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Activation of small conductance Ca2+-activated K+ channels suppresses Ca2+ transient and action potential alternans in ventricular myocytes

Giedrius Kanaporis and Lothar A. Blatter
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The referees have opted to remain anonymous.

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1st Editorial Decision **04-Oct-2022**

Dear Dr Kanaporis,

Re: JP-RP-2022-283870 "Activation of small conductance Ca2+-activated K+ channels suppresses Ca2+ transient alternans in ventricular myocytes" by Giedrius Kanaporis and Lothar A. Blatter

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert Referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

Please advise your co-authors of this decision as soon as possible.

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Yours sincerely,

Bjorn Knollmann
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EDITOR COMMENTS

Reviewing Editor:

We thank the authors for submitting your manuscript for consideration of publication to the Journal of Physiology. Your submission has been reviewed by two experts, and it was viewed as being quite influential. Each reviewer highlighted several positive aspects of your work. However, they also identified several opportunities to strengthen the hypothesis and increase the impact of the work. This includes performing additional studies. We ask that the authors address each one of these issues by providing a point-by-point response to all of Reviewer 1 and Reviewer 2's comments/critiques and modify the manuscript and its presentation accordingly. This includes addressing Reviewer 1's suggestion to provide new action potential data, and Reviewer 2's suggestion to expand the study to include pharmacological experiments studies that mimic Long QT Syndrome Type 2.

Senior Editor:

I concur with the reviewing editor's favorable assessment. Please address all concerns raised in the review. Please note that a responsive revisions will have to include additional data as requested by the reviewers and the reviewing editor. Please also include the appropriate animal approvals in the revised MS.

REFEREE COMMENTS

Referee #1:

Cardiac alternans has been identified as a significant risk factor for arrhythmias. Cardiac alternans originate from electrical alternans or calcium alternans. This study examined whether SK channel activation can suppress cardiac alternans. Dynamics of calcium alternans and action potentials were monitored in the absence or presence of SK channel enhancer NS309 or blocker apamin using single cell confocal calcium imaging and action potentials were recorded using microelectrode recordings. SK activation by NS309 resulted in significant shortening of APD and reduced pacing-induced calcium alternans, which was reversed by SK blocker, apamin. SR calcium content was significantly reduced by enhancing NS309, which underlies reduction in calcium alternans. The study shows that SK channel enhancement can reduce proarrhythmic calcium alternans through APD shortening that reduces SR calcium content.

Major comments:

This manuscript reports that calcium alternans can be suppressed by SK channel enhancers through modulating APD and SR Ca content. Suppression of calcium alternans by SK channel is an important discovery and the high quality data from calcium and action potential recording supports this. The experimental design such as changes in calcium transients from control to SK enhancer and washout or SK blocker from the same cells are very impressive and strongly back up the important roles of SK channels in modulating APD. However, the mechanisms solely attributing to SR Ca content may not be accurate. Here are my concerns.

• This paper suggests that reduced Ca content through shortening of APD is the major mechanism underlying reduction in alternans by SK enhancement. Previous theoretical work and experimental data suggest that Ca alternans originate from several important factors including RyR Ca release (gain function or recruitment), RyR refractoriness from previous beat, etc. The proposed SR Ca content through APD may not fully explain the genesis of Ca alternans. Simulation studies indicate that Ca alternans disappear in both low or high SR Ca load. It is also possible that the prolonged APD causes RyR refractoriness so rather than APD and SR Ca content but diastolic interval might be important in genesis of alternans. The current data do not fully explain how calcium alternans can be suppressed by SK enhancers.

• This paper links APD shortening as a major cause behind reduced SR Ca load. Alternatively, SK channel works as if transient outward K current to bring the plateau Vm lower, which causes larger peak L-type Ca current to trigger larger Ca release, which eventually deplete the SR Ca load. I think the discussion should clarify what mechanisms the data presented in this paper support and whether there are limitations in the interpretation of the current data.

• Although the discussion section mentions electrical alternans, the paper mainly focuses on calcium alternans. It is more

interesting how SK enhancement can influence electrical alternans because of SK channel's main feature - negative feedback from calcium to voltage. Since Ca alternans in this study disappeared in the presence of NS309, electrical alternans may disappear as well regardless of negative feedback. I think adding new data of APD alternans before and after NS309 can strengthen this paper.

Minor comments:

• Please explain why only male rabbits were used and briefly discuss how sex may affect the results. Chen's group reported that SK channel expression is much greater in female rabbits (ref: J Physiol. 2018, PMCID: PMC6138290).

• I am struggling to understand the cause of APD shortening at a rapid rate under NS309. If NS309 reduces SR Ca content to have smaller CaT amplitudes, SK amplitude will be also smaller to affect minimal contribution to APD, right? How can SK channels be activated under small SR Ca content?

• Why has this study used HMR1556 to prolong APD through blocking IKs and study Ca alternans? Most drug-induced QT prolongation is through IKr block and IKr blocker such as E4031 can be more clinically relevant.

• I think Figure 7 can be put in Fig 1 to show the impact of NS309 on CaT under normal pacing and then rapid pacing for alternans.

• Fig 3 includes APD at control rate and Ca alternans amplitudes in the presence of SK channel blocker at fast pacing rate. Comparing two at the same CL would be ideal.

• PKA activation can enhance SK channel under sympathetic stimulation and its roles can have greater impact on alternans but this study only focuses on the normal condition when SK channel is least effective in modulating cardiac repolarization. The discussion may add a limitation section to include sympathetic or sex differences.

Referee #2:

In this article "Activation of small conductance Ca2+-activated K+ channels suppresses Ca2+ transient alternans in ventricular myocytes", Giedrius Kanaporis and Lothar Blatter tested whether pharmacological modulation of SK channels affects the development of cardiac alternans in isolated ventricular rabbit cardiomyocyes. They demonstrated no effects of SK channel blockers on APD, but a pronounced AP shortening by SK channel activators. These also abolished or reduced the degree of pacing induced alternation in Ca2+ release by lowering sarcoplasmic reticulum Ca2+ content and Ca2+ release.

This article is well written and addresses an important issue, as calcium and AP alternans is a well-known pro-arrhythmic mechanism. And novel approaches tackling the underlying mechanisms may provide novel anti-arrhythmic tools.

There are a couple of points that need to be addressed:

Major comments:

1) In their key messages and at the end of the abstract, the authors state "The data suggest SK activation as a potential intervention to avert development of alternans with important ramifications for arrhythmia prevention and therapy for patients with LQT syndrome." - without indicating that they have performed some experiments with (drug-induced) LQTS cardiomyocytes. The latter result should be included into the abstract; otherwise, the conclusion is purely speculative.

Also, it would be important to expand these experiments a bit more, as the mechanisms of alternans may differ in healthy vs. LQT cardiomyocytes. It has for example been demonstrated that particularly in LQT2 calcium alternans drives AP alternans. Hence adding drug-induced LQT2 experiments in addition to the experiments with HMR (drug-induced LQT1) (Figure 6) would be very interesting.

2) Methods:

"Ventricular myocytes were isolated from male New Zealand White rabbits"

Why were only male rabbits investigated? According to the ARRIVE guidelines, ideally animals of both sexes should be used; and if this is not possible for a given scientific question, the scientific rationale that is in favour of only using one sex should be clearly stated.

3) Methods:

"Rabbits were anaesthetized with an intravenous injection of sodium pentobarbital (100 mg/kg)"

This is the dosage that is usually given for euthanasia rather than anesthesia. Were the rabbits anesthetized or sedated prior to the lethal application of pentobarbital to avoid any distress?

4) Methods:

"CaT alternans was induced by incrementally increasing the pacing frequency until stable alternans was observed (typical range where stable CaT were observed was 1.6 - 2.5 Hz)."

This is a very low frequency, in which there was already alternans. Particularly as the normal heart rate in awake rabbits is in the range of 2.5-3.5 Hz. Have the authors an explanation for this?

5) Results / Figure 2:

The normal AP in the representative figure is extremely long (~650ms) for a wildtype rabbit. Usually, one would expect an APD of around 400-500ms at 1Hz and body temperature. (And even for RT that APD is still very long.) Why have the authors chosen to go for a very unphysiologically slow pacing rate of 0.5 Hz (e.g., 30 beats/min)? It would be good to perform some experiments at a more physiological pacing rate of 1 and 2 Hz.

6) Results / Figure 2:

Also, the shortening by SK activator is extreme big ~300ms; this is much more than one would expect with IKs or IKr activators - despite IKs and IKr being the main repolarizing ion currents in rabbit cardiomyocytes. How do the authors explain this extremely pronounced effect? Also here, it would be good to perform some experiments at a more physiological pacing rate of 1 and 2 Hz - to investigate how pronounced the SK activating effect would then be.

7) Results / Figure 3:

There seems to have been one cell with already very long APD at baseline that demonstrated pronounced APD prolongation; and one outlier, which seems to be the only cell that demonstrated an SK blocker induced APD shortening (while all other individual cells were prolonging with SK blocker). What were the specifics of these two cells / experiments? Were they derived from different animals?

8) Results / Figure 6:

"Furthermore, we tested if activation of SK channels during KV7.1 channel inhibition can normalize APD.."

In line with my comment regarding the pronounced shortening effect of SK activation (see comment 6), SK activation does not lead to a normalization of the APD prolongation induced by IKs-blockade but to a much more pronounced shortening of the APD (nearly significantly shorter than baseline even in presence of HMR). This would suggest that SK activation has a much more pronounced effect on the APD than changes in the physiologically most relevant currents IKs and IKr; most likely due to extremely high dosages of SK channel activators. This could cause severe pro-arrhythmic effects ("drug-induced SQTS") when being given clinically.... Hence it would be interesting to see whether similar beneficial alternans suppressing effects can be seen in case a lower dosage of SK activator is used that does not shorten APD to such an extreme degree (or normalizes it in drug-induced LQTS).

Is it known how selective SK activators are and whether they may also activate other repolarizing ion currents?

END OF COMMENTS

Confidential Review **15-Sep-2022**

REFEREE COMMENTS

Referee #1:

Major comments:

This manuscript reports that calcium alternans can be suppressed by SK channel enhancers through modulating APD and SR Ca content. Suppression of calcium alternans by SK channel is an important discovery and the high quality data from calcium and action potential recording supports this. The experimental design such as changes in calcium transients from control to SK enhancer and washout or SK blocker from the same cells are very impressive and strongly back up the important roles of SK channels in modulating APD. However, the mechanisms solely attributing to SR Ca content may not be accurate. Here are my concerns.

1) This paper suggests that reduced Ca content through shortening of APD is the major mechanism underlying reduction in alternans by SK enhancement. Previous theoretical work and experimental data suggest that Ca alternans originate from several important factors including RyR Ca release (gain function or recruitment), RyR refractoriness from previous beat, etc. The proposed SR Ca content through APD may not fully explain the genesis of Ca alternans. Simulation studies indicate that Ca alternans disappear in both low or high SR Ca load. It is also possible that the prolonged APD causes RyR refractoriness so rather than APD and SR Ca content but diastolic interval might be important in genesis of alternans. The current data do not fully explain how calcium alternans can be suppressed by SK enhancers.

We completely agree with the reviewer that Ca alternans mechanisms may not be solely attributed to SR content. We apologize for the inadequate and misleading phrasing. Our intention was to suggest that changes in SR Ca content is one of the contributing factors to Ca alternans. Indeed, our group has made, what we think are important, contributions to the understanding of CaT alternans and we have previously addressed the roles of L-type Ca currents, SR Ca content and refractoriness of the SR Ca release mechanism (e.g. (Huser *et al.*, 2000; Shkryl *et al.*, 2012; Kanaporis & Blatter, 2017). We have thoroughly revised our discussion section to clearly state that other factors besides SR Ca are likely significant contributors to the development of CaT alternans.

2) This paper links APD shortening as a major cause behind reduced SR Ca load. Alternatively, SK channel works as if transient outward K current to bring the plateau Vm lower, which causes larger peak L-type Ca current to trigger larger Ca release, which eventually deplete the SR Ca load. I think the discussion should clarify what mechanisms the data presented in this paper support and whether there are limitations in the interpretation of the current data.

The discussion was modified as suggested to address these points.

3) Although the discussion section mentions electrical alternans, the paper mainly focuses on calcium alternans. It is more interesting how SK enhancement can influence electrical alternans because of SK channel's main feature - negative feedback from calcium to voltage. Since Ca alternans in this study disappeared in the presence of NS309, electrical

alternans may disappear as well regardless of negative feedback. I think adding new data of APD alternans before and after NS309 can strengthen this paper.

As suggested, we have conducted additional experiments. In the revised manuscripts we now provide Fig. 4 that demonstrate effects of NS309 on APD alternans.

Minor comments:

4) Please explain why only male rabbits were used and briefly discuss how sex may affect the results. Chen's group reported that SK channel expression is much greater in female rabbits (ref: J Physiol. 2018, PMCID: PMC6138290).

Historically, for the past two decades we have conducted all our alternans studies in myocytes isolated from male animals. Thus, we have generated a substantial body of data on that topic which allows us to put current results in a broader context of previous studies. We now address the issue of sex of animals in the 'Limitations' section.

5) I am struggling to understand the cause of APD shortening at a rapid rate under NS309. If NS309 reduces SR Ca content to have smaller CaT amplitudes, SK amplitude will be also smaller to affect minimal contribution to APD, right? How can SK channels be activated under small SR Ca content?

Our data show that NS309 leads to APD shortening regardless of pacing rate (Figs 1 and 4). Also, please note that the effect of NS309 on SR Ca load, while statistically significant, is not dramatic. The CaT amplitude is reduced by ~25% and the remaining Ca release might still be of sufficient magnitude to activate SK channels. In addition, NS309 was shown to increase Ca sensitivity of SK channel activation (Hougaard *et al.*, 2007) which would tend to compensate for the reduced CaT amplitude**.**

6) Why has this study used HMR1556 to prolong APD through blocking IKs and study Ca alternans? Most drug-induced QT prolongation is through IKr block and IKr blocker such as E4031 can be more clinically relevant.

We have chosen to use HMR1556 to mimic LQTS type-1 which is the most frequent congenital LQTS type. In the revised manuscript we now provide new data obtained under LQTs type-2 conditions induced by E4031 (Fig. 7E).

7) I think Figure 7 can be put in Fig 1 to show the impact of NS309 on CaT under normal pacing and then rapid pacing for alternans.

We are not quite sure whether we completely understand the reviewer's concern here. In both figures data on pacing induced CaT alternans are shown.

Nonetheless, in the revised manuscript we have rearranged and reorganized several figures with the goal to improve readability of the manuscript.

8) Fig 3 includes APD at control rate and Ca alternans amplitudes in the presence of SK channel blocker at fast pacing rate. Comparing two at the same CL would be ideal.

We have conducted additional experiments and present Fig. 4 with simultaneous AP and $[Ca]_i$ measurements.

9) PKA activation can enhance SK channel under sympathetic stimulation and its roles can have greater impact on alternans but this study only focuses on the normal condition when SK channel is least effective in modulating cardiac repolarization. The discussion may add a limitation section to include sympathetic or sex differences.

A 'Limitations' section was added as suggested and issues of sympathetic stimulation and sex differences are discussed there.

Referee #2:

Major comments:

1) In their key messages and at the end of the abstract, the authors state "The data suggest SK activation as a potential intervention to avert development of alternans with important ramifications for arrhythmia prevention and therapy for patients with LQT syndrome." - without indicating that they have performed some experiments with (drug-induced) LQTS cardiomyocytes. The latter result should be included into the abstract; otherwise, the conclusion is purely speculative.

In the original abstract we mentioned the use of HMR1556 to mimic LQTS type-1. We now also mention the new experiments where we induced LQTS type-2 using the drug E4031.

Also, it would be important to expand these experiments a bit more, as the mechanisms of alternans may differ in healthy vs. LQT cardiomyocytes. It has for example been demonstrated that particularly in LQT2 calcium alternans drives AP alternans. Hence adding drug-induced LQT2 experiments in addition to the experiments with HMR (drug-induced LQT1) (Figure 6) would be very interesting.

In the revised manuscript (Fig. 7E) we provide data that SK activation by NS309 also reduces CaT alternans in cells treated with E4031 (drug-induced LQTS type-2).

2) Methods:

"Ventricular myocytes were isolated from male New Zealand White rabbits"

Why were only male rabbits investigated? According to the ARRIVE guidelines, ideally animals of both sexes should be used; and if this is not possible for a given scientific

question, the scientific rationale that is in favour of only using one sex should be clearly stated.

Historically, for the past two decades we have conducted all our alternans studies in myocytes isolated from male animals. Thus, we have generated a substantial body of data on that topic which allows us to put current results in a broader context of previous studies. We now address the issue of sex in the 'Limitations' section.

3) Methods:

"Rabbits were anaesthetized with an intravenous injection of sodium pentobarbital (100 mg/kg)"

This is the dosage that is usually given for euthanasia rather than anesthesia. Were the rabbits anesthetized or sedated prior to the lethal application of pentobarbital to avoid any distress?

Under our experimental protocol rabbits were euthanized by exsanguination through the heart excision procedure (the heart is still beating during this procedure). Sodium pentobarbital, which is the most common anesthetic agent used before laboratory animal euthanasia by exsanguination, was used to induce deep anesthesia of the rabbit. Such pentobarbital dosage is commonly used, and it was also recommended by our veterinarian staff and approved by Institutional Animal Care and Use Committee of Rush University. Prior to the injection of pentobarbital a local ectopic anesthetic (lidocaine) was applied to the injection site.

4) Methods:

"CaT alternans was induced by incrementally increasing the pacing frequency until stable alternans was observed (typical range where stable CaT were observed was 1.6 - 2.5 Hz)."

This is a very low frequency, in which there was already alternans. Particularly as the normal heart rate in awake rabbits is in the range of 2.5-3.5 Hz. Have the authors an explanation for this?

Experiments were performed at room temperature which significantly lowers the pacing threshold for alternans. This now is addressed in the 'Limitations' section of the revised manuscript.

5) Results / Figure 2:

The normal AP in the representative figure is extremely long (~650ms) for a wildtype rabbit. Usually, one would expect an APD of around 400-500ms at 1Hz and body temperature. (And even for RT that APD is still very long.) Why have the authors chosen to go for a very unphysiologically slow pacing rate of 0.5 Hz (e.g., 30 beats/min)? It would be good to perform some experiments at a more physiological pacing rate of 1 and 2 Hz.

We have used 0.5 Hz pacing as a standard pacing rate for CaTs properties under control conditions at room temperature and in the *absence* of CaT alternans. To be

consistent with previous our studies we used the same pacing rate here. At room temperature APs are prolonged. Pacing at 2 Hz at room temperature is within the range where we typically observed stable CaT alternans. Nonetheless, in Fig. 4 of the revised manuscript we now show simultaneous CaT and AP recordings obtained at higher pacing rates (1.6 Hz in the example shown). The new data show a much shorter AP at higher pacing rates and still substantial degree of AP shortening by NS309.

6) Results / Figure 2:

Also, the shortening by SK activator is extreme big ~300ms; this is much more than one would expect with IKs or IKr activators - despite IKs and IKr being the main repolarizing ion currents in rabbit cardiomyocytes. How do the authors explain this extremely pronounced effect? Also here, it would be good to perform some experiments at a more physiological pacing rate of 1 and 2 Hz - to investigate how pronounced the SK activating effect would then be.

Indeed, such a prominent effect of SK activation on AP morphology is not easily predictable. However, our results demonstrating that effects of NS309 can be prevented by two different SK channels blockers (apamin and UCL1684) demonstrate that effects are caused by SK activation. In the revised manuscript we include Fig. 4 showing effects of NS309 at higher pacing rates. In this set of experiments pacing rate varied from 1.6 to 2 Hz and cells exhibited alternans. Mean APD_{90} for the longer of alternating APs was 445 ± 89 ms in control and shortened to 179 ± 27 ms in the presence of NS309. APD_{90} of shorter APs was 339 ± 47 ms and shortened to 170 ± 23 in NS309, i.e. the effect of NS309 was comparable to the effects at low pacing rate (shortening of ~45%). Please see also our response to concern #5 above.

7) Results / Figure 3:

There seems to have been one cell with already very long APD at baseline that demonstrated pronounced APD prolongation; and one outlier, which seems to be the only cell that demonstrated an SK blocker induced APD shortening (while all other individual cells were prolonging with SK blocker). What were the specifics of these two cells / experiments? Were they derived from different animals?

We have run Grubb's (with α=0.05) and ROUT (both with GraphPad Prism 9.0) statistical tests to identify possible outliers in APD data sets. Both tests indicated the same cell as an outlier. This cell with much longer APD was obtained from the different animal. We decided to exclude this data point from the analysis in the revised manuscript. Also, in Fig. 2A we now provide new example of AP traces and APD measurements over the time that better represent this set of data. We have no clear explanation why one of the cells showed APD shortening in the presence of apamin, but we have no reason to eliminate these data from the sample.

8) Results / Figure 6: "Furthermore, we tested if activation of SK channels during KV7.1 channel inhibition can normalize APD."

In line with my comment regarding the pronounced shortening effect of SK activation (see comment 6), SK activation does not lead to a normalization of the APD prolongation induced by IKs-blockade but to a much more pronounced shortening of the APD (nearly significantly shorter than baseline even in presence of HMR). This would suggest that SK activation has a much more pronounced effect on the APD than changes in the physiologically most relevant currents IKs and IKr; most likely due to extremely high dosages of SK channel activators. This could cause severe pro-arrhythmic effects ("drug-induced SQTS") when being given clinically.... Hence it would be interesting to see whether similar beneficial alternans suppressing effects can be seen in case a lower dosage of SK activator is used that does not shorten APD to such an extreme degree (or normalizes it in drug-induced LQTS).

We refrain from using the term 'normalization' in this context in the revised manuscript. Also, we agree with the reviewer that for future studies it would be necessary to establish an SK activator dosage that avoids the risk of extreme APD shortening. This issue is now discussed in the new 'Limitations' section. However, in this manuscript we aimed to demonstrate that SK channels can be activated in principle in ventricular myocytes (a controversial issue as mentioned in the manuscript) and SK channel activation has the potential to the reduce risk for ventricular alternans.

9) Is it known how selective SK activators are and whether they may also activate other repolarizing ion currents?

Selectivity of pharmacological agents is always a valid concern. We tried to address this question by the simultaneous application of NS309 with two different SK blockers (apamin and UCL1684). Our results show (Figs. 5 and 6) that both blockers of SK channels largely abolish the effects of NS309 on APD and on CaT alternans, strongly indicating that the observed effects are indeed due to SK channel activation.

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Hougaard C, Eriksen BL, Jorgensen S, Johansen TH, Dyhring T, Madsen LS, Strobaek D & Christophersen P. (2007). Selective positive modulation of the SK3 and SK2 subtypes of small conductance Ca^{2+}-activated K^{+} channels. *Br J Pharmacol* **151,** 655-665.

Huser J, Wang YG, Sheehan KA, Cifuentes F, Lipsius SL & Blatter LA. (2000). Functional coupling between glycolysis and excitation-contraction coupling underlies alternans in cat heart cells. *J Physiol* **524 Pt 3,** 795-806.

Kanaporis G & Blatter LA. (2017). Membrane potential determines calcium alternans through modulation of SR Ca^{2+} load and L-type Ca^{2+} current. *J Mol Cell Cardiol* **105,** 49-58.

Shkryl VM, Maxwell JT, Domeier TL & Blatter LA. (2012). Refractoriness of sarcoplasmic reticulum Ca release determines Ca alternans in atrial myocytes. *Am J Physiol Heart Circ Physiol* **302,** H2310-2320.

Dear Dr Kanaporis,

Re: JP-RP-2022-283870R1 "Activation of small conductance Ca2+-activated K+ channels suppresses Ca2+ transient and action potential alternans in ventricular myocytes" by Giedrius Kanaporis and Lothar A. Blatter

We are pleased to tell you that your paper has been accepted for publication in The Journal of Physiology.

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Authors should note that it is too late at this point to offer corrections prior to proofing. The accepted version will be published online, ahead of the copy edited and typeset version being made available. Major corrections at proof stage, such as changes to figures, will be referred to the Editors for approval before they can be incorporated. Only minor changes, such as to style and consistency, should be made at proof stage. Changes that need to be made after proof stage will usually require a formal correction notice.

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Yours sincerely,

Bjorn Knollmann
Senior Editor
The Journal of Physiology

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EDITOR COMMENTS

Reviewing Editor:

The authors have responded and satisfied the concerns of Reviewers 1 and 2. Both reviewers agree that this important work will make quite an influential impact on the field.

The manuscript should be edited by the authors to include a statement about animal food/housing/water.

Senior Editor:

The MS is now acceptable for publication. Excellent work!

REFEREE COMMENTS

Referee #1:

The revised version addressed all of my comments and concerns and the importance of SK channels in modulating alternans and its potential mechanisms are backed up with additional data and modified discussion. One comment about reorganizing figure panels are not necessary for the flow of the current manuscript. I have no further questions.

Referee #2:

The authors have adequately addressed all my comments and, particularly, have conducted the requested additional experiments (E4031 to mimic LQT2 and stimulation at different rates). The manuscript has improved considerably.

1st Confidential Review **10-Nov-2022**