

Mechano-electrical interactions and heterogeneities in wild-type and drug-induced long QT syndrome rabbits

Raphaela Diana Lewetag, Saranda Nimani, Nicolò Alerni, Tibor Hornyik, Simon Friedrich Jacobi, Robin Moss, Marius Menza, Nicolas Pilia, Teo Puig Walz, Amir HajiRassouliha, Stefanie Perez-Feliz, Manfred Zehender, Gunnar Seemann, Callum Michael Zgierski-Johnston, Ruben Lopez, and Katja E Odening

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The referees have opted to remain anonymous.

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Dear Dr Odening,

Re: JP-RP-2023-284604 "Mechano-electrical interactions and heterogeneities in wild-type and drug-induced long QT syndrome rabbits" by Raphaela Diana Lewetag, Saranda Nimani, Nicolò Alerni, Tibor Hornyik, Simon Jacobi, Robin Moss, Marius Menza, Nicolas Pilia, Teo Puig Walz, Amir HajiRassouliha, Stefanie Perez-Feliz, Manfred Zehender, Gunnar Seemann, Callum Michael Zgierski-Johnston, Ruben Lopez, and Katja E Odening

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Yours sincerely,

Peter Kohl
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The Journal of Physiology

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-The contact information provided for the person responsible for 'Research Governance' at your institution is an author on this paper. Please provide an alternative contact who is not an author on this paper or confirm that the author whose email was provided has sole responsibility for research governance. This is the person who is responsible for regulations, principles and standards of good practice in research carried out at the institution, for instance the ethical treatment of animals, the keeping of proper experimental records or the reporting of results.

-You must start the Methods section with a paragraph headed [Ethical Approval](#). A detailed explanation of journal policy and regulations on animal experimentation is given in [Principles and standards for reporting animal experiments in The Journal of Physiology and Experimental Physiology](#) by David Grundy J Physiol, 593: 2547-2549. doi:10.1113/JP270818.). A checklist outlining these requirements and detailing the information that must be provided in the paper can be found at: <https://physoc.onlinelibrary.wiley.com/hub/animal-experiments>. Authors should confirm in their Methods section that their experiments were carried out according to the guidelines laid down by their institution's animal welfare committee, and conform to the principles and regulations as described in the Editorial by Grundy (2015). The Methods section must contain details of the anaesthetic regime: anaesthetic used, dose and route of administration and method of killing the experimental animals.

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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)

-The most appropriate summary statistic (e.g. mean or median and standard deviation) must be used. Standard Error of the Mean (SEM) alone is not permitted.

-Exact p values must be stated. Authors must not use 'greater than' or 'less than'. Exact p values must be stated to three significant figures even when 'no statistical significance' is claimed.

-Statistics Summary Document completed appropriately upon revision

EDITOR COMMENTS

Reviewing Editor:

Your paper has been reviewed by two experts who agree that the writing, design, and presentation of this study is impressive and represents a valuable contribution to the field. They raised a few major concerns that ought to be addressed or clarified to enhance the overall impact of the paper.

Senior Editor:

Please address the comments of the reviewers in a revised manuscript.

Please comply with our statistics policy. You must use SD (rather than SEM) and you must also state precise p -values.

REFEREE COMMENTS

Referee #1:

This study by Lewetag & Nimani examines electromechanical reciprocity in rabbits with acquired LQTS. The concept of mechanical influences in channelopathies is a topical and important area. The study showcases novel in vivo methodologies (cardiac MR and body surface mapping which has recently been developed by this group for mapping) which are impressive and likely to be of use to others in the field. They also include preliminary data from motion-corrected optical mapping of beating hearts, which has not been fully optimised.

The choice of rabbits is fully justified as is the choice of aLQTS rather than utilising transgenic animals. The study design is appropriate and the experimental data are robust.

The results describe changes (i) expected changes in ECG parameters (increased RT interval as well as spatial and temporal heterogeneity of RT interval) induced by E4031 infusion in vivo (ii) associated changes in indices of mechanical function occurring with RT prolongation and (iii) increases in RT with elevated preload (by bolus NaCl injection) which were greater in aLQTS and associated with increases in RT-dispersion.

The conclusion that acute MEC (as distinct from negative EMW) may play a role in arrhythmogenesis is speculative (and is stated as such).

The manuscript is concise and well-written in clear and comprehensible language with clear and appropriate figures.

My main concerns are:

1. The incremental information derived from the study is somewhat limited (eg vs. prior studies of EMC in drug-induced LQTS in rabbits Odening et al 2013).
2. The novel insight of this study lies in the BSM data from the vest. Quantification of these data is required. Can the authors demonstrate that the additional regional BSM data tell us something that the ECG RT dispersion does not? How different are these BSM indices between animals? I find the described apex-base difference difficult to appreciate from the example given. A statistically significant result is alluded to in the text but no numbers given. Please also quantify the RV-LV gradient described.

Minor issues:

Figure 1 C & D should these data not be presented as paired (as in 1B)?

The BSM data are a little hard to grasp from the visualisations alone. Clearer heart outlines in the images and indication of which electrode pairs are assigned to which region would be useful in Fig 1.

Some of the text in Fig 4 is small and hard to read.

Referee #2:

With this article "Mechano-Electrical Interactions and Heterogeneities in Wild-Type and Drug-Induced Long QT Syndrome Rabbits" Lewetag et al treat us on the novel insight that drug-induced QT prolongation renders the rabbit heart more sensitive to further -mechano-induced- repolarization prolongation. QT prolongation was induced by administering the IKr blocker E-4031, and acute mechanical changes were superimposed by intravenous infusion of 6 mL/kg bodyweight saline in the in-vivo experiments or volume load in the Langendorff working-heart configuration. Thus, these new results by Lewetag et al suggest that acute mechano-electrical effects may play an additional role in long-QT-related arrhythmogenesis, but -fair to say- arrhythmia was not demonstrated in these experiments. The authors ought to be complemented with their important data and for combining modern approaches of recording and analysis, including tissue-phase mapping MRI, ECG body mapping (in vivo) and optical mapping in the Langendorff working-heart configuration.

This original study follows after previous work of the Odening group on both experimental and clinical long-QT syndrome, and after the publication of a seminal review article on electromechanical reciprocity and arrhythmogenesis by Odening et al in the European Heart Journal.

Major Comments

1. Whereas it becomes clear from these results how acute mechanical load further impacts on repolarization that is already

prolonged by IKr blockade, Lewetag et al do not provide a direct demonstration of how this evolves into arrhythmia. Did the investigators find any (tendency towards) ventricular ectopy / non-sustained / sustained tachycardia) during superimposed repolarization prolongation? If yes, show the data! If no, please explain (e.g., in the Discussion) how you interpret this.

2. The experiments to study mechano-induced repolarization changes in the presence of sympathetic and parasympathetic blockade remain somewhat underexposed (shown in Supplemental Figure 4), whereas they are crucially important. I suggest to bring these data and figure in the main body of the text while demonstrating via various autonomic-responsive parameters (blood pressure, heart rate, other) what the impact of sympathetic and parasympathetic stimulation was. Personally, I am not yet convinced -based on their data- that the authors can discard any autonomic modulation of the mechano-induced repolarization changes. The authors should discuss their arguments of the opposite more thoroughly.

Minor comments

Abstract figure, panel C: in the myocardial tissue velocity curves the time to peak diastole appears little different between "control" and "aLQTS". I suggest to add the full electrical activation-repolarization curve to this panel to accentuate that any mechanical difference between "control" and "aLQTS" occurs while repolarization is normal and prolonged under these conditions.

The continuous infusion of ketamine S / xylazine during the in-vivo experiments raises the question, at least with this reviewer, of what is known about the effects of ketamine S / xylazine on the autonomic nervous system, and as such on the results of this study. This is in view of literature reports stating e.g., that ketamine/xylazine anesthesia increased parasympathetic activity, and suppressed sympathetic and baroreceptor activity independently of the light-dark cycle (Prague Med Rep. 2013;114:72-80), and that ketamine and propofol had different effects on autonomic cardiovascular function, but attenuated the baroreflex sensitivity of heart rate and renal sympathetic nerve activity in a dose-dependent manner (Auton Neurosci. 2001;87:201-8).

Results, p. 15: typo: the effect of electrical changes (e.g., prolongation of cardiac repolarization in aLQTS) on mechanical features "was" (not "were") assessed.

END OF COMMENTS

Confidential Review

27-Feb-2023

Response to Referees

We thank the referees and the reviewing and senior editor for their appreciation of our work, their thorough assessment of our manuscript and the encouraging comments on how to improve the manuscript.

According to the editors' comments, we have adjusted the method section, starting with a paragraph headed Ethical Approval. We furthermore included the exact p-values of all comparisons in the manuscript and the figures and adjusted the latter to the statistic policy showing SD instead of SEM.

According to the referees' comments, we have added information about the sympathetic and parasympathetic blockade to the main text and figures, quantified the LV-RV heterogeneity and added outlines to the vECG figures to clarifying both RV and apical region.

Please find our detailed responses to the reviewer comments below. Changes in the manuscript are presented in **bold** text. We believe that the review process has strengthened the manuscript and we hope that you will find it acceptable for publication in The Journal of Physiology.

EDITOR COMMENTS

Reviewing Editor:

Your paper has been reviewed by two experts who agree that the writing, design, and presentation of this study is impressive and represents a valuable contribution to the field. They raised a few major concerns that ought to be addressed or clarified to enhance the overall impact of the paper.

Response: We thank the Reviewing Editor for kind comment on our study.

Senior Editor:

Please address the comments of the reviewers in a revised manuscript. Please comply with our statistics policy. You must use SD (rather than SEM) and you must also state precise p-values.

Response: We apologize for the discrepancy and thank the senior editor for referencing to the statistics policy. We adjusted the description of our data to the statistics policy, corrected the presentation of the affected figures to SD and stated precise p-values.

REFEREE COMMENTS

Referee #1

This study by Lewetag & Nimani examines electromechanical reciprocity in rabbits with acquired LQTS. The concept of mechanical influences in channelopathies is a topical and important area. The study showcases novel in vivo methodologies (cardiac MR and body surface mapping which has recently been developed by this group for mapping) which are impressive and likely to be of use to others in the field. They also include preliminary data from motion-corrected optical mapping of beating hearts, which has not been fully optimised.

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prolongation and (iii) increases in RT with elevated preload (by bolus NaCl injection) which were greater in aLQTS and associated with increases in RT-dispersion.

The conclusion that acute MEC (as distinct from negative EMW) may play a role in arrhythmogenesis is speculative (and is stated as such).

The manuscript is concise and well-written in clear and comprehensible language with clear and appropriate figures.

Response: We thank the reviewer for the thorough assessment of our study, the appreciation of our work, and the helpful suggestions on how to further improve our study.

Major comments:

1. The incremental information derived from the study is somewhat limited (eg vs. prior studies of EMC in drug-induced LQTS in rabbits Odening et al 2013).

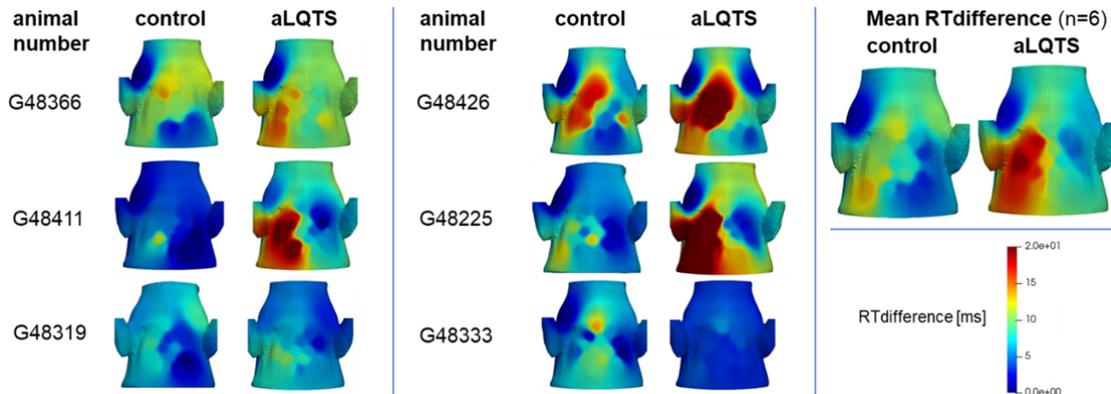
Response 1: We agree with the reviewer that the novelty regarding EMC in aLQTS is somewhat limited as we have previously demonstrated EMC in aLQTS and in transgenic LQT2 rabbits in prior studies from *Odening et al. 2013* and *Ziupa et al. 2019*, as also already indicated in the discussion section of our manuscript. The major novelty of our work certainly lies in the insights in MEC. Nonetheless, for the first time we were able to demonstrate significant EMC-induced changes in apico-basal heterogeneity in Vz AMPdia and Vz TTPdia, seen in Figure 3 E,F. These alterations in mechanical apico-basal heterogeneity in aLQTS were not addressed before and therefore complement the previously acquired knowledge of mechanical dysfunction in aLQTS. To clarify this in the manuscript, we added an additional sentence to the discussion part (page 14):

Substantiating the mounting evidence that LQTS is an 'electro-mechanical' – rather than a 'purely electrical' – disease, we observed impaired diastolic peak velocities (AMPdia) in longitudinal direction (Vz) in the overall base and 4/6 mid segments and significantly prolonged time-to-diastolic peak duration (TTPdia), a marker for contraction duration. Moreover, we complemented these data with a novel aspect by revealing significant EMC-induced changes in apico-basal heterogeneity in Vz AMPdia and Vz TTPdia.

2. The novel insight of this study lies in the BSM data from the vest. Quantification of these data is required. Can the authors demonstrate that the additional regional BSM data tell us something that the ECG RT dispersion does not? How different are these BSM indices between animals? I find the described apex-base difference difficult to appreciate from the example given. A statistically significant result is alluded to in the text but no numbers given. Please also quantify the RV-LV gradient described.

Response 2: We thank the reviewer for this thoughtful and detailed comment. Indeed, the novelty of our study lies in the MEC data collected from 12-lead ECG and vest ECG. The importance of the vECG is justified in the insights in spatial distribution in repolarization. Other than RT dispersion, the visualization of the heart-rate corrected RTn370 and RTdifference on the rabbits' thoraces allows an appreciation of the actual regional differences in repolarization – while RT dispersion only estimates the overall regional heterogeneity in repolarization but does not give any information about affected regions themselves. Therefore, vECG enables the spatial allocation of impaired repolarization, which 12-lead RT dispersion does not. To highlight the alteration in apico-basal and RV-LV heterogeneity between control and aLQTS, we added white labeling of the regions to Fig. 2 I,J and Fig. 4 D.

The differences of the BSM indices in the single rabbits and the corresponding mean RTdifference from those n=6 rabbits are shown in the following Figure.



Response Figure 1: Visualization of regional RTdifference (color-coded as differences compared to minimum RT) on rabbits' torsos in aLQTS compared to control.

Response Figure 1 presents the alteration in regional repolarization using color-coded RTdifference. RTn370-prolonging effects were more pronounced in apical regions (see Fig. 2. I). To be able to compare spatial allocation in repolarization between control and aLQTS, RTdifference was calculated. The significant increase in RTdifference in the apical region led to an increased apico-basal heterogeneity in repolarization, seen in 4/5 animals (G48411, G48319, G48426, G48225). For animal G48333, the apical leads 21-24 are sadly missing due to ECG signal distortion. Response Figure 1 therefore shows an interpolation of this region from the upper leads in G48333. We hope that showing the individual animals and adjusting Figures 2 and 4 helped to outline the increase apico-basal heterogeneity in aLQTS. The increase in RV-LV RT-heterogeneity was seen in 4/6 animal (see Response Figure 1), but there was no significant increase in RTdifference in the RV depicting leads 10, 15, 17 and 23. To clarify this fact, we changed the corresponding part as followed:

*Moreover, in aLQTS the change in regional RT-interval was **visually** noticeable in the leads depicting the right ventricle (lead 10, 15, 17, 23) with particularly pronounced changes in RTdifference (indicated in red in Fig. 2I-J) in 4 out of 6 animals. In these, the RV-LV difference was 3.46 ± 1.73 ms in control and increased to 9.09 ± 5.28 ms in aLQTS ($p=0.13$, paired t-test, Figure 2 E,I,J).*

Minor comments:

Figure 1 C & D should these data not be presented as paired (as in 1B)?

Response: We thank the reviewer for spotting this. We have now also indicated the individual paired values in the Figures C and D.

The BSM data are a little hard to grasp from the visualisations alone. Clearer heart outlines in the images and indication of which electrode pairs are assigned to which region would be useful in Fig 1.

Response: This is a very good suggestion. We added white outlines and regional labeling to Figures 2 and 4 (Figure numbers have been switched to include the Suppl. Figures into the manuscript). To increase the visibility of the RV heart outline in Figure

2 G, we changed its color to orange. Unfortunately, further changes of the heart's outline would distort the CT generated data presented in this Figure.

Some of the text in Fig 4 is small and hard to read.

Response: We thank the reviewer for pointing this out. We have modified the font size accordingly.

Referee #2

With this article "Mechano-Electrical Interactions and Heterogeneities in Wild-Type and Drug-Induced Long QT Syndrome Rabbits" Lewetag et al treat us on the novel insight that drug-induced QT prolongation renders the rabbit heart more sensitive to further -mechano-induced- repolarization prolongation. QT prolongation was induced by administering the IKr blocker E-4031, and acute mechanical changes were superimposed by intravenous infusion of 6 mL/kg bodyweight saline in the in-vivo experiments or volume load in the Langendorff working-heart configuration. Thus, these new results by Lewetag et al suggest that acute mechano-electrical effects may play an additional role in long-QT-related arrhythmogenesis, but -fair to say- arrhythmia was not demonstrated in these experiments. The authors ought to be complemented with their important data and for combining modern approaches of recording and analysis, including tissue-phase mapping MRI, ECG body mapping (in vivo) and optical mapping in the Langendorff working-heart configuration.

This original study follows after previous work of the Odening group on both experimental and clinical long-QT syndrome, and after the publication of a seminal review article on electromechanical reciprocity and arrhythmogenesis by Odening et al in the European Heart Journal.

Response: We thank the reviewer for the thoroughness in reviewing our study, the appreciation of our work, and the enriching suggestions and comments on how to further improve our study.

Major comments:

1. Whereas it becomes clear from these results how acute mechanical load further impacts on repolarization that is already prolonged by IKr blockade, Lewetag et al do not provide a direct demonstration of how this evolves into arrhythmia. Did the investigators find any (tendency towards) ventricular ectopy / non-sustained / sustained tachycardia) during superimposed repolarization prolongation? If yes, show the data! If no, please explain (e.g., in the Discussion) how you interpret this.

Response 1: We thank the reviewer for this thoughtful comment. We have indeed not provided evidence on whether the mechano-induced prolongation of repolarization can further evolve into arrhythmias in our model. During the short monitoring periods, we have not observed any mechano-induced arrhythmias in the drug-induced LQTS rabbits. This is the reason, why we have thus far only presented this as a potential further cause for long-QT-mediated arrhythmia formation.

There are several reasons that may account for the lack of any arrhythmic events in our acute drug-induced LQTS model. Not only is the IKr-blocker E-4031 a short acting drug, but we also monitor the rabbits for a short period of time and have only performed one single change in preload during 12-lead ECG and one during vest ECG in each aLQTS animal, which might be too little to observe ME-induced arrhythmic events. Moreover, the rabbits are also under anaesthesia with ketamine/xylazine during the ME interventions, making it highly unlikely for arrhythmic events to occur/get captured due to an overall relatively low sympathetic tone with parasympathetic predominance.

However, this might be different in transgenic rabbits with mutations in the HERG/ I_{Kr} channel, which are in general more prone to arrhythmias than aLQTS rabbits. Along those lines, in another ongoing project, in which we also perform mechanical changes under anesthesia in our various transgenic LQTS and SQTs rabbit models, we do indeed see occasionally single ventricular extra beats or bigeminy after changes in the preload or afterload. Examples of the occurrence of bigeminy in two different SQT1 rabbits upon increased afterload are shown below.



Response Figure 2: Two exemplary ECG recordings from two SQT1 rabbits after an acute increase in the afterload due to balloon occlusion in the proximal aorta.

We have added this limitation of lack of arrhythmias into the discussion (page 16), which now reads as follows:

Importantly, the mechano-induced electrical alterations were particularly pronounced in aLQTS, in which not only an overall RT prolongation occurred, but also an increase in regional RT-dispersion in 12-lead ECG and a changed pattern of RT heterogeneity. These data indicate that acute changes in (global) myocardial stretch may cause additional alterations of electrical function in aLQTS. When these changes exert regionally divergent effects - as observed in our study in aLQTS – they may potentially increase proarrhythmic APD heterogeneity and thereby facilitate arrhythmia formation in acquired QT-prolongation. However, no direct evidence was provided in our model on whether these alterations in the electrical function can further evolve into arrhythmias. There are several reasons that may account for the lack of arrhythmic events in our acute drug-induced LQTS model. Not only is the I_{Kr} -blocker E-4031 a short acting drug, but we also monitor the rabbits for a short period of time and have only performed one single change in preload during 12-lead ECG and one during vECG in each aLQTS animal, which might be too little to observe ME-induced arrhythmic events. Moreover, the rabbits are also under anaesthesia with ketamine/xylazine during the ME interventions, which may further contribute to a lack of arrhythmic events due to an overall relatively low sympathetic tone with parasympathetic predominance.

2. The experiments to study mechano-induced repolarization changes in the presence of sympathetic and parasympathetic blockade remain somewhat underexposed

(shown in Supplemental Figure 4), whereas they are crucially important. I suggest to bring these data and figure in the main body of the text while demonstrating via various autonomic-responsive parameters (blood pressure, heart rate, other) what the impact of sympathetic and parasympathetic stimulation was. Personally, I am not yet convinced -based on their data- that the authors can discard any autonomic modulation of the mechano-induced repolarization changes. The authors should discuss their arguments of the opposite more thoroughly.

Response 2: We agree with the reviewer on the importance of these experiments, and we have implemented the suggestion of moving this figure to the main body of the text.

Regarding the reviewer's request to show autonomic-responsive parameters such as blood pressure or heart rate: we have not performed invasive blood measurements while assessing the effect of pharmacological blockade of the sympathetic and parasympathetic nervous system on the extent of MEC-induced QT changes. However, we would like to add the findings from the heart rate measurements. While we observed no changes in heart rate during betablockade alone – likely due to the parasympathetic predominance in rabbits anaesthetized with xylazine and the consecutively already pretty slow heart rate for a rabbit – we observed an increase in heart rate when the parasympathetic blocker was also added (HR before vs after blockade [bpm±SD]: baseline 151.3±23.8 vs. blockers 173.2±20.9, p=0.0162, n=8).

We have added this information to the result section (page 11), which now reads as follows:

*To further investigate whether the observed changes in the electrical function (RT) are mainly due to intrinsic mechano-induced electrical changes or whether (some parts of it) are mediated secondarily by autonomic reflexes, we performed additional experiments in a subset of control rabbits before and after complete blockade of the parasympathetic and sympathetic system. **Heart rate measurements were performed to validate the pharmacological autonomic blockade. While no changes in heart rate were observed due to betablockade alone – likely due to parasympathetic predominance in rabbits anaesthetized with xylazine and the consecutive slow heart rate – we observed an increase in heart rate after the parasympathetic blocker was also added (Figure 5B.3).** In these experiments, the bolus-induced changes in RTn370 did not differ between baseline and autonomic-blockade experiments (Fig. 5B), suggesting a direct role of myocardial stretch caused by increased preload on the observed electrical alterations.*

In response to the reviewer's comment on discarding autonomic modulation of MEC: we would like to highlight the fact that we do not claim that autonomic modulation of MEC can or should be discarded. However, we suggest that the observed MEC are not solely due to autonomic modulation / reflex loops, but are partly driven by cardiac-intrinsic mechanisms, or at least a combination of both. This suggestion is based on the observation of a similar extent of bolus-induced RT-prolongation before and after the pharmacological blockade of the autonomic system in control and aLQTS rabbits. We thus claim that there is (also) a direct role of bolus-induced myocardial stretch on the electrical alterations.

To make this clearer, we have rephrased the text in the discussion (page 15) as follows:

This prolongation occurred to a similar extent with intact autonomic activity and after pharmacological blockade of both the sympathetic and parasympathetic nervous system, suggesting that this mechano-induced electrical alteration **is not solely due to autonomic**

modulation / reflex loops, but may indeed be **caused by** cardiac-intrinsic mechanisms such as electrical alterations caused by bolus-induced changes in myocardial stretch, **or a combination of both.**

Minor comments:

Abstract figure, panel C: in the myocardial tissue velocity curves the time to peak diastole appears little different between "control" and "aLQTS". I suggest to add the full electrical activation-repolarization curve to this panel to accentuate that any mechanical difference between "control" and "aLQTS" occurs while repolarization is normal and prolonged under these conditions.

Response: Indeed, this is a very good suggestion that would appreciate the differences in the electro-mechanical window between control and aLQTS. Unfortunately, we could not acquire proper ECG measurement of the rabbits while they are in the MRI. The MRI-ECG used for the tissue phase measurements only detects R to generate heart rate dependent recordings and the T-waves are usually very distorted by the MR-compatible ECG devices. Moreover, with our MRI system, there was no option to either extract nor to analyze these ECG data. Therefore, we have no opportunity to include these data in the abstract figure.

The continuous infusion of ketamine S / xylazine during the in-vivo experiments raises the question, at least with this reviewer, of what is known about the effects of ketamine S / xylazine on the autonomic nervous system, and as such on the results of this study. This is in view of literature reports stating e.g., that ketamine/xylazine anesthesia increased parasympathetic activity, and suppressed sympathetic and baroreceptor activity independently of the light-dark cycle (Prague Med Rep. 2013;114:72-80), and that ketamine and propofol had different effects on autonomic cardiovascular function, but attenuated the baroreflex sensitivity of heart rate and renal sympathetic nerve activity in a dose-dependent manner (Auton Neurosci. 2001;87:201-8).

Response: We thank the reviewer for this valuable remark. Indeed, the xylazine in our anaesthesia regimen increases the parasympathetic tone, and therefore, might not be the best anaesthesia to use when investigating the effect of autonomic blockade on MEC. We however usually use this ketamine/xylazine anaesthesia combination as it has no effects on cardiac repolarizing ion currents (Odening et al. AJP 2008), while most alternative anaesthetics such as propofol or isoflurane do block various repolarizing ion channels, rendering it suboptimal in our experimental setting, in which we are interested in changes in cardiac repolarization.

We have added this into the discussion (page 16), which now reads as follows:

This prolongation occurred to a similar extent with intact autonomic activity and after pharmacological blockade of both the sympathetic and parasympathetic nervous system, suggesting that this mechano-induced electrical alteration is not solely due to autonomic modulation / reflex loops, but may indeed be caused by cardiac-intrinsic mechanisms such as electrical alterations caused by bolus-induced changes in myocardial stretch, or a combination of both.

Of note, considering that xylazine increases the parasympathetic tone, our anaesthetic regimen might not be ideal in the setting of investigating the effect of pharmacological autonomic blockade on MEC. However, as we are interested in changes in cardiac repolarization, and it is shown that the ketamine/xylazine combination has no effect on

cardiac repolarizing ion currents (Odening et al., 2008), it is of advantage to utilize this regimen as opposed to the alternative options such as propofol or isoflurane, which do block various repolarizing ion channels.

Results, p. 15: typo: the effect of electrical changes (e.g., prolongation of cardiac repolarization in aLQTS) on mechanical features "was" (not "were") assessed.

Response: We would like to thank the reviewer for the very accurate revision of our manuscript - the error has been corrected.

Dear Dr Odening,

Re: JP-RP-2023-284604R1 "Mechano-electrical interactions and heterogeneities in wild-type and drug-induced long QT syndrome rabbits" by Raphaela Diana Lewetag, Saranda Nimani, Nicolò Alerni, Tibor Hornyik, Simon Friedrich Jacobi, Robin Moss, Marius Menza, Nicolas Pilia, Teo Puig Walz, Amir HajiRassouliha, Stefanie Perez-Feliz, Manfred Zehender, Gunnar Seemann, Callum Michael Zgierski-Johnston, Ruben Lopez, and Katja E Odening

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

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EDITOR COMMENTS

Reviewing Editor:

Thank you to the authors for their thoughtful engagement in the revision process which has greatly strengthened the manuscript. Congratulations on this excellent paper!
