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| To Dr. Vineeta Bajaj  Editor of *Jove* |  |
| Copenhagen May 2nd 2018  JournalNr |
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# Resubmission of a manuscript

Dear Dr. Baja

I hereby submit an updated version of the manuscript with the title “Cochlear implant surgery and electrically-evoked auditory brainstem response recordings in C57BL/6 mice by *CA Navntoft et. al*..

We thank all reviewers for their thorough reading and detailed comments on our original manuscript. It is gratifying to hear that our study is “… a useful investigation” (Rev#1) and that our topic “…will draw attention by contemporary auditory neuroscientists, cochlear implant researchers and neuroanatomists interested in structural/functional properties in the inner ear and technology interventions related to hearing loss and deafness in humans” (Rev#1). We were also happy to read that the reviewers found that “the description and photos are instructive” (Rev#2).

Our manuscript has been expanded and improved as suggested by the reviewers and the editor. Specifically, we have now added 1) a Summary; 2) a short section in the Discussion on how the mouse CI surgery compares to other rodents who have been used with CIs; and 3) thorough proofreading.

We hope that the manuscript is now suitable for *Jove*.

With best regards,

Charlotte Amalie Emdal Navntoft

***<Editor>***

1. *Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.*

We agree with the Editor. The updated manuscript has been thoroughly proofread.

1. *Please submit the figures as a as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 pixels x 1080 pixels or 300dpi.*

All figures in the original manuscript were submitted as .ai files. The updated figures are also submitted as .ai files.

1. *Figure 1: Please delete RPvsdEX MAtlab and RZ6 to avoid commercial language and products.*

Corrected as requested.

1. *Figures 2/7/8: Please use the Greek symbol mu for the microvolt abbreviation instead of the letter u.*

Corrected as requested.

1. *Figure 5: Should panels A and B be switched? Panel A is the magnification of the red dotted box of Panel B? What is the d?*

We agree with the Editor that Panel A and B should be switched. It is now corrected. *d* is the diameterof the electrodes. The denotation of *d* has been added to the figure text of **Figure 5**.

1. *Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.*

Corrected as requested.

1. *Title: Please simplify the title by deleting “Protocol for”.*

Corrected as requested.

1. *Keywords: Did you mean C57BL mouse?*

Yes, we did. It is now corrected.

1. *Please include a Summary that clearly describes the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”*

Provided as requested.

1. *Line 99: Please use numbered superscripted references whenever possible.*

Corrected as requested.

1. *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: TDT, Tucker Davis Technologies, Otoform, Horizon, BioSigRZ, etc.*

Corrected as requested.

1. *The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.*

We only find action items that direct the reader to do something in the Protocol. If certain sentences are specified as not related to action items by the Editor, we will be happy to move them to the Discussion.

1. *In the JoVE Protocol format, “Notes” should be concise and used sparingly. They should only be used to provide extraneous details, optional steps, or recommendations that are not critical to a step. Any text that provides details about how to perform a particular step should either be included in the step itself or added as a sub-step. Please consider moving some of the notes about the protocol to the discussion section.*

We agree. In the manuscript, “Notes” is only used in four places. They provide extraneous details and recommendation, such as “..the steps can be done with even higher microscopic magnification” in Step 3.8.

1. *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.*

We have provided more details in our protocol steps where we found a need, such as “Shave the fur behind the left ear” has been changed to “Cut the fur behind the left ear with a scissor (or shave it with a shaver) to expose the skin” in Step 3.2. We have also added a reference on the speaker calibration in Step 2.2. If more details in certain specified steps are needed, we will be happy to provide them.

1. *2.1: How much foam is used?*

0.1-0.2 ml foam delivered via a 1 ml syringe is used to cover the whole ear canal with foam. This has been elaborated in Step 2.1.

1. *2.2: How was the calibration done? Citation?*

The calibration was done according to the calibration protocol from Tucker Davis Technologies. The reference for the protocol has been added to Step 2.2.

1. *2.3? 70% ethanol? Or absolute?*

Yes, we meant 70 % ethanol solution. This has been elaborated in Step 2.3.

1. *2.7: How is the filter applied? Manually or via software?*

The filtering is applied offline via a custom-made matlab script. This has been elaborated in Step 2.7.

1. *3.2: What is used to shave the fur? What percentage of ethanol is used?*

The fur is cut behind the left ear with a scissor or shaved with a shaver. 70 % ethanol solution is used. This has been elaborated in Step 3.2.

1. *3.5: Blunt dissection with what?*

The blunt dissection is performed with forceps. This has been elaborated in Step 3.5.

1. *3.10: Stapes?*

The stapes is the middle ear bone that is connected to the oval window. This has been elaborated in Step 3.10.

1. *4.2: What is the salt dissolved in? Water or PBS, etc.*

The salt is dissolved in PBS. This has been added to Step 4.2.

1. *6.5: How small of an incision? What is used to incise?*

The incision in the neck is 0.5 mm long and is made using a tissue scissor. This has been elaborated in Step 4.2.

1. *Can the matlab script be provided as a supplementary file?*

Yes, we have provided a simple matlab script and an example of a data recording as a supplementary file.

1. *Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.*

Provided (in grey highlight) as requested.

1. *Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.*

Provided (in grey highlight) as requested.

1. *Please include a Disclosures section, providing information regarding the authors’ competing financial interests or other conflicts of interest. If authors have no competing financial interests, then a statement indicating no competing financial interests must be included.*

Provided as requested.

1. *Please do not abbreviate journal titles.*

Corrected as requested.

***<Reviewer #1>***

1. *Line 338: "CBA/Ca" should be "CBA/CaJ".*

We agree and have corrected the typo.

1. *Discussion: explain briefly how the mouse CI surgery compares to other rodents who have been used with CIs, and what parts are more difficult due to the small size of the mouse.*

Provided as requested.

1. *Methods: How many observers determined the ABR thresholds for Figure 3? And were they "blind" to the experimental condition of each animal. State if this was so.*

In this manuscript, the ABR thresholds in Figure 3 were determined by two observes, one blind and one biased to the experimental condition of each animal. In the future, when we want to address specific scientific questions we will use two blind observes.

***<Reviewer #2>*** *Minor Concerns:*

1. *Line 72 Suggest "was an "effective" deafening procedure"*

We agree and have corrected the sentence.

1. *Line 88 "animal" should be "animals"*

We agree and have corrected the sentence.

1. *Line 123 Does FHC or ME stand for anything?*

FHC, Inc. is a company and ME is an abbreviation for the state Maine in the US.

1. *Line 134 - Comment - Can the authors detail what soundproof booth is used?*

The soundproof booth used is model Mac-2 Enclosure RF Shielded Box 2A from IAC Acoustics, Winchester, UK. This has been added to the Material list.

1. *Line 142 - Comment - Is the Otoform sufficient for masking the free field speaker?*

In a pilot study, we have shown that occluding the contralateral ear produces a similar small increase in the ABR threshold as deafening the contralateral ear does. Therefore, we argue that Otoform is sufficient for masking the free field speaker in our setup with respect to the contralateral ear.

1. *Line 170 - Comment - Ethanol is ototoxic. How is this accounted for?*

Ethanol and betadine are used as antiseptics to sterilize the skin. We agree that ethanol is ototoxic in large quantities and when applied to the cochlea but not when it is applied in small quantitates and topically on the skin, as in our study.

1. *Line 194 - Suggest "small bleeds" rather than "small bleedings"*

We agree and have corrected the sentence.

1. *Line 347 - Suggest "does not increase" instead of "do not increase"*

We agree and have corrected the sentence.