated based on experimental data (**Figure 8B**). The corresponding 'overnight' and 'week' parameter sets can be found in **Table** 7. Subsequently, we performed similar simulations to those in **Figure 5D** to evaluate the combined acute and long-term (trafficking) effects of hypokalaemia. After 24 hours, the [K^+] was reduced from 5.4 mmol/L to 2.5 mmol/L, reflecting a clinically-relevant hypokalaemia. The 'overnight' parameter set resulted in an approximately 20% reduction in I_{Kr}, however, the amount of K_v11.1 membrane channels remained stable, reflecting the acute effects of hypokalaemia on channel gating over time (**Figure 8C**). For the 'week' parameter set, the reduction in I_{Kr} was much more pronounced (e.g., approximately 45%) due to an additional 25% reduction in K_v11.1 membrane channels (**Figure 8C**). This is also reflected in differences between APD prolongation immediately after extracellular [K^+] was reduced to 2.5 mmol/L (**25th hour; Figure 8D**) and towards the end of the hypokalaemic period (47th hour). With the 'overnight' parameters, APD remained mostly stable after the first hour of*

*hypokalaemia (**Figure 8D, left panel**). By contrast, the APD related to the 'week' parameters substantially increased during hypokalaemia (**Figure 8D, right panel**)."*

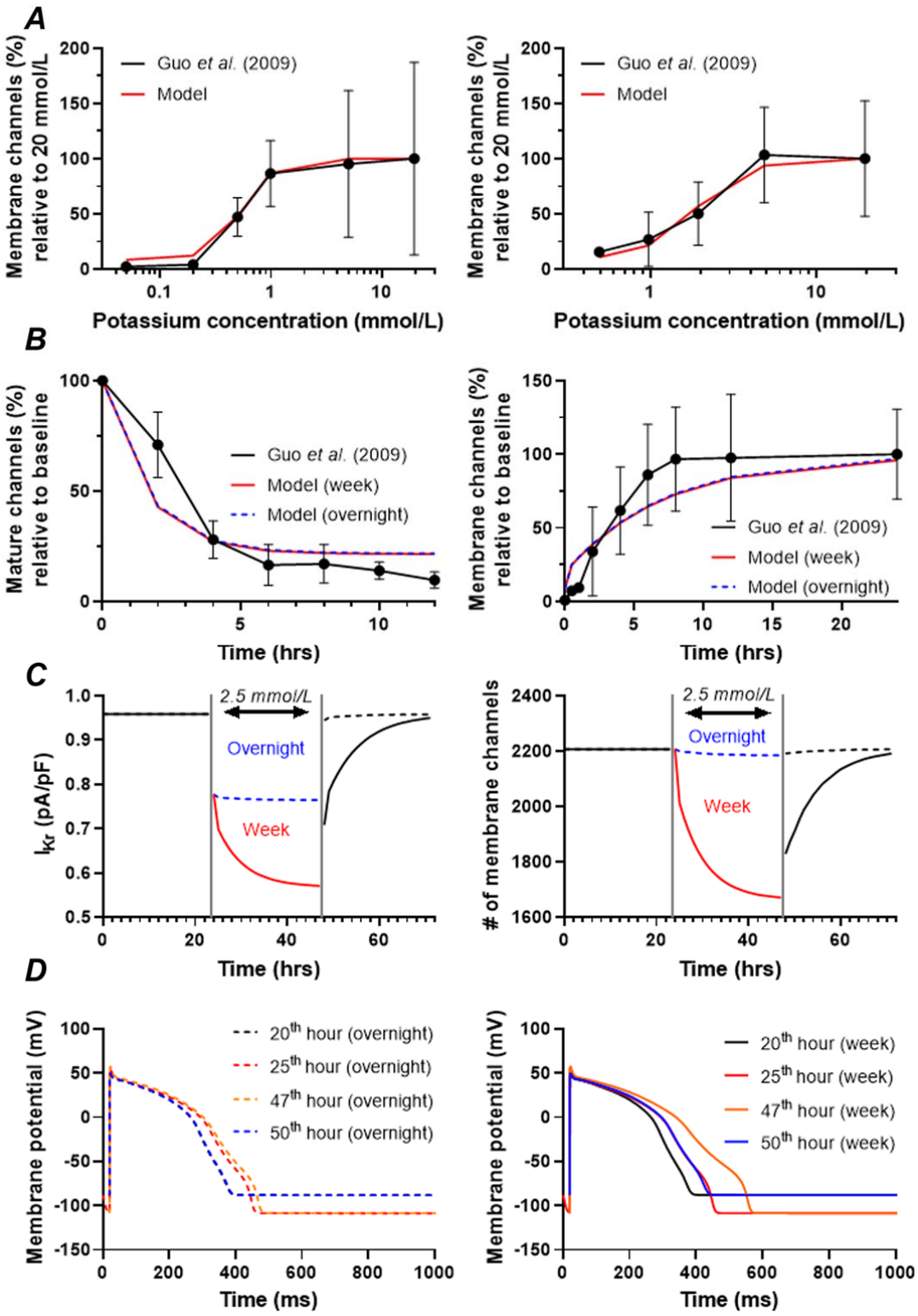


Figure 8. Modelling the effects of hypokalaemia. *A)* Concentration-dependence of extracellular [K^+] on Kv11.1 membrane levels based on overnight incubation (i.e., 12 hours; left) or incubation for one week (right) in experimental data (Guo *et al.*, 2009) (symbols) and

corresponding model versions (lines). Experimental data were based on I_{Kr} recordings in 5 mmol/L [K^+] after incubation at the indicated concentration for 12 hours or one week, which were used as a proxy for $K_v11.1$ membrane levels. *B)* Time course of reduction in $K_v11.1$ membrane levels in response to incubation in low (0 mmol/L in experiments, 0.1 mmol/L in model) extracellular [K^+] (left) or recovery after 12 hours at low extracellular [K^+] following re-exposure to 5 mmol/L extracellular [K^+] (right) in experimental data (Guo *et al.*, 2009) as well as 'overnight' and 'week' model configurations. *C)* Simulated time course of I_{Kr} (left) and $K_v11.1$ membrane levels (right) during 24 hours at baseline (i.e., 5.4 mmol/L [K^+]), followed by 24 hours with hypokalaemia (2.5 mmol/L), and 24 hours at baseline, revealing acute inhibition for both the 'overnight' and 'week' model configurations, and long-term decrease of $K_v11.1$ membrane levels for the 'week' parameters. The dashed vertical lines (grey) indicate the start and end of hypokalaemia. *D)* Action-potential morphology at various time points from the simulations in panel C for the 'overnight' parameters (dashed lines) and 'week' parameters (solid lines), showing the acute prolongation of repolarization duration for both parameter sets and subsequent additional APD prolongation for the 'week' parameters, which remains present after cessation of hypokalaemia due to the decrease in $K_v11.1$ membrane levels (compare blue vs. black curves in right panel).

Finally, we also discussed the simulation results and their relation to cardiac arrhythmogenesis in lines 566-576 as:

*"Hypokalaemia affects several key repolarizing K^+ channels and is a known risk factor for cardiac arrhythmogenesis (Pezhouman et al., 2015). Our simulations show an acute prolongation of APD and hyperpolarization of the resting membrane potential in response to hypokalaemia (**Figure 8**), in line with experimental data (Pezhouman et al., 2015). In addition, severe hypokalaemia may induce additional APD prolongation over time due to a decrease in $K_v11.1$ membrane levels. Whether this effect occurs at clinically relevant concentrations depends on the affinity of $K_v11.1$ trafficking for extracellular [K^+]. Guo et al. (2009) identified a half-maximal effect on $K_v11.1$ internalisation of 0.5 mmol/L for 12-hour incubation and 2.1 mmol/L for 1-week incubation. Thus, while short periods of clinically relevant hypokalaemia are unlikely to affect $K_v11,1$ membrane levels, longer periods may reduce $K_v11.1$ levels, potentially contributing to excessive APD prolongation."*

Is it significant that Kanner et al., also reported two other mutations (N33T and R56Q), which disrupt forward trafficking like A57P but also decrease surface stability, express similar

KV11.1 channel protein levels at the cell surface member. From a modeling perspective- what might this mean/suggest?

We deliberately chose a mutation with an isolated forward trafficking phenotype, because we wanted to investigate whether our model could identify the rate(s) responsible for this mutation. Our hypothesis was that the model would identify ψ and α as the primary parameters involved. However, after the optimization based on the mutant data, we found that this relatively straightforward phenotype could be reproduced with different parameter sets. Given that this 'simple' phenotype was already difficult to identify, the model will not be able to identify the relative contribution of individual rates for more complex phenotypes.

This important aspect has now been addressed in the revised discussion at lines 481-485: *"Despite the model's simplicity, our sensitivity analyses (**Figures 3-4**) revealed that similar phenotypic behaviour of LQTS2 mutations can be obtained through markedly different parameter combinations, even for a mutation (p.(A57P)) for which experimental data indicate that only forward trafficking is impaired. Mutations with more complex phenotypes (Kanner et al., 2018) are even less likely to provide a unique parameter set."*

Referee #2:

Summary Comments:

• The authors present a framework for incorporating ion-channel trafficking into computational models of cardiac electrophysiology, which currently do not incorporate them. This model uses Kv11.1 (hERG), the channel for which the most experimental data are available, as a proof-of-concept. This model incorporates temperature- and drug-dependent effects on channel kinetics and trafficking to enable a more sophisticated approach to modeling channel behavior. After optimization and parameter sensitivity analyses, the authors identified key differences between the acute and chronic electrophysiology of temperature changes and drugs that modify channel kinetics and trafficking. Moreover, this provides a potential framework for future work investigating the role of channel trafficking and its modulation to arrhythmogenesis.

• Overall, the paper flowed relatively well. There were a few points of confusion:

We would like to thank the referee for the positive assessment, useful comments and the proposed changes/additions. A point-by-point response to the referee's comments is provided below.

o In the Methods section, it was not immediately clear how certain parameter modifications were explicitly integrated into the model. Additional explanatory text and updates to Figure 1 would strengthen the paper.

We added the temperature- (i.e., θ), drug- (i.e., λ), and hypokalaemia (i.e., κ_b & κ_d) parameters to the model overview in **Figure 1A** and **Figure 1C**. The corresponding changes to the text are addressed in greater detail in response to the referee's 'specific suggestions' below.

o The "inverse" or "opposite" effects of temperature increases and decreases are referenced before the precise effects are described in detail near the end of the Results section. Clarifying the language in these locations would be helpful.

We carefully addressed these issues below in response to the referee's 'specific suggestions'. In brief, we updated these sentences by explicitly explaining the effects of lower temperature,

rather than referring to the opposite to higher temperatures.

• Overall, model predictions of experimental data were fairly robust, save for a few minor discrepancies. These may not significantly impact the results, but a discussion of their potential impact (and/or tweaking the model to verify this) would strengthen the paper. There were a few places where the figures could be modified to resolve ambiguities in presentation and clarify the methods. See specific comments below.

We modified the figures based on the referees suggestions as detailed in our responses below.

• Discussion and Limitations were robust and comprehensive. The model predictions with respect to temperature contradicted observed clinical impacts, but potential reasons for this discrepancy were discussed in detail. It may be beneficial to include a brief discussion on fever as a trigger for arrhythmia in those with a vulnerable substrate (e.g., Brugada Syndrome).

We agree that this would provide relevant context and have incorporated this information in the revised discussion at lines 538-540: *"It is known that fever can be an important trigger of arrhythmias when a vulnerable substrate is present, e.g., in Brugada Syndrome (Adler et al., 2013; Roterberg et al., 2020) or for certain LQT2 mutations (Amin et al., 2008)."*

• This is an important contribution to the modeling literature and suggests several future directions for further research.

We thank the referee for their positive assessment.

Specific Suggestions:

• 85-87

o Please clarify this sentence: "Dofetilide substantially increased Kv11.1 membrane levels at supraphysiological concentrations that produce significant channel block, but not at clinically-relevant concentrations." Does "supraphysiologic concentrations" refer to dofetilide or Kv11.1? Does this mean that subclinical levels of dofetilide increased Kv11.1 membrane levels AND produced significant channel block?

We tried to clarify this in lines 72-74, which now read: *"Supraphysiological dofetilide concentrations substantially increased $K_v11.1$ membrane levels while also producing significant channel block, while clinically-relevant concentrations did not affect trafficking".*

• 89-98

o "The opposite was true" is unclear. Does this mean that lower temperature slowed kinetics, decreased IKr acutely, had increased trafficking after 24 hours, and increased IKr chronically?

Although the referee's interpretation of this sentence is correct, we agree that the phrasing is somewhat complex. Unfortunately, given the word limit of the abstract and the new extensive data obtained during the revision that had to be incorporated, we were unable to expand the explanation. In the main text we have now more extensively explained what this 'opposite' effect is (see below).

• 129

o "The opposite is true for hypothermia."

o Same comment as above. I think this would benefit from additional clarification.

At lines 117-119, we re-formulated the sentence: *"The opposite is true for hypothermia, which acutely decreased I_{Kr} due to slower $K_v11.1$ channel gating, but on the long term increased I_{Kr} due to increased $K_v11.1$ trafficking."*

• 169

o The notation is confusing. What do "M#(1)" and "S#(2)" denote? Is this a typo?
o On second look, this appears to be a formatting error for equation numbering that is carried through the document?

We thank the referee for this comment and apologise for these errors. This was a formatting error for the equation numbering caused by the pdf-converter of the journal. We were unfortunately unable to avoid this issue when re-uploading the revised submission, but will

make sure that this is implemented correctly during the proof-phase when the manuscript has been accepted.

• 209 (Equation 5)

o Additional clarity on how the lambda parameter is explicitly embedded within the model would be helpful.

We have updated Figure 1 and added equations 10-12 on lines 244-255 to the revised manuscript to explain how the modulators (i.e., temperature and drugs), as well as the new modifier extracellular [K^+], which was added in response to comments from Referee 1, affect the trafficking rates:

"The trafficking effects of temperature, drugs, and extracellular [K^+] were introduced as scaling factors to the appropriate rates in the trafficking model:

$$\psi = \psi_{base} \cdot \lambda \cdot \theta \#(10)$$

*where ψ_{base} is the baseline production rate as shown in **Table 1**, λ represents the opposing effects of pentamidine and dofetilide, and θ represents the temperature-dependent regulation of $K_v11.1$ channel trafficking. In addition,*

$$\beta = \beta_{base} \cdot \left(\frac{\kappa_b}{\kappa_{bref}}\right) \#(11)$$

*where β_{base} is the baseline internalisation rate (**Table 1**), κ_b is the effect of extracellular [K^+] on β, and κ_{bref} is the reference value of κ_b at 5.4 mmol/L extracellular [K^+]. Similarly,*

$$\delta = \delta_{base} \cdot \left(\frac{\kappa_d}{\kappa_{dref}}\right) \#(12)$$

*where δ_{base} is the baseline degradation rate (**Table 1**), κ_d is the effect of extracellular [K^+] on δ, and κ_{dref} is the reference value of κ_d at 5.4 mmol/L extracellular [K^+]."*

• 254-255

o Figure 2B: At 3 minutes, model appears to imply substantially less recycling than Dennis et

al. (2011)? This is potentially important for acute phenomena. While authors acknowledge this, an explanation of potential impacts of this discrepancy (either here, or in the Discussion/Limitations section) would strengthen the paper. Overall, the model predictions were in line with experimental data.

We agree with the referee that this is one component that has been challenging to reproduce. We have acknowledged this limitation in the revised discussion on lines 494-501: *"In particular, the experimental data from Dennis et al. (2011) in* ***Figure 2B*** *showed approximately 60% K_v11.1 recycling within 3 minutes after 30 minutes of experimental channel internalisation. Thereafter, the amount of channel recycling remains stable. Our model shows a more sigmoidal increase in channel recycling, with the model recycling rate falling within the experimental standard deviation after 10 minutes. This difference might lead to an underestimate of the short-term (occurring within 5 minutes) effects of modulators of channel recycling and should be taken into consideration when interpreting our findings."*

• 334-341

o These sentences help clarify the confusion in lines 89-98 and 129 regarding the effects of temperature on kinetics, channel trafficking, and acute vs. chronic IKr current. The earlier sections would benefit from this level of clarity, since they precede this section.

We hope that our changes in response to the previous comments have helped to clarify these aspects.

• 406-407

o This section would benefit from an explanation of how the rapid kinetics in Kanner et al. (2018) (minutes vs. hours in other literature) could potentially impact the conclusions of this paper, in addition to clinical implications. It is clear from the methods section (288-292) that this was compensated for by taking relative differences in dynamics between the wild-type and mutant Kv11.1, but what impact would the timescale of kinetics have clinically?

While the faster time course identified by Kanner *et al.* (2018) is noteworthy and we are keen to see whether additional experimental studies with novel methodologies will support these findings, we believe that the general conclusions of our manuscript about the complex

combined effects of acute (gating) and more long-term (trafficking) regulation of electrophysiology would remain intact. However, the definition of what constitutes 'long-term' would likely change in this case. We implemented this in the discussion at lines 473-477: *"Moreover, the distinct acute and long-term effects identified in the present study would likely still apply even if future experiments would show faster time courses, just for different time points (e.g., 1 vs. 12 hours could be 10 vs. 60 minutes). The 'long-term' effects of trafficking modulators would then be observable after a couple of hours instead of 24-48 hours."*

• Figure 1:

o The paper would benefit from a modification to Figure 1A/1B (or an additional subfigure) to make explicit how temperature and drug modifications of model parameters affect the model. While there is text in the figure description that discusses this, a visual representation would make the methods easier to follow.

We have added θ, λ, κ_b, and κ_d (temperature, drugs and extracellular potassium, respectively) to the ψ, β and δ rates in **Figure 1A** and **Figure 1C** and we added the δ rate as degradation rate in **Figure 1C,** which was missing in the original version. The revised figure is shown below. In the figure legend, we have provided additional text explaining these scalars: *"The temperature (θ), drugs (λ), and extracellular [K^+] (κ_b and κ_d) parameters are used to scale the ψ, β, and δ rates."*

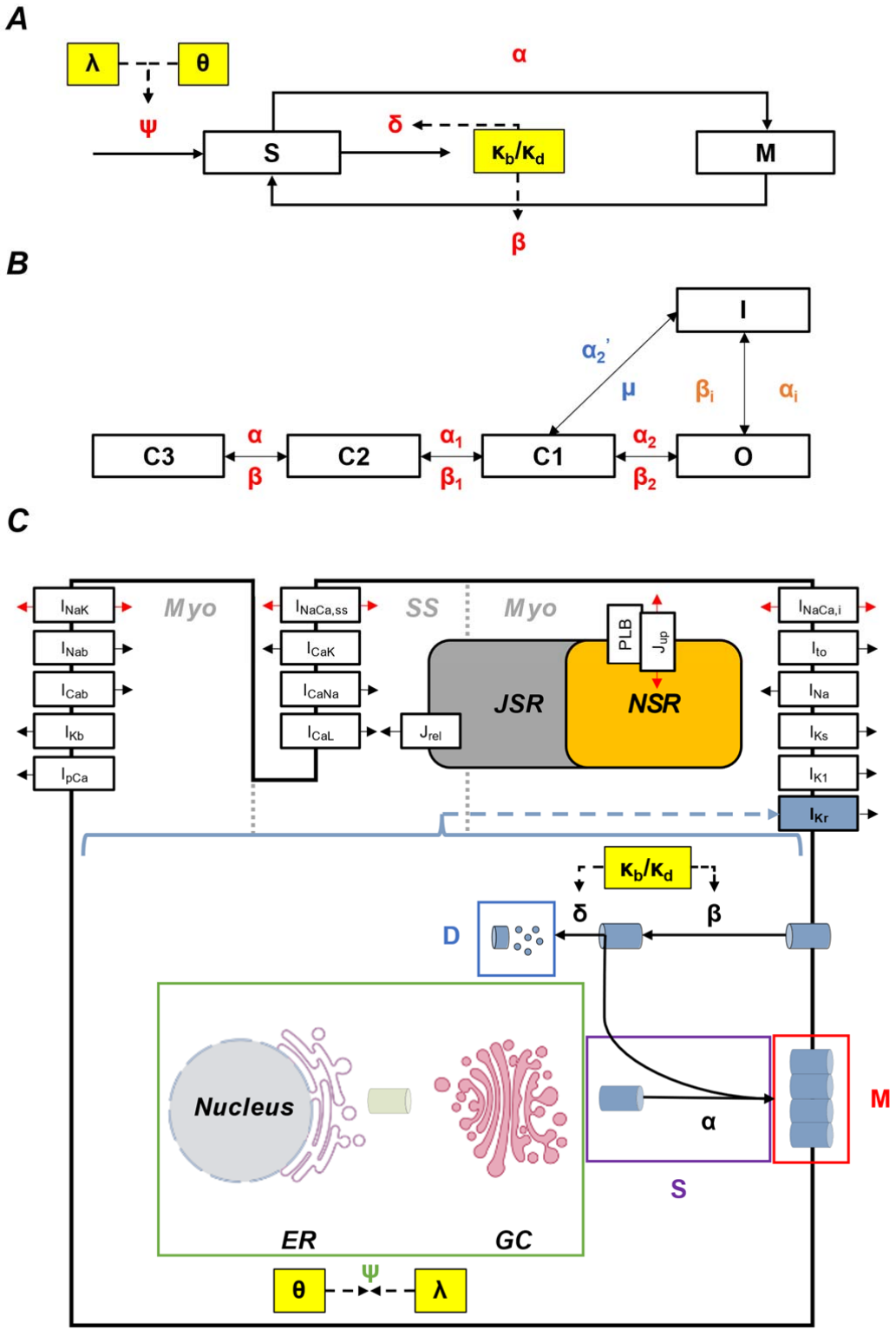


Figure 1. Model components required to simulate regulation of $K_v11.1$ trafficking and gating, and its effects on ventricular cardiomyocyte electrophysiology. *A,* The $K_v11.1$ trafficking model consist of two-states (M: Membrane, S: Sub-membrane) with four rates (ψ:

production rate, α: forward trafficking rate, β: internalisation rate, and δ: degradation rate). The temperature (θ), drugs (λ), and extracellular $[K^+]$ (κ_b and κ_d) parameters are used to scale the ψ, β, and δ rates. *B,* The Clancy and Rudy (2001) I_{Kr} Markov model was used to create a temperature-sensitive model of I_{Kr} gating by shifting the voltage dependence of all the rates and scaling each rate with their respective Q_{10} values (Clancy & Rudy, 2001). In particular, α_n, β_n, α_2', and μ rates were scaled with $Q_{10Activation}$ and $Q_{10Deactivation}$, while α_i and β_i were scaled with $Q_{10Inactivation}$ and $Q_{10Recovery}$, respectively. *C,* The trafficking, temperature, drug, and extracellular $[K]^+$ components controlling I_{Kr} were embedded in the O'Hara-Rudy (ORd) human ventricular action potential model (O'Hara et al., 2011). Adapted from O'Hara et al. (2011) and the nucleus, endoplasmic reticulum (ER), and Golgi complex (GC) were created with BioRender.com.

• Figure 2:

o Overall, the model fit experimental data well. One exception is the acute phase of recycling in Figure 2B, in which model underpredicts Denis et al. (2011) at 3 minutes. See discussion of lines 254-255.

We addressed this comment above.

• Figure 5:

o Overall, the model fit experimental data well. There is a minor underprediction of mature channel trafficking at low concentrations of dofetilide (Figure 5B). A small description of possible impacts in the Discussion could be beneficial.

The model has been updated to incorporate regulation by extracellular $[K^+]$ in response to comments from Referee 1. The parameter optimization in the presence of these additional data resulted in a better fit for several components, although a slight underestimation at the lower dofetilide concentrations (0.01 – 0.03 μmol/L) remains (**Figure 5B**). Given that the experimental studies primarily used higher dofetilide concentrations (e.g., in Figure 5C), these concentrations received a larger weight during optimization. This aspect has been briefly mentioned in the revised discussion in lines 501-505:

"*The model also slightly underestimated the effect of dofetilide on Kv11.1 trafficking at low doses (**Figure 5B**), since we emphasised the 1 µmol/L concentration, which is what is primarily used experimentally. As such, the effect of clinically relevant concentrations may have been slightly underestimated.*"

o In Figure 5D, the upper and lower graphs are quite close together. In combination with the color change in the middle section (drug application), this makes the graphs somewhat confusing, on first glance. Separating the upper and lower graphs (there appears to be sufficient space above Figure 5E) would make this clearer. Alternatively/additionally, a break in the dashed lines would help the reader to distinguish the graphs from one another.

We added a break in the dashed lines and increased the space between the upper and lower graph.

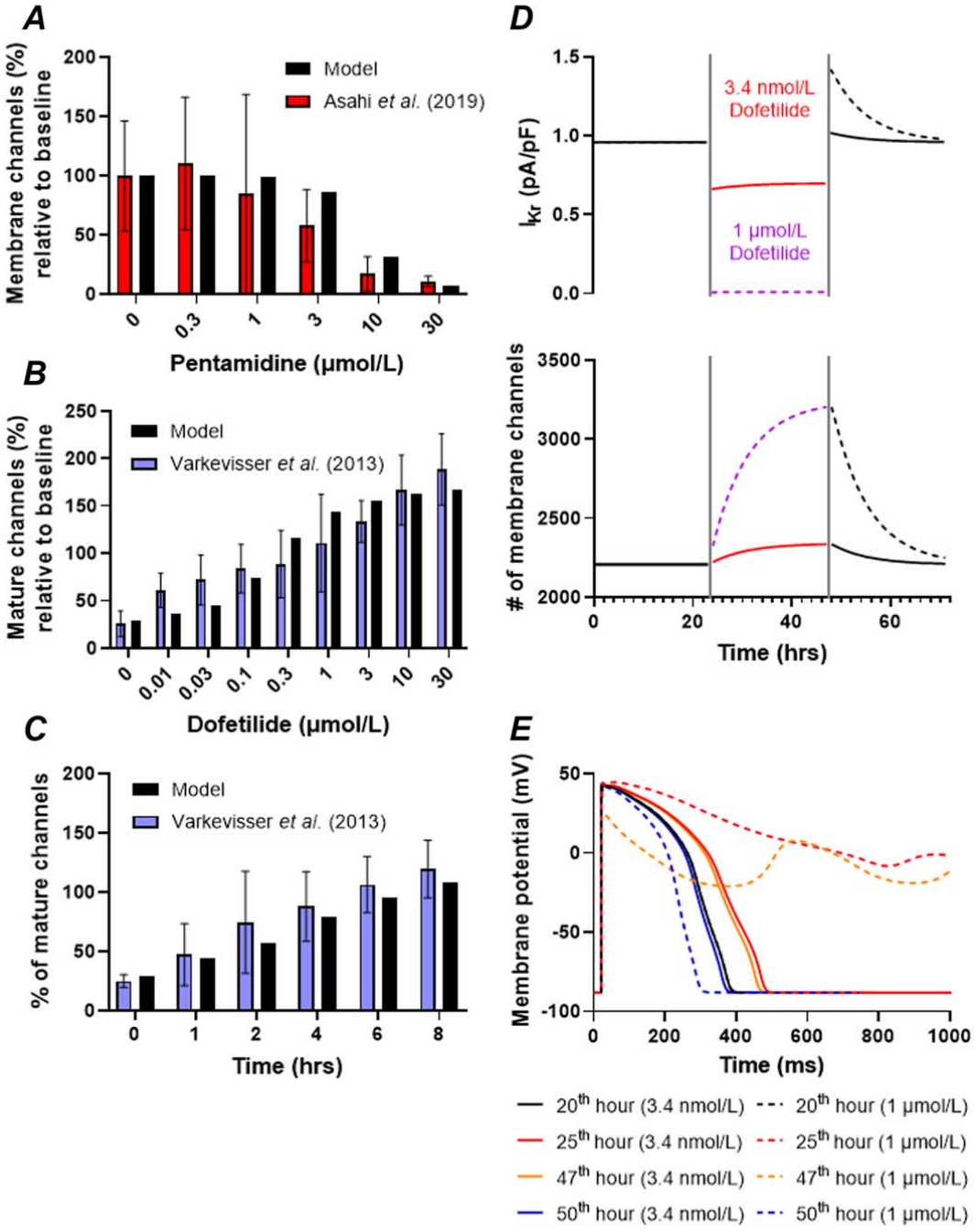


Figure 5. Modelling the effects of pentamidine and dofetilide on K_v11.1 gating and trafficking. *A*, The pentamidine concentration dependence of K_v11.1 membrane levels after 24 hours incubation in experimental data from Asahi *et al.* (2019) (red bars) and model (black bars). *B*, The concentration dependence of dofetilide-induced rescue of mature K_v11.1 (155 kDa) levels in the presence of pentamidine (10 μM for 48 hours) after 48 hours of incubation in experimental data (Varkevisser *et al.*, 2013) and model. *C*, The temporal dynamics of

dofetilide (1 μM)-induced rescue of mature $K_v11.1$ levels after 48-hours pretreatment with pentamidine in experimental data (Varkevisser *et al.*, 2013) and model. *D*, Time course of I_{Kr} (top) and $K_v11.1$ membrane levels (bottom) during 24 hours at baseline, followed by 24 hours with simulated dofetilide application (3.4 nmol/L or 1 μmol/L) and 24 hours at baseline, revealing acute inhibition and long-term rescue of $K_v11.1$ membrane levels. The dashed vertical lines (grey) indicate the start and end time of dofetilide application. Note, these simulations were performed without pentamidine. *E*, Action-potential morphology at various points in the time course shown in panel D for the simulations with 3.4 nmol/L dofetilide (solid lines) and 1 μmol/L dofetilide (dashed lines), showing the acute prolongation of repolarization duration and shortening of repolarization after cessation of simulated dofetilide application due to the increase in $K_v11.1$ membrane levels. Note, these simulations were performed without pentamidine.

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Dear Dr Heijman,

Re: JP-RP-2022-283976R1 "In-silico analysis of the dynamic regulation of cardiac electrophysiology by Kv11.1 ion-channel trafficking" by Stefan Meier, Adaïa Grundland, Dobromir Dobrev, Paul GA Volders, and Jordi Heijman

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Natalia Trayanova
Senior Editor
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EDITOR COMMENTS

Reviewing Editor:

Reviewer 1 deems the study highly influential, but suggests that the concept that wild type KV11.1 channel trafficking is increased with drugs the block IKR is not well supported experimentally and these data should be removed. The authors are invited to review the references provided in Reviewer 1's comments.

REFEREE COMMENTS

Referee #1:

The authors have been very responsive to my concerns. This manuscript will advance the computational modeling of cardiac electrophysiology. However, major concerns regarding the simplifying assumptions used for the modeling in Figure 5D-5E remain. The concept that drugs which rescue the trafficking of mutations also increases the trafficking of wild type KV11.1 channels is not well supported and whether dofetilide increases the trafficking of wild type KV11.1 channels has not been demonstrated. These data should be removed from the manuscript. The reason is the majority of experimental evidence does not support the concept that drugs which block IKR can also increase the trafficking and functional expression of wild-type KV11.1 channels.

Major concern:

The authors justify modeling the increase of wild type KV11.1 channel protein in Figure 5D-5E based on the results in Al-Moubarak et al., 2020 studying cells expressing wild type KV11.1 channels in E-4031 (not dofetilide). The simplifying assumption is the effect that dofetilide will have on trafficking of wild type KV11.1 channels is similar. This needs to be demonstrated at the functional level.

The data the authors cite to support the concept that wild type KV11.1 channel trafficking is increased with drugs the block IKR is indirect (using an on cell protein labeling method). It does not show incubating cells in E-4031 increases the functional expression of wild type KV11.1 channels measuring currents. The concept, of how different drugs that affect block of IKR and/or impact the trafficking of wild type and mutant Kv11.1 channels, has been extensively explored in PMID 15950494. This later study does not support the idea that drugs that rescue mutant channels also increase the trafficking of wild type KV11.1 channels. Moreover, studies cited by the authors also show drugs that increase the trafficking of mutant channels do not increase the functional expression of wild type KV11.1 channels (including E-4031). This includes Amin et al., 2008. These are not isolated findings, please see: PMID 11741928 and PMID 16432067.

Referee #2:

All of the comments from the previous review were thoroughly addressed.

END OF COMMENTS

1st Confidential Review **05-Dec-2022**

Responses to Referees on the paper:

'In-silico analysis of the dynamic regulation of cardiac electrophysiology by Kv11.1 ion-channel trafficking' by Stefan Meier, Adaïa Grundland, Dobromir Dobrev, Paul G.A. Volders, and Jordi Heijman.

Referee #1:

The authors have been very responsive to my concerns. This manuscript will advance the computational modeling of cardiac electrophysiology. However, major concerns regarding the simplifying assumptions used for the modeling in Figure 5D-5E remain. The concept that drugs which rescue the trafficking of mutations also increases the trafficking of wild type KV11.1 channels is not well supported and whether dofetilide increases the trafficking of wild type KV11.1 channels has not been demonstrated. These data should be removed from the manuscript. The reason is the majority of experimental evidence does not support the concept that drugs which block IKR can also increase the trafficking and functional expression of wild-type KV11.1 channels.

We thank the referee for their positive assessment and are grateful for the additional explanation and suggestions. A detailed response is provided below.

The authors justify modeling the increase of wild type KV11.1 channel protein in Figure 5D-5E based on the results in Al-Moubarak et al., 2020 studying cells expressing wild type KV11.1 channels in E-4031 (not dofetilide). The simplifying assumption is the effect that dofetilide will have on trafficking of wild type KV11.1 channels is similar. This needs to be demonstrated at the functional level.

The data the authors cite to support the concept that wild type KV11.1 channel trafficking is increased with drugs the block IKR is indirect (using an on cell protein labeling method). It does not show incubating cells in E-4031 increases the functional expression of wild type KV11.1 channels measuring currents. The concept, of how different drugs that affect block of IKR and/or impact the trafficking of wild type and mutant Kv11.1 channels, has been extensively explored in PMID 15950494. This later study does not support the idea that drugs that rescue mutant channels also increase the trafficking of wild type KV11.1 channels. Moreover, studies cited by the authors also show drugs that increase the trafficking of mutant channels do not increase the functional expression of wild type KV11.1 channels (including E-4031). This includes Amin et al., 2008. These are not isolated findings, please see: PMID 11741928 and PMID 16432067.

Based on the referee's suggestion, we adapted the drug simulations to only rescue $K_v11.1$ channels when both pentamidine and dofetilide are present (as demonstrated in Varkevisser et al. 2013), but not in the presence of dofetilide alone. In particular, we have modified the amplitude (a) term of our 'drug-effect' equation (eq. 5) to a', defined as:

$$a' = \frac{a}{\left(1 + exp\left(-([P] - b)\right)\right)} \quad (7)$$

with a the magnitude of the dofetilide-induced promotion of trafficking in the presence of pentamidine, [P] is the pentamidine concentration in µmol/L, b is the midpoint of the pentamidine dependence."

We have added the additional parameter (b) to **table 4**:

Table 4. *Calibrated parameters for the drug effects on trafficking.*

Parameter	**Value**
H	2.196566
$\boldsymbol{h_D}$	0.525717
A	0.730636
$\boldsymbol{km_D}$	0.329207
km'	6.628938
R	0.126642
b	6

This formulation ensures that dofetilide only has a rescuing effect when pentamidine is present. We subsequently separated the calibration and the modelling of the pentamidine/dofetilide effects shown in **Figure 5** of the original manuscript into **Figure 5** (calibration) and **Figure 6** (modelling/simulation). Most importantly, we now show two simulations for each concentration of dofetilide in **Figure 6**: in the presence of 5 µmol/L pentamidine (showing the rescuing effect) and without pentamidine (showing a negligible effect of dofetilide on $K_v11.1$ trafficking). The revised figures are shown below. We have adapted the results section accordingly (Lines 344-366):

"*After parameter optimization, the model could accurately reproduce the concentration-dependent effect of pentamidine on $K_v11.1$ membrane levels (**Figure 5A**), as well as the concentration and time-dependent rescue of mature $K_v11.1$ levels (155 kDa) by dofetilide in the presence of 10 µmol/L pentamidine (**Figures 5B-C**). We subsequently employed the model to investigate the combined effect of acute channel inhibition and long-term trafficking promotion by dofetilide in the presence of 5 µmol/L pentamidine (**Figure 6**, dashed lines) and absence of pentamidine (**Figure 6, solid lines**). A supraphysiological (1 µmol/L) concentration of dofetilide acutely completely inhibited I_{Kr}, while promoting an approximately 50% increase in membrane channels over 24 hours in the presence of pentamidine (**Figure 6A, dashed lines**). By contrast, in the absence of pentamidine, dofetilide had a negligible effect on $K_v11.1$ membrane trafficking (**Figure 6A, solid lines**). The acute inhibition resulted in repolarization failure during dofetilide treatment (25^{th} hour; **Figure 6A, bottom panel**). However, the rescue of $K_v11.1$ membrane channels after 24 hours of dofetilide caused a slight AP shortening shortly*

after dofetilide application was stopped, counteracting the effects of pentamidine (50th hour; ***Figure 6A, bottom panel****). By contrast, a more clinically relevant dofetilide concentration (3.4 nmol/L) produced a modest acute I_{Kr} inhibition that prolonged AP duration (APD), while having a minimal rescuing-effect on membrane channel numbers after 24 hours independent of the presence of pentamidine (****Figure 6B****). As such, there was only a minimal rebound in APD after cessation of simulated dofetilide application. Thus, while dofetilide can rescue $K_v11.1$ trafficking under pathological conditions (e.g., trafficking blocker pentamidine), this effect appears negligible at clinically relevant concentrations. However, other drugs and trafficking-modulators such as temperature may alter $K_v11.1$ membrane levels over a physiological range."*

In addition, we added the following to the Discussion on Lines 531-543:

"Previous experimental work has shown that high concentrations of dofetilide can rescue $K_v11.1$-trafficking deficiencies induced by pentamidine (Varkevisser et al., 2013). Similarly, our model only rescues channels when pentamidine is present, however, our results also suggest that the impact of dofetilide on trafficking is likely limited for clinically relevant concentrations, even in the presence of pentamidine. In general, drugs that rescue ion-channel trafficking primarily seem to have an effect during aberrant conditions (e.g., trafficking deficient mutations, trafficking blockers), but may not be able to increase $K_v11.1$-trafficking under physiological conditions (Wible et al., 2005; Anderson et al., 2006; Varkevisser et al., 2013). However, a recent study has shown an approximately 60% increase in WT $K_v11.1$ levels after 24 hours of incubation with 5 µmol/L E-4031 (Al-Moubarak et al., 2020) in the absence of pentamidine or other compounds impairing $K_v11.1$ trafficking. More research on the effects of trafficking rescuers under physiological conditions is therefore warranted. Moreover, distinct short- and long-term effects of dofetilide…

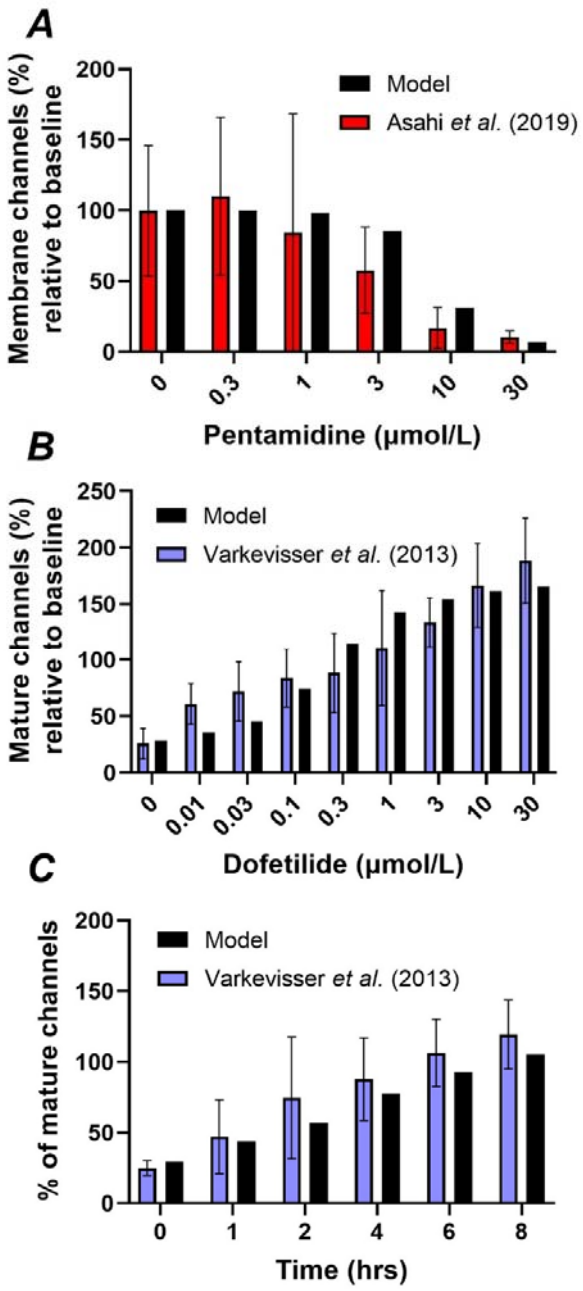

Figure 5. Calibration of the effects of pentamidine and dofetilide on K_v11.1 trafficking. *A, The pentamidine concentration dependence of K_v11.1 membrane levels after 24 hours incubation in experimental data from Asahi et al. (2019) (red bars) and model (black bars). B, The concentration dependence of dofetilide-induced rescue of mature K_v11.1 (155 kDa) levels in the presence of pentamidine (10 µmol/L for 48 hours) after 48 hours of incubation in experimental data (Varkevisser et al., 2013) and model. C, The temporal dynamics of dofetilide (1 µmol/L)-induced rescue of mature K_v11.1 levels after 48-hours pre-treatment with 10 µmol/L pentamidine in experimental data (Varkevisser et al., 2013) and model.*

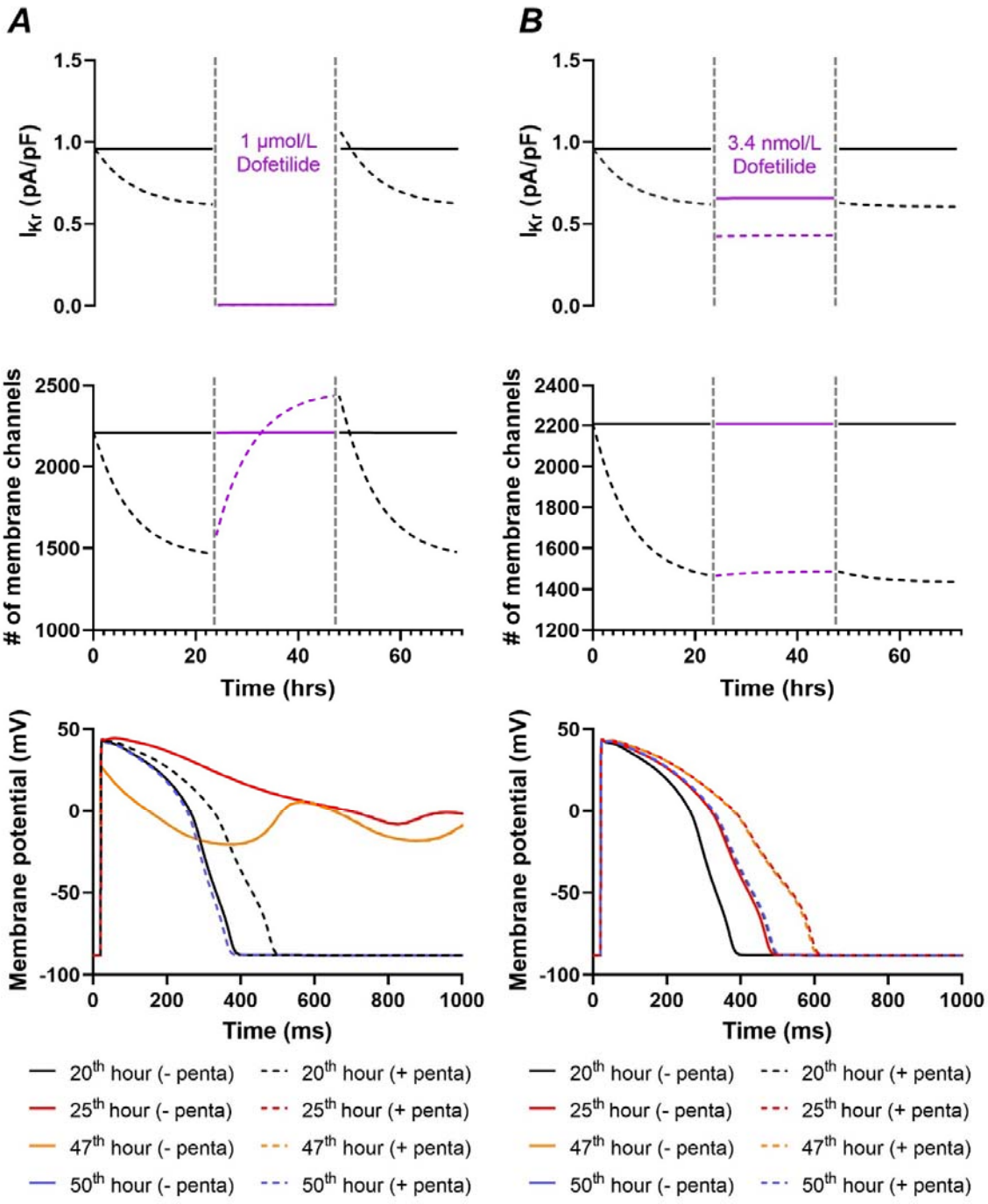

Figure 6. Simulated effects of pentamidine and dofetilide on K_v11.1 gating and trafficking. *A, Time course of I_{Kr} (top panel) and K_v11.1 membrane levels (middle panel) during 24 hours at baseline (solid lines) or with 5 µmol/L pentamidine (dashed lines), followed by 24 hours with simulated dofetilide application (1 µmol/L) and 24 hours at baseline, revealing acute inhibition and long-term rescue of K_v11.1 membrane levels only during the presence of pentamidine. The dashed vertical lines (grey) indicate the start and end time of dofetilide application. The bottom panel shows the action-potential morphology at various points in the time course shown in the other panels for the simulations with 1 µmol/L dofetilide with pentamidine (solid lines) and*

without pentamidine (dashed lines), showing acute prolongation of repolarization duration for both conditions, but only a slight shortening of repolarization after cessation of simulated dofetilide application due to the complete rescue and minor increase in $K_v11.1$ membrane levels. B, Similar to panel A, for 3.4 nmol/L dofetilide. The bottom panel shows action potential prolongation when pentamidine is present due to a reduction of the number of membrane channels. The subsequent simulated dofetilide administration (3.4 nmol/L) had minimal effects on $K_v11.1$ trafficking."

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Dear Dr Heijman,

Re: JP-RP-2023-283976R2 "In-silico analysis of the dynamic regulation of cardiac electrophysiology by Kv11.1 ion-channel trafficking" by Stefan Meier, Adaïa Grundland, Dobromir Dobrev, Paul GA Volders, and Jordi Heijman

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EDITOR COMMENTS

Reviewing Editor:

Thank you for addressing the reviewer's critiques. Congratulations!

REFEREE COMMENTS

Referee #1:

The authors have satisfactorily addressed all of my concerns.

2nd Confidential Review **26-Jan-2023**