

Editorial comments:

Changes to be made by the Author(s):

- 1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.**

We have thoroughly proofread the manuscript and all supplemental files to ensure that there are no grammar and spelling issues. We defined all abbreviations at first use. We have made all corrections in the track changes mode.

- 2. Please revise the following lines to avoid overlap with previously published work: 185-188, 242 (position..)-245, 392 (The applied methods...)-396 (..HR); 397 (This kind...)-401 (...sequence), Figure 2 and 3 legends, 578-581 (..alterations), 625-628, 633-6363.**

We have revised the specified sections to avoid overlap with previously published work.

- 3. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: Dataquest A.R.T software, ecgAUTO software etc.**

We did remove commercial language from the manuscript.

- 4. Being a video based journal, JoVE authors must be very specific when it comes to the humane treatment of animals. Regarding animal treatment in the protocol, please add the following information to the text: please specify the euthanasia method, but do not highlight it. Similarly, please do not highlight anesthesia steps.**

We now specify the euthanasia method in step 2.5.4. It now reads: At the end of the experiment, euthanize the mouse by carbon dioxide (CO₂) inhalation. Note: we do not recommend cervical dislocation or decapitation as euthanasia method since this could damage parts of the ECG and BP transmitter device.

- 5. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.**

We have made the changes as requested and now use the imperative tense throughout the manuscript.

6. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g., button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

We added more details to the protocol steps and made the changes as requested. We have already completed the video of the surgery (Supplemental Video 1).

7. Please specify which mouse strain was used here. Do you mean C57B/L6?

For the production of the video and the illustrations, as well as for the generation of the representative data, we used mice of the C57BL/6J mouse strain.

The animals of the sick sinus syndrome mouse model (Hcn4^{tm3(Y527F;R669E;T670A)}Biel) displaying increased BRS sensitivity were maintained on a mixed C57BL/6N and 129/SvJ background (Fenske S., Nat Commun, 2020).

We now provide this information in the manuscript as a “Note” at the beginning of the Protocol section.

8. Please include a one line space between each protocol step and then highlight up to 3 pages of protocol text for inclusion in the protocol section of the video.

We now include a one-line space between each protocol step. In addition, we have highlighted in yellow all text passages that are shown in our video (Supplemental video 1).

9. Please include a scale bar and define the scale in the Figure Legend for Figure 2.

We added a scale bar to the images shown in Figure 2 D-L and we define the scale in the Figure Legend for Figure 2 as follows: Scale bar in D-L shows 4 mm.

10. Please do not abbreviate journal names in the reference list.

We have corrected this accordingly. The journal names in the reference list are no longer abbreviated.

11. Please provide timecodes or markers with the footage that correspond to the script steps to aid our editors

We now provide a detailed time code (Supplemental file Timecode) to the script steps.

Reviewers' comments:

Reviewer #1:

Manuscript Summary: This paper aims to present a surgical technique for implantation of an ECG and BP measurement device. Furthermore, they described data processing procedure for assessment of baroreflex sensitivity. The paper is well written and only few issues should be discussed before considering it for publication in JoVE.

We would like to thank the reviewer for his/her positive feedback.

Major Concerns: None

Minor Concerns:

- 1) Section 2.8: parameters for data processing were extensively described. However, it is not clear if these parameters can fit all rodents' models used in preclinical field. In other words, some specific experimental models (as an example, with physiological extremely high/low heartbeat and/or pressure values) could require adaptation of these parameters to their specific physiological features. Accordingly, authors should add a comment about the need of carefully review data processing parameters in order to check that they fit the specific model under study.**

We agree with the reviewer and added the following passage to section 2.8:

“Note: the following data processing parameters are optimized for data acquired from wildtype mice and should in principle fit all mouse models used in preclinical field. However, adaption of these parameters might be necessary when working with specific experimental models, e.g. mice with extremely high or low HR and/or BP values, or different rodent species. In any case, data processing parameters need to be carefully reviewed in order to assure that they fit the specific model under study.”

- 2) Section 2.8.8 and 2.8.9: sorting of data should be better described. In particular, MATLAB code should be mandatory added. Furthermore, screen capture of this processing step could be usefully added to the paper.**

We now provide an Excel file in the supplement (TemplateBRS, Supplemental file 8) to easily sort for up- and down-sequences by copying the data from the exported Results File into it. We also provide screen captures containing a step-by-step explanation on how to analyze data with the TemplateBRS Excel file (Supplementary files 9-13) and we describe all corresponding steps in the protocol section of the manuscript (section 2.8.7 – 2.8.8). Using Excel has two major advantages compared to a MATLAB script. First, it is an inexpensive standard software available in every lab. Second, it is easy to use even for the less experienced experimenter.

Reviewer #2:

Manuscript Summary: The paper describes a relatively novel way to determine baroreceptor sensitivity using radio telemetry recordings of blood pressure and ECG in mice. This approach will be useful for models of chronic disease in genetic mouse models.

We would like to thank the reviewer for his/her positive feedback.

Major Concerns:

The paper would be stronger if it included a chronic model in which BRS changes to better demonstrate the utility and sensitivity of this method. This issue might also be addressed in the discussion.

We thank the reviewer for this suggestion to improve the manuscript. We have now added BRS data from a mouse model with sick sinus syndrome. In this mouse model, BRS sensitivity is significantly increased. For reference please see (Fenske S., Nat Commun, 2020)

Minor Concerns:

It is not clear whether Dataquest ART is still available. Would a new be able to do the same analysis with Ponemah software? This should be addressed.

We agree with the reviewer on this point. The Dataquest A.R.T. recording software from DSI has been discontinued and replaced by the Ponemah software. But since Dataquest A.R.T has been the gold standard software for decades it is still very common and used in many labs, like ours. That is why we have described the protocol using this software. Furthermore, the Ponemah raw data can similarly be imported into the ecgAUTO analysis software for subsequent BRS analysis.

As the editor told us during the revision process to remove all trademarks and company names from the manuscript, since JoVE does not publish manuscripts that contain commercial language, we have now included Ponemah software in the "table of materials and reagents" in the supplements as potential recording software.

References

Fenske, S., K. Hennis, R. D. Rotzer, V. F. Brox, E. Becirovic, A. Scharr, C. Gruner, T. Ziegler, V. Mehlfeld, J. Brennan, I. R. Efimov, A. G. Pauza, M. Moser, C. T. Wotjak, C. Kupatt, R. Gonner, R. Zhang, H. Zhang, X. Zong, M. Biel and C. Wahl-Schott (2020). "cAMP-dependent regulation of HCN4 controls the tonic entrainment process in sinoatrial node pacemaker cells." Nat Commun **11**(1): 5555.