Journal of Visualized Experiments

Cochlear implant surgery and electrically-evoked auditory brainstem response recordings in C57BL/6 mice

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58073R1
Full Title:	Cochlear implant surgery and electrically-evoked auditory brainstem response recordings in C57BL/6 mice
Keywords:	Cochlear implant; mouse model; auditory nerve; electrically auditory brainstem response; C57B mouse; ototoxic deafening; neomycin; round window
Corresponding Author:	Charlotte Amalie Navntoft Danmarks Tekniske Universitet Kgs. Lyngby, DENMARK
Corresponding Author's Institution:	Danmarks Tekniske Universitet
Corresponding Author E-Mail:	charlotte.navntoft@unibas.ch
First Author:	Charlotte Amalie Navntoft
Other Authors:	Jeremy Marozeau
	Tania Rinaldi Barkat
Author Comments:	No comments.
Additional Information:	
Question	**Response**
If this article needs to be "in-press" by a certain date, please indicate the date below and explain in your cover letter.	

Title:

Cochlear implant surgery and electrically-evoked auditory brainstem response recordings in C57BL/6 mice

Authors:
Charlotte Amalie Navntoft[1,2], Jeremy Marozeau[1], Tania Rinaldi Barkat[2]

[1]Hearing Systems Group, Department of Electrical Engineering, Technical University of Denmark, Kgs. Lyngby, Denmark
[2]Brain and Sound Lab, Department of Biomedicine, Basel University, Basel, Switzerland

Corresponding Author
Charlotte Amalie Navntoft
charlotte.navntoft@unibas.ch

Email Address of Co-authors
Jeremy Marozeau (jemaroz@elektro.dtu.dk)
Tania Rinaldi Barkat (tania.barkat@unibas.ch)

Keywords:
Cochlear implant, mouse model, auditory nerve, electrically auditory brainstem response, C57B mouse, ototoxic deafening, neomycin, round window

Summary:
Animal models of cochlear implants can advance knowledge of the technological bases of treating permanent sensorineural hearing loss with electrical stimulation. This study presents a surgical protocol for acute deafening and cochlear implantation of an electrode array in mice as well as the functional assessment with auditory brainstem response.

Abstract:
Cochlear implants (CIs) are neuroprosthetic devices that can provide a sense of hearing to deaf people. However, a CI cannot restore all aspects of hearing. Improvement of the implant technology is needed if CI users are to perceive music and perform in more natural environments, such as hearing out a voice with competing talkers, reflections, and other sounds. Such improvement requires experimental animals to better understand the mechanisms of electric stimulation in the cochlea and its responses in the whole auditory system. The mouse is an increasingly attractive model due to the many genetic models available. However, the limited use of this species as a CI model is mainly due to the difficulty of implanting small electrode arrays. More details about the surgical procedure are therefore of great interest to expand the use of mice in CI research.

In this report, we describe in detail the protocol for acute deafening and cochlear implantation of an electrode array in the C57BL/6 mouse strain. We demonstrate the functional efficacy of this procedure with electrically-evoked auditory brainstem response (eABR) and show examples

of facial nerve stimulation. Finally, we also discuss the importance of including a deafening procedure when using a normally hearing animal. This mouse model provides a powerful opportunity to study genetic and neurobiological mechanisms that would be of relevance for CI users.

Introduction

Cochlear implants (CIs) are electronic devices that can provide a sense of hearing to people with severe and profound hearing loss. It uses electrodes surgically implanted in the cochlea of the inner ear to directly stimulate the auditory nerve. To date, the CI is the most successful sensory prosthesis and has helped more than 600,000 people worldwide[1]. However, the device has shortcomings. First, the benefits provided by the device vary greatly among recipients. Second, speech in noisy environments and music are still poorly perceived by most CI users.
For many years, animal models have been used to better understand these issues in CI research and to continuously improve safety and efficacy of the devices. The models have given valuable insight into several phenomena, such as plastic changes in the brain taking place following CI implantation[2], the effect of applying gene therapy to preserve residual hearing[3], and biophysical properties of the electrically stimulated auditory nerve[4], among many other examples.

Mice are a powerful model organism due to the large availability of genetic models of deafness. Other advantages include the ability to manipulate the mouse genome (*e.g.,* via the CRISPR-Cas system), the opportunity to use advanced imaging techniques to study mechanisms, particularly in the brain, the high reproduction rate, rapid development and easy breeding and handling. The main technical challenges in performing CI surgeries in mice are the small size of the cochlea and the presence of a large stapedial artery (SA). The SA usually disappears during embryonic development in humans but persists throughout life in a number of rodents, including mice, rats, and gerbils. The SA runs below the round window niche, which complicates access to the cochlea and increases surgical risk.

Previous studies have shown the feasibility of CI implantation in mice[5-7]. Irving et. al demonstrated that chronic intracochlear electrical stimulation can be achieved for up to one month. Acute stimulation was also performed but the recordings were not presented. They showed that cauterizing the stapedial artery had no significant effect on the hearing threshold or the number of spiral ganglion neurons and that topical application of the aminoglycoside neomycin, an ototoxic drug, was an effective deafening procedure in mice [5]. Soken *et al.* described a modified dorsal approach to the mouse cochlea through the round window to better preserve hearing status[6]. Following insertion of a platinum-iridium wire, substantial residual hearing was observed with an increased auditory brainstem response (ABR) threshold of 28 dB. Otoacoustic emissions (OAE) were lost in animals with large ABR threshold shifts[6]. Mistry *et al.* tested the functional and histopathological effects of implantation in the absence of electric stimulation[7]. Even though hearing was preserved in both 3- and 6-months-old implanted mice at low frequencies, implantation resulted in fibrosis-like tissue around the implant and osteoneogenesis around the bullostomy[7].

In short, out of the three studies on CIs in mice, only one demonstrates functional recording of CI stimulation. Irving and colleagues performed both acute and chronic eABR recordings but only showed data from chronic CI stimulation[5]. However, the chronic model with a fully implantable device developed by Irving *et al.* is technically challenging. It is not yet known if acute CI stimulation, both less challenging and faster, can achieve similar results.

CIs are used by people with severe and profound hearing loss who no longer benefit from hearing aids. Animal models for CI users should therefore include a deafening procedure when normally hearing animals are used. Another reason to deafen hearing animals is that the electrical stimulation of a deaf or hearing cochlea produces different neural responses[4,8-12]. Electrical stimulation of a deaf cochlea directly activates the auditory nerve fibers and generates an electroneural response (α). It is characterized by short latency and a small dynamic range in the periphery[8,10]. On the other hand, electrical stimulation of a hearing cochlea also excites the hair cells in an electrophonic response (β) that is characterized by longer latencies and larger dynamic range[4,11]. The electrophonic response is attributed to normal excitation of nerve fibers by inner hair cells, electrically induced contraction of outer hair cells, and generation of a travelling wave[4]. Electroneural and electrophonic responses also result in two different activity patterns in the central nervous system[9]. Sato *et al.* recorded midbrain neurons of a CI implanted guinea pig before and after deafening with neomycin, which eliminates the electrophonic contribution. They showed that the slope of the rate-level function was steeper and firing rates higher in the deafened condition compared to the hearing condition[9]. Therefore, depending on the research question stated, it is important to consider including deafening to separate electrophonic and electroneural responses upon electric stimulation of the auditory nerve.

Here, we describe the procedure for acute deafening and the cochlear implantation of an electrode array in a mouse as well as the functional recording of intracochlear electric stimulation with electrically-evoked auditory brainstem response (eABR).

Protocol:

All procedures were carried out according to Basel University, Switzerland, animal care and guidelines. They were licensed by the Veterinary Office of the Canton of Basel, Switzerland.

Note: C57BL/6 adult mice, aged 8-12 weeks (weight 20-30g), were used in this study.
The left ear is used as the experimental ear. The right ear serves as an intra-animal control and is not surgically altered.

1. Preoperative Procedures

1.1. Anaesthetize the animal 30 min prior to surgery via intra-peritoneal (i.p.) injection of ketamine/xylazine (80 mg/kg ketamine, 16 mg/kg xylazine, i.p., volume injected at 10 μL/g body weight).

1.1.1. Supplement anesthesia as necessary, as judged by a positive pedal and palpebral (toe-pinch) reflex and movement of the whiskers, with a lower dose of ketamine (45 mg/kg, i.p., injected at 10 μL/g body weight). Agents and dose regimes can be substituted per institutional guidelines.

Notes: In general, the animal will need an injection every 45-60 min with this agent and dose regime.

1.2. Check for full sedation of the animal marked by a regular breathing rate and a lack of toe-pinch reflexes. Maintain this level of anesthesia.

1.3. Maintain the animal's body temperature at 36.6 °C with a closed-loop heating pad. Apply eye ointment to avoid dehydration of the cornea. This will also suppress the animal's blink reflex, which can add noise to the recoding.

1.4. Administer local analgesic via subcutaneous injection (s.c.) of bupivacaine/Lidocaine (0.1 mg/mL bupivacaine and 0.4 mg/mL lidocaine, 0.1 mL administered s.c.) along the intended incision line to minimize any surgical discomfort. Agents and dose regimes can be substituted per institutional guidelines.

1.5. Administer the muscarinic antagonist atropine (atropinesulfate amino, 0.1 mg/mL, 20 μL administered s.c., dissolved in PBS) in the neck to reduce mucus secretion and to facilitate breathing. Agents and dose regimes can be substituted per institutional guidelines.

2. Pre-deafening Acoustic Auditory Brainstem Response (aABR)

Note: aABR is used to measure the status of hearing before and after deafening. Testing is performed on the left ear and in a soundproof electrically shielded booth. We recommend to test and later implant the left ear for a right-handed person. Further details on ABR in mice can be found in[13,14]. Tucker Davis Technologies (TDT) hardware and software (BioSig) are used to record ABR but other systems can be used.

2.1. Block the contralateral (right) ear with acoustic foam to isolate the ABR response from the ipsilateral (left) ear. Put the foam in a 1 mL syringe and inject it into the right ear canal of the mouse to cover the whole ear canal with foam (0.1-0.2 mL of foam). Make sure the syringe seals closely to the ear so that the foam gets all the way into the ear canal.

2.2. Place the speaker 10 cm from the left ear.

Notes: The speaker for this setup was calibrated using a PCB microphone as described in[15].

2.3. Clean the ABR electrodes with 70% ethanol solution. Place the electrodes under the skin: active (Ch1) on the vertex, reference (-) below the pinna of the ipsilateral ear, and ground in the hind leg (**Figure 1**).

2.4. Connect the head-stage and pre-amplifier to the auditory processor via the optic fiber port.

2.5. Check the impedance of the active and reference electrode.

2.5.1. If the impedance is over 3 Ohm, re-arrange them and re-take the measurement. The best recordings are obtained when the electrodes have the same impedance. Close the sound-proof booth.

2.6. Present click stimulation and record ABR in a free-field condition with a complex auditory processor and software. Standardize the click stimulus in the software: 0.1 ms single-channel monophasic clicks are presented at 21 Hz; Click level decreases from 90 dB SPL to 10 dB SPL in 10 dB steps; 10 ms recording window. Average a total of 512 responses at each dB level.

2.7. Apply a 2000 Hz lowpass filter and a 300 Hz highpass filter offline to reduce noise in the recording using a custom-made Matlab script.

2.8. Determine the ABR threshold as the lowest dB level with a recognizable ABR wave response (**Figure 2**, **Figure 3**).

3. Surgery

Note: Typical instruments used include a scissor, a scalpel, a pair of metallic forceps with straight or curved tips, a tissue retractor tool, several suction wedges and absorbable paper points. The surgery is performed on the left ear.

3.1. Put the mouse on its right side. Avoid undue torsional stress on the cervical vertebrae. Make sure to keep the body straight to keep the airways open.

3.2. Cut the fur behind the left ear with a scissor (or shave it with a shaver) to expose the skin. Sterilize the skin with 70% ethanol solution and betadine (povidone/iodine).

3.3. Under microscopic magnification (16X), make a 1-1.5 cm post-auricular incision with the scalpel.

3.4. Switch to higher microscopic magnification (25X).

3.5. Perform blunt dissection through the subcutaneous fat layer, which can be of variable thickness, with forceps.

Notes: Be careful when dissecting as the external jugular vein traverses this area. Damage to this structure can cause excessive bleeding.

3.6. Retract the sternocleidomastoid muscle to reveal the tympanic bulla periosteum. Use the facial nerve as a key anatomical landmark to aid identification of the auditory bulla. The facial nerve wraps around the posterior/dorsal edge of the sternocleidomastoid muscle and runs rostrally along the ear canal towards the pinna. Gently place the self-retaining retractor tool in the incision to ease access to the bulla (**Figure 4**).

3.7. Remove the tissue overlying the medio-dorsal area of the bulla to allow clear visualization of the ridge between the bulla and the mastoid process.

3.8. Gently rotate a 30G needle to pierce the bulla and make a hole (bullostomy) on the posterior-superior side of the ridge (the bone is thinner on this side). Alternatively, use a dental surgical drill.

Notes: This and the following steps can be done with even higher microscopic magnification (40X) if preferred. Also, change the position of the microscope if needed. It is important to maximize the surgical view of the middle ear space.

3.9. Widen the bullostomy by pinching small bone pieces using fine tipped forceps to expose the middle ear cavity. Extend the bullostomy dorsally towards the mastoid process until the round window niche is clear of overlying bone. The stapedial artery, a branch of the internal carotid artery, runs ventral to the round window niche.

3.9.1. Be careful not to damage the vessel as excessive bleeding may be fatal. Small bleeds can be stopped by pressing a small piece of spongostan in the inner ear cavity.

3.9.2. Extend the bullostomy toward the anterior-superior direction to visualize the stapes, the middle ear bone connected to the oval window.

3.10. Remove the stapes with forceps to expose the oval window.

4. Round Window Application of Ototoxic Agent

4.1. Gently perforate the round window and oval window membranes using a blunted 30G needle. Check that perilymph runs out.

4.2. Slowly perfuse 5% weight/volume neomycin dissolved in PBS (adjusted to pH 7.4) through the oval window. Liquid should flush out of the round window. Repeat the same procedure on the round window. Be careful not to damage the window bone structures with the needle used to perfuse.

4.3. Place a small piece (1 mm^2) of spongostan soaked in neomycin within the round window and oval window niche.

4.4. Remove the retractor tool, close the incision and wait 30 min.

5. Post-deafening acoustic ABR

5.1. Record aABR in a similar way as before deafening (Steps 2.2 to 2.8) (**Figure 2b**, **Figure 3**).

6. Insertion of CI electrode array

Note: The intracochlear electrode array consists of four platinum bands (Ø0.2 mm) with platinum/iridum parylene insulated wire shielded in a silicone tube (**Figure 5**).

6.1. Place the retractor tool in the incision to re-access the bulla.

6.2. Insert the electrode array into the round window (scala tympani) at a depth where the 4th platinum ring is located just inside the round window. This gives an insertion depth of ~2 mm, corresponding to an intracochlear position at ~ 30 kHz[16].

6.3. Coil the lead wire inside the bulla and glue the wire to the tissue above the bulla. Coiling the wire helps to keep the array in place throughout the experiment.

6.4. Carefully remove the retractor and close the insertion with tissue glue.

6.5. Make a small incision (0.5 mm) in the neck perpendicular to the line between where the active and reference ABR electrodes will be using a tissue scissor. Place the platinum ground ball in the subcutaneous pocket and close the small incision with tissue glue (**Figure 6**).

6.6. Connect the electrode array board to the Animal Stimulator Platform.

7. Electric auditory brainstem response (eABR)

Note: An Animal Stimulator Platform (ASP) is used to electrically stimulate the electrode array. Other current sources and software systems can be used.

7.1. Place the ABR electrodes as before (Steps 2.3 to 2.5) (**Figure 6**).

7.2. Open the ASP software and define the electric pulse stimulation paradigm. We use a charge-balanced biphasic pulses with 50µs/phase and 10µs interphase gap presented at 23.3 pulses per second (pps). The electric stimulation is delivered in monopolar electrode configuration with increasing current levels. A total of 400 responses are averaged at each current level.

7.3. Present the electric pulse trains and record the evoked eABR response continuously via the TDT headstage, pre-amplifier and auditory processor.

7.4. Plot and analyze the eABR data via a custom-made matlab script (**Figure 7**). The script and an example of a recording are provided in the Supplementary.

8. End of experiment

8.1. At the end of the experiment, euthanize the animal according to institutional guidelines.

8.2. Carefully open the incision and remove the implant.

8.3. Ultra-sonicate the electrode array in distilled water for 10 minutes to remove tissue debris.

Note: The implant can be reused several times if the electrodes are intact and properly conducting. To check this, measure the impedance of the electrodes with a multimeter when the array is dry.

8.4. Store the electrode array in a dry place.

Representative results:

The purpose of this study was to describe a reliable model for acute CI stimulation in the deafened mouse. Pre- and post-surgical hearing thresholds served as a functional readout of the deafening procedure. Topical application of 5% neomycin in the oval and round window significantly increased click-evoked hearing thresholds by 46 dB $\pm$ 6 (pre- vs post-neomycin: 30.0 dB $\pm$ 3.8 vs 75.7 dB $\pm$ 3.7, p = 0.0003, paired t-test, n= 7) (**Figure 3**). The mouse-sized electrode array was hereafter inserted into the round window (**Figure 4, Figure 5**). Electric simulation of an intracochlear electrode could reliably generate eABR activity. (**Figure 7**). In some cases, CI stimulation activated the facial nerve and produced a high amplitude wave with either short or long latency (**Figure 8A** and **Figure 8B**, respectively). The short latency response was characterized by a rapid amplification of wave IV around 3 ms and is likely to be a direct response of the facial nerve. The long latency response appeared around 5-6 ms and is likely to be a non-auditory muscle (myogenic) response evoked indirectly by the facial nerve. Facial nerve responses are rarely reported in animal studies in literature but is a well-known complication in human CI users[17-19]. In **Figure 8**, facial nerve stimulation appeared at relatively medium current levels (150-200 μA) and in two different animals. In other cases, both responses could appear in the same animal at very high current levels (not shown). We recommend limiting the current level to levels below the appearance of facial nerve stimulation.

Figure 1. Auditory Brainstem Response (ABR) setup. Subdermal electrodes are placed at the vertex (active/channel 1 [Ch1]), behind the ipsilateral ear (reference [Ref]) and at the hind leg (ground [Gnd]) of the anesthetized mouse. Electrode signals are amplified and then recorded by a TDT system. Acoustic and electric stimulation are presented via a microphone and an Animal Stimulator Platform, respectively.

Figure 2. Representative aABR waves to click stimulation from a wild-type mouse before and after deafening with 5% neomycin. **(A)** The normal-hearing aABR pattern is characterized by waves labelled I-V and a low hearing threshold, here 30 dB SPL (arrow). **(B)** The deafened aABR pattern shows an increased hearing threshold, here 70 dB SPL (arrow). The waves have a longer latency and more temporal jitter.

Figure 3. aABR threshold before and after deafening. Application of neomycin significantly increased aABR thresholds by 46 dB $\pm$ 6. Pre- vs post-neomycin: 30.0 dB $\pm$ 3.8 vs 75.7 dB $\pm$ 3.7, p = 0.0003, paired t-test, n = 7. Errors are standard error of the means.

Figure 4. The surgery. (A) Exposure to the auditory bulla. The bullostomy is performed (white dotted circle) along the ridge on the tympanic bulla (black dotted line). **(B)** The bullostomy allows visualization of the round window, stapedial artery, and oval window. Neomycin is gently flushed through first the oval window, then the round window. **(C)** The electrode array is inserted until the 4th electrode is located just inside the round window niche. The electrode wire is coiled inside the bulla to keep the array in place before the incision is closed. CN VII = cranial nerve VII (facial nerve), OW = oval window, RW = round window, SA = stapedial artery, SCM = sternocleidomastoid muscle, TB = tympanic bulla.

Figure 5. The mouse cochlear implant. (A) The intracochlear electrode array consists of four platinum bands spaced at a 0.4 mm interval with a diameter *d*: 0 [tip] (d=0.21), 1 (d=0.23), 2 (d=0.25), 3 (d=0.27). The width of each electrode is 0.2 mm. The four platinum/iridium (90/10) parylene insulated wires are shielded in a silicone tube. **(B)** Magnification of the electrode array tip (red dotted square). The electrode array and a platinum reference ball are connected to a print board. Scale bar: 1 mm.

Figure 6. Electrically-evoked ABR (eABR) setup. The CI platinum ground ball (Gnd, red) is placed in a subcutaneous pocket in the neck of the mouse. The line between active (Ch1(+) at the vertex) and reference (Ref (-) at the ipsilateral ear) ABR electrodes is perpendicular to the line between the electrode array and the ground in order to obtain the best eABR response. The eABR ground electrode (Gnd, black) is placed in the hind leg.

Figure 7. Representative eABR waves to CI stimulation in a deafened mouse. A biphasic pulse train is presented to electrode #1 in monopolar configuration at 23.3 pulses per second (pps) with 400 repetitions. Stimuli level 0-175 μA is shown in 25 μA steps (see stimulation details in Step 7.2). Roman numerals denote eABR wave number. The wave amplitudes and latency increase and decrease, respectively, with increasing current level. In this example, wave II appeared around 1 ms, wave III around 2 ms, wave IV around 3 ms, wave V around 4 ms.

Figure 8. Example of facial nerve stimulation. In some cases, CI stimulation can activate the facial nerve and evoke a direct response with short latency **(A)** (arrow) or indirect response with longer latency **(B)** (arrow). The examples shown are from two CI-implanted animals stimulated with a biphasic pulse train using 0-300 μA in 50 μA steps (see stimulation details in Step 7.2).

Roman numerals denote eABR wave numbers. * denotes clipping of the eABR wave due to saturation of the amplifier.

Discussion:

This manuscript describes the surgical approach for acute deafening and cochlear implantation in the mouse, as well as the functional assessment of CI stimulation with auditory brainstem response. Although the mouse cochlea is small and the surgery challenging, the CI mouse model is feasible and serves as a valuable tool in auditory research.

The stapedial artery is present in the middle ear of the mouse. The artery enters the bulla posterior-medially, and runs inferiorly to the round window niche and then superiorly to the oval window niche. In the initial development of the mouse model, we experienced fatal intra-operative bleeding following trauma to the stapedial artery, mainly while accessing the bulla. As a consequence, we adapted a more limited approach and accessed the bulla in smaller, refined dissection steps. No further complications due to bleeding were thereafter observed. Despite the fact that stapedial artery cauterization has no significant effect on hearing threshold or number of spiral ganglion neurons in mice[5], in our opinion, it is unnecessary so long as great care and attention are taken during the surgery. We suggest taking the time needed to develop fine psychomotor skills and reach technical proficiency. The average time from initial incision to closure around the implanted electrode array is typically 1-1.5 hours.

The described acute CI surgery in mice is similar to the “ventral” procedure and round window insertion used in other rodents, including rats and gerbils[20-22]. Other rodent studies have used the “dorsal approach” with a basal turn cochleostomy instead of a round window insertion, avoiding the SA entirely and inserting the array more deeply[6,23,24]. The implantation of a chronic stimulation assembly in mice follows the same steps as described in this protocol with the addition of a Dacron mesh to fix the implant and postoperative care[5].

The main technical challenges when performing CI surgeries in mice are the small size of the cochlea compared to the cochlea of rats and gerbils, and the presence of a large SA. The SA is also present in rats but not in gerbils. In addition, since mice are smaller than rats and gerbils, they are more vulnerable to surgical procedures.

To eliminate electrophonic responses in eABR recordings and to mimic hair cell loss found in most CI users, we deafened the animals before CI insertion. Mice are difficult to deafen ototoxically *in vivo*[25] because the systemic concentrations of aminoglycosides required to cause ototoxicity has a narrow dose window: lower doses given over several days results in no hair cell loss whereas a single injection of a higher dose can be lethal[26]. Also, susceptibility to aminoglycosides is strain dependent[26]. However, it has been shown that a single dose of aminoglycosides in combination with a loop diuretic can produce excessive outer hair cell loss in CBA/CaJ mice without fatal consequences[27]. Delayed inner hair cell death was reported in half of all cochleae examined[27].

In this manuscript, we used topical application of the aminoglycosides neomycin inspired by the protocol recently established for C57BL/6 mice[5]. Acute application of neomycin significantly increased the click-evoked hearing threshold by 46 dB ± 6.1. Although this increase is larger than the 35 dB increase reported by Irving *et al.* (pre- vs post-surgery: 41.6 dB ± 3.3 vs 76.6 dB ± 4.4, p = 0.02, n = 3)[5], we achieved the same post-deafening threshold (75.7 dB ± 3.7 vs 76.6 dB ± 4.4). 5% neomycin is thought to cause a partial loss of hearing, mainly by rapid outer hair cell death, as inner hair cell loss takes longer to occur[27]. It is therefore possible that electrophonic response, which is generated both by inner and outer hair cells[4,8-12], is only partially eliminated in deafened animals with residual hearing. Even though 5% (weight/volume) neomycin does not decrease the number of spiral ganglion neurons 4 weeks post-deafening[5], it is yet unknown if neomycin in our acute setup affects the auditory nerve fibers or promotes synaptopathy (loss of synapses between inner hair cells and type I auditory nerve fibers). Another uncertainty is that the topical neomycin treatment may not produce a uniform distribution of hair cell loss along the length of the cochlea. Future studies are required to answer these questions.

In summary, the growing number of genetic models for human deafness and the biochemical tools available make the mouse an attractive animal model for auditory research, including the field of CIs.

Disclosure:

No competing financial interests. The authors have nothing to disclose.

Acknowledgement:

The authors would like to thank Pierre Stahl, Oticon Medical, Nice, France, for providing the Animal Stimulation Platform and advice on stimulation paradigms, and James B. Fallon and Andrew K. Wise from Bionics Institute, Melbourne, Australia, for surgical advice. This work was supported by a grant from the Swiss National Science Foundation (ERC transfer grant to T.R.B.).

References

1 The-Ear-Foundation. *Cochelar Implants Update*, 2018).

2 Fallon, J. B., Irvine, D. R. F. & Shepherd, R. K. Cochlear Implants and Brain Plasticity. *Hearing Research.* **238** (1-2), 110-111 (2008).

3 Pfingst, B. E. *et al.* Neurotrophin Gene Therapy in Deafened Ears with Cochlear Implants: Long-term Effects on Nerve Survival and Functional Measures. *Journal of the Association for Research in Otolaryngology.* **18** (6), 731-750 (2017).

4 Miller, C. A. *et al.* Electrical excitation of the acoustically sensitive auditory nerve: single-fiber responses to electric pulse trains. *Journal of the Association for Research in Otolaryngology.* **7** (3), 195-210 (2006).

5 Irving, S. *et al.* Cochlear implantation for chronic electrical stimulation in the mouse. *Hearing Research.* **306** 37-45 (2013).

6 Soken, H. *et al.* Mouse cochleostomy: a minimally invasive dorsal approach for modeling cochlear implantation. *Laryngoscope.* **123** (12), E109-115 (2013).

7 Mistry, N., Nolan, L. S., Saeed, S. R., Forge, A. & Taylor, R. R. Cochlear implantation in the mouse via the round window: effects of array insertion. *Hearing Research.* **312** 81-90 (2014).

8 Hartmann, R., Topp, G. & Klinke, R. Discharge patterns of cat primary auditory fibers with electrical stimulation of the cochlea. *Hearing Research.* **13** (1), 47-62 (1984).
9 Sato, M., Baumhoff, P. & Kral, A. Cochlear Implant Stimulation of a Hearing Ear Generates Separate Electrophonic and Electroneural Responses. *The Journal of Neuroscience.* **36** (1), 54-64 (2016).
10 Pfingst, B. E., Spelman, F. A. & Sutton, D. Operating ranges for cochlear implants. *Annals of Otology, Rhinology & Laryngology .* **89** (2) (1980).
11 Miller, C. A., Hu, N., Zhang, F., Robinson, B. K. & Abbas, P. J. Changes across time in the temporal responses of auditory nerve fibers stimulated by electric pulse trains. *Journal of the Association for Research in Otolaryngology.* **9** (1), 122-137 (2008).
12 Shepherd, R. K. & Javel, E. Electrical stimulation of the auditory nerve. I. Correlation of physiological responses with cochlear status. *Hearing Research.* **108** (1-2), 112-144 (1997).
13 Akil, O., Oursler, A. E., Fan, K. & Lustig, L. R. Mouse Auditory Brainstem Response Testing. *Bio Protocol.* **6** (6) (2016).
14 Willott, J. F. Measurement of the auditory brainstem response (ABR) to study auditory sensitivity in mice. *Current Protocols in Neuroscience.* **Chapter 8** (Unit 8.21B) (2006).
15 TDT. ABR User Guide: A Guide to ABR Testing with the System 3 RZ6. Updated August 2017. Florida, USA.
16 Muller, M., von Hunerbein, K., Hoidis, S. & Smolders, J. W. A physiological place-frequency map of the cochlea in the CBA/J mouse. *Hearing Research.* **202** (1-2), 63-73 (2005).
17 Cushing, S. L., Papsin, B. C. & Gordon, K. A. Incidence and characteristics of facial nerve stimulation in children with cochlear implants. *Laryngoscope.* **116** (10), 1787-1791 (2006).
18 Berrettini, S., Vito, D. A., Bruschini, L., Passetti, S. & Forli, F. Facial nerve stimulation after cochlear implantation: our experience. *Acta Otorhinolaryngologica Italica.* **31** (1), 11-16 (2011).
19 Hu, H., Kollmeier, B. & Dietz, M. Reduction of stimulation coherent artifacts in electrically evoked auditory brainstem responses. *Biomedical Signal Processing and Control.* **21** 74-81 (2015).
20 Wiegner, A., Wright, C. G. & Vollmer, M. Multichannel cochlear implant for selective neuronal activation and chronic use in the free-moving Mongolian gerbil. *Journal of Neuroscience Methods.* **273** 40-54 (2016).
21 Hessel, H. *et al.* Meriones unguiculatus (Gerbil) as an animal model for the ontogenetic cochlear implant research. *American Journal of Otolaryngology.* **18** (S21) (1997).
22 Pinilla, M., Ramirez-Camacho, R., Jorge, E., Trinidad, A. & Vergara, J. Ventral approach to the rat middle ear for otologic research. *Otolaryngology Head Neck Surgery.* **124** (5), 515-517 (2001).
23 King, J., Shehu, I., Roland, J. T., Jr., Svirsky, M. A. & Froemke, R. C. A physiological and behavioral system for hearing restoration with cochlear implants. *Journal of Neurophysiology.* **116** (2), 844-858 (2016).
24 Lu, W., Xu, J. & Shepherd, R. K. Cochlear implantation in rats: a new surgical approach. *Hearing Research.* **205** (1-2), 115-122 (2005).
25 Poirrier, A. L. *et al.* Ototoxic drugs: difference in sensitivity between mice and guinea pigs. *Toxicology Letters.* **193** (1), 41-49 (2010).

26 Wu, W. J. *et al.* Aminoglycoside ototoxicity in adult CBA, C57BL and BALB mice and the Sprague-Dawley rat. *Hearing Research.* **158** (1-2), 165-178 (2001).
27 Taylor, R. R., Nevill, G. & Forge, A. Rapid hair cell loss: a mouse model for cochlear lesions. *Journal of the Association for Research in Otolaryngology.* **9** (1), 44-64 (2008).

Figure1

Click here to download Figure Figure1.ai

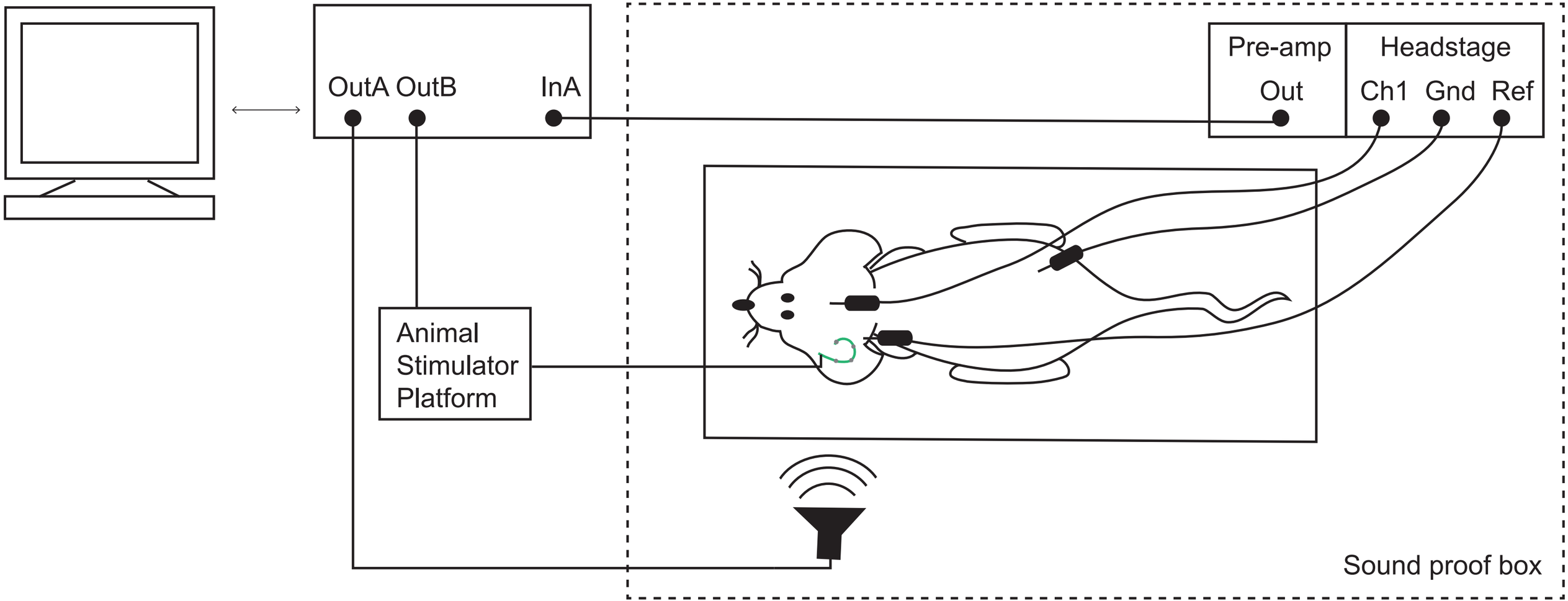


Figure2

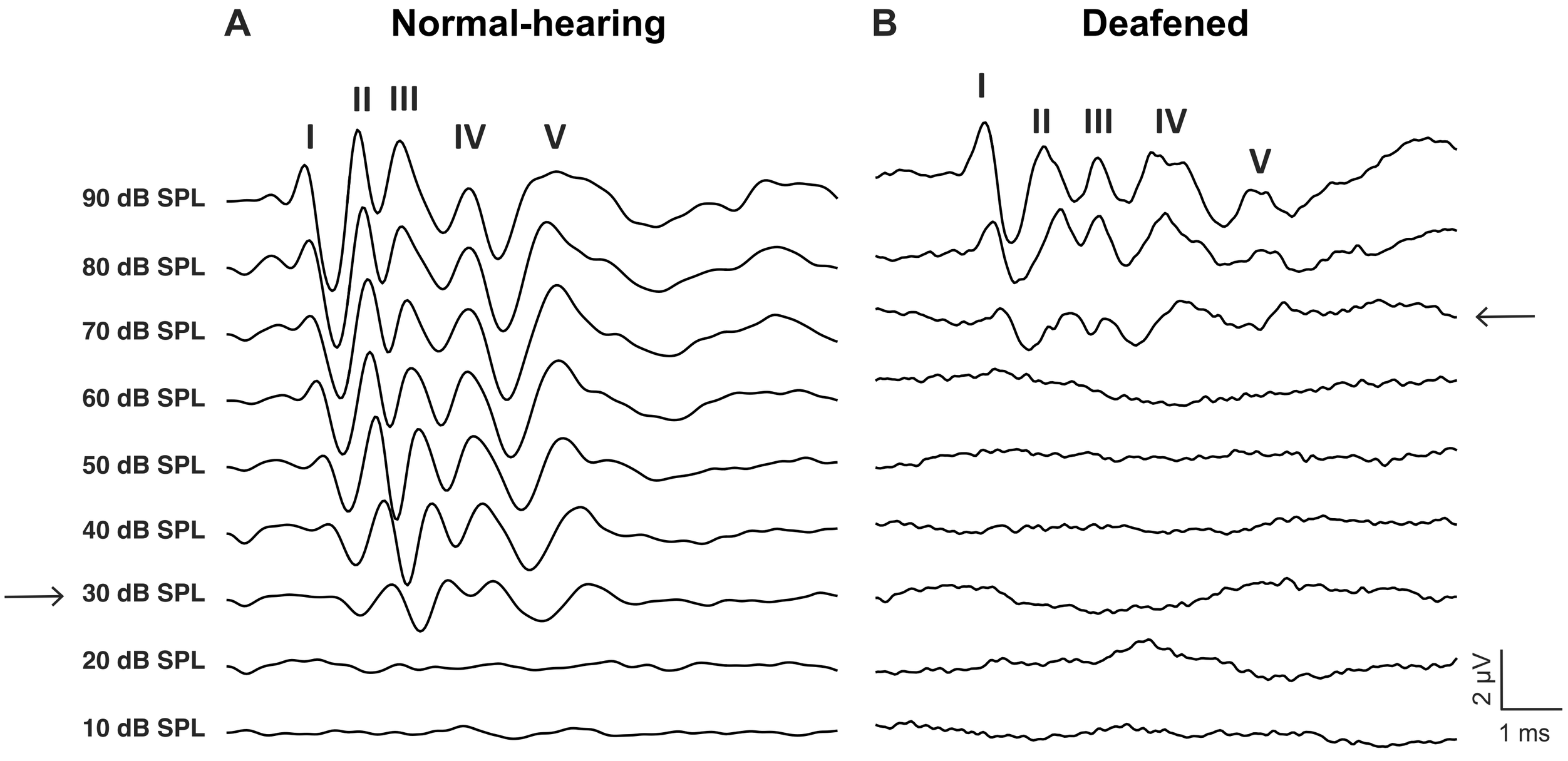


Figure3

Click here to download Figure Figure3.ai

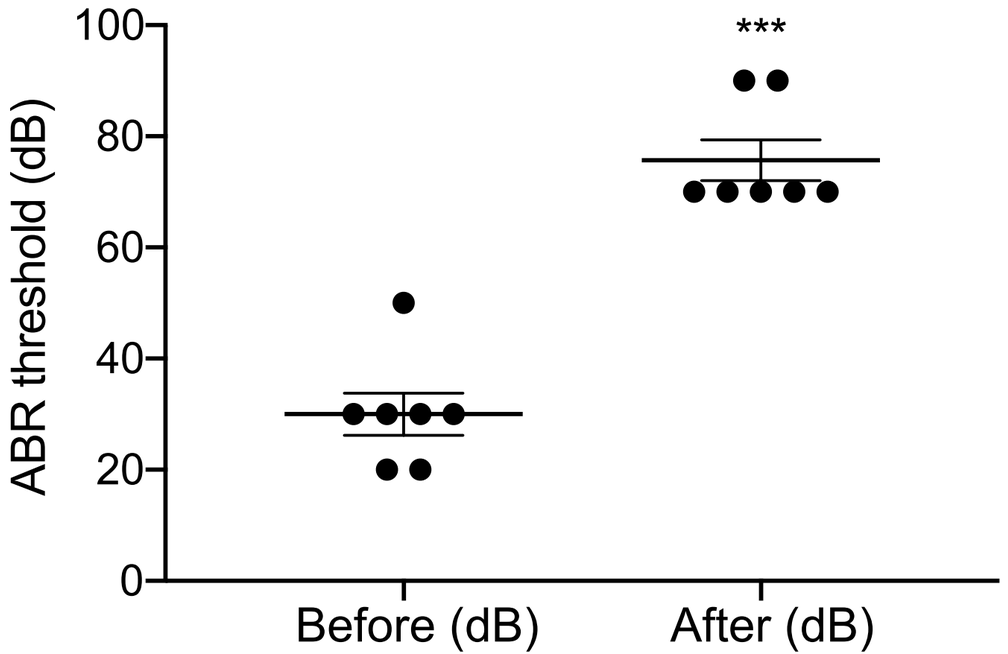
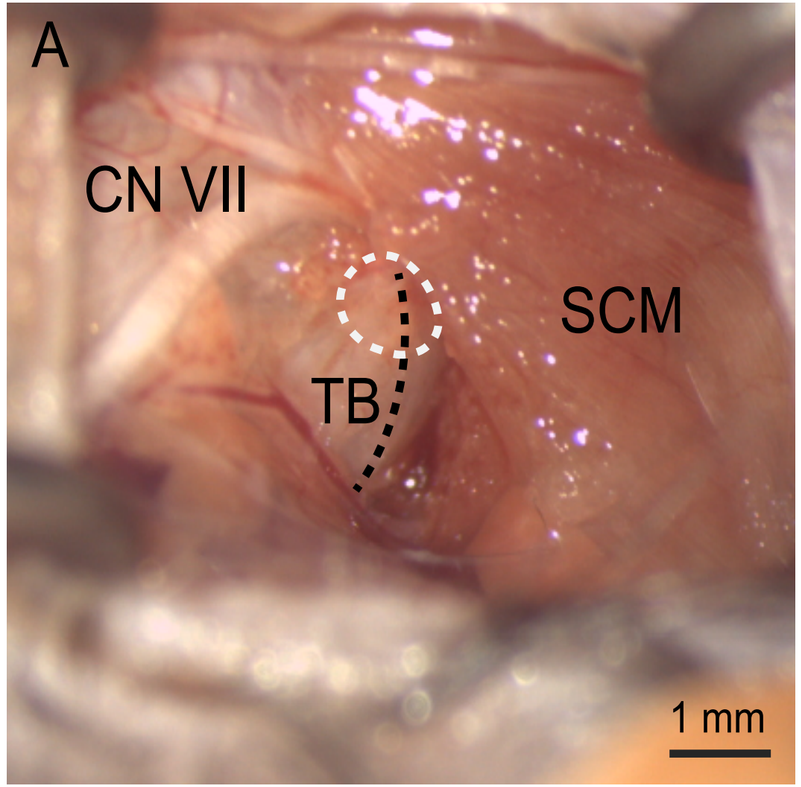
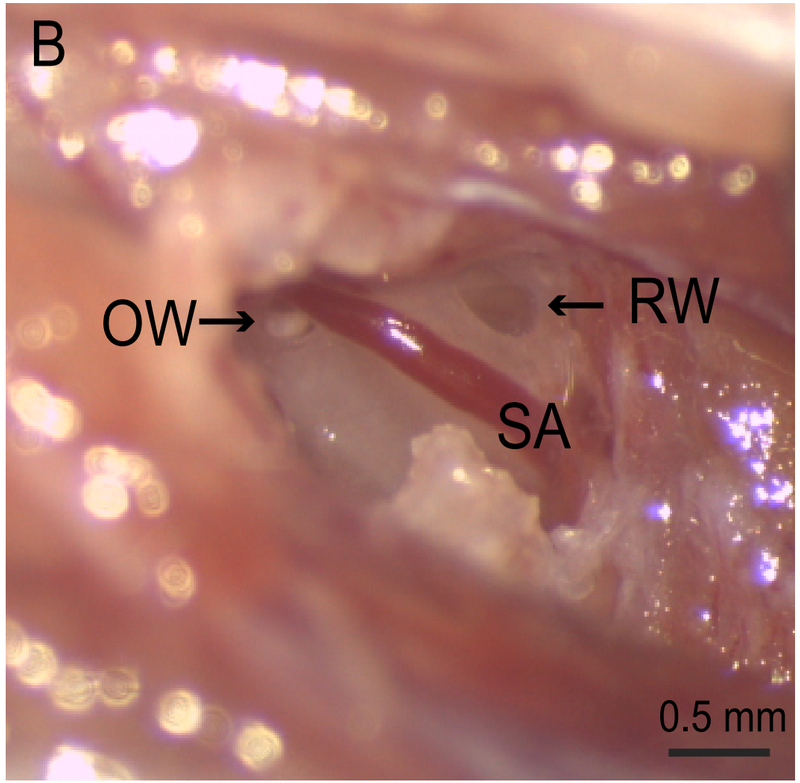


Figure4

Click here to download Figure Figure4.ai





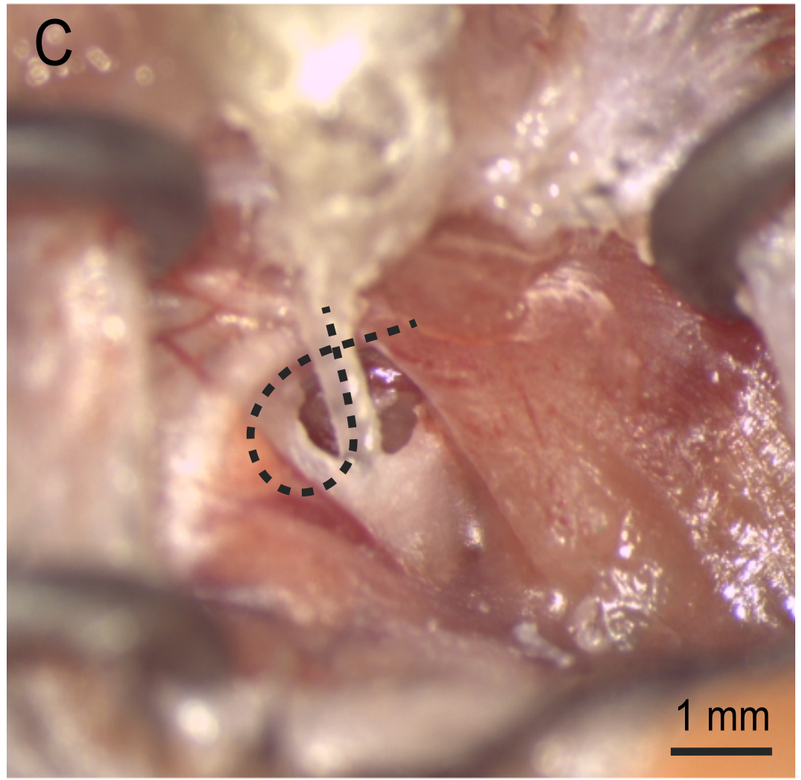
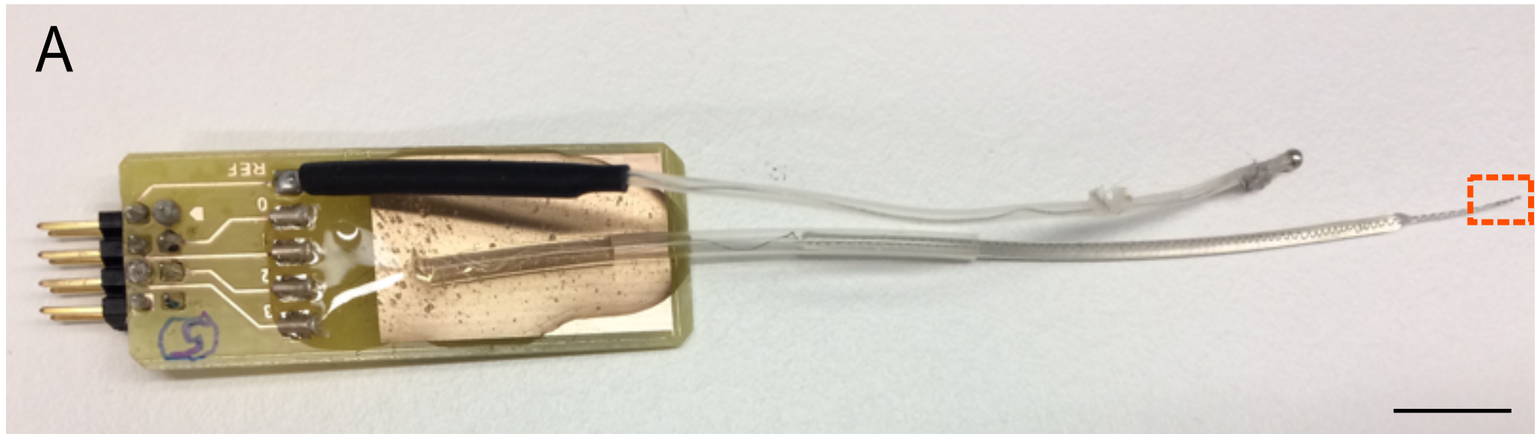


Figure5

Click here to download Figure Figure5.ai



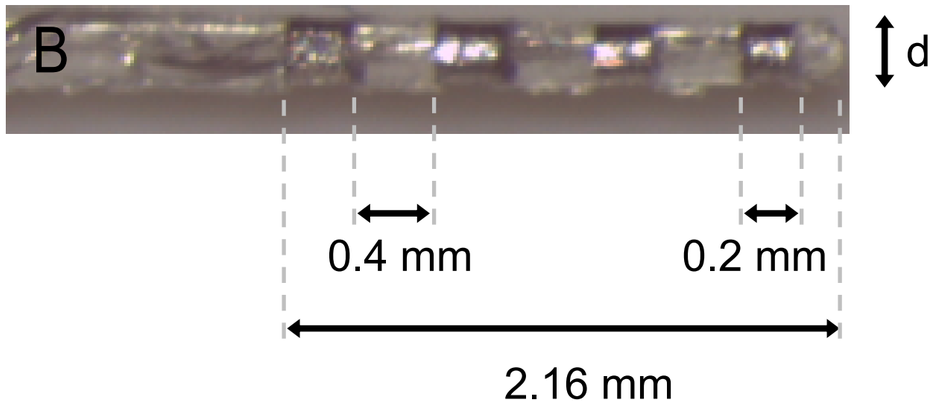


Figure6

Click here to download Figure Figure6.ai

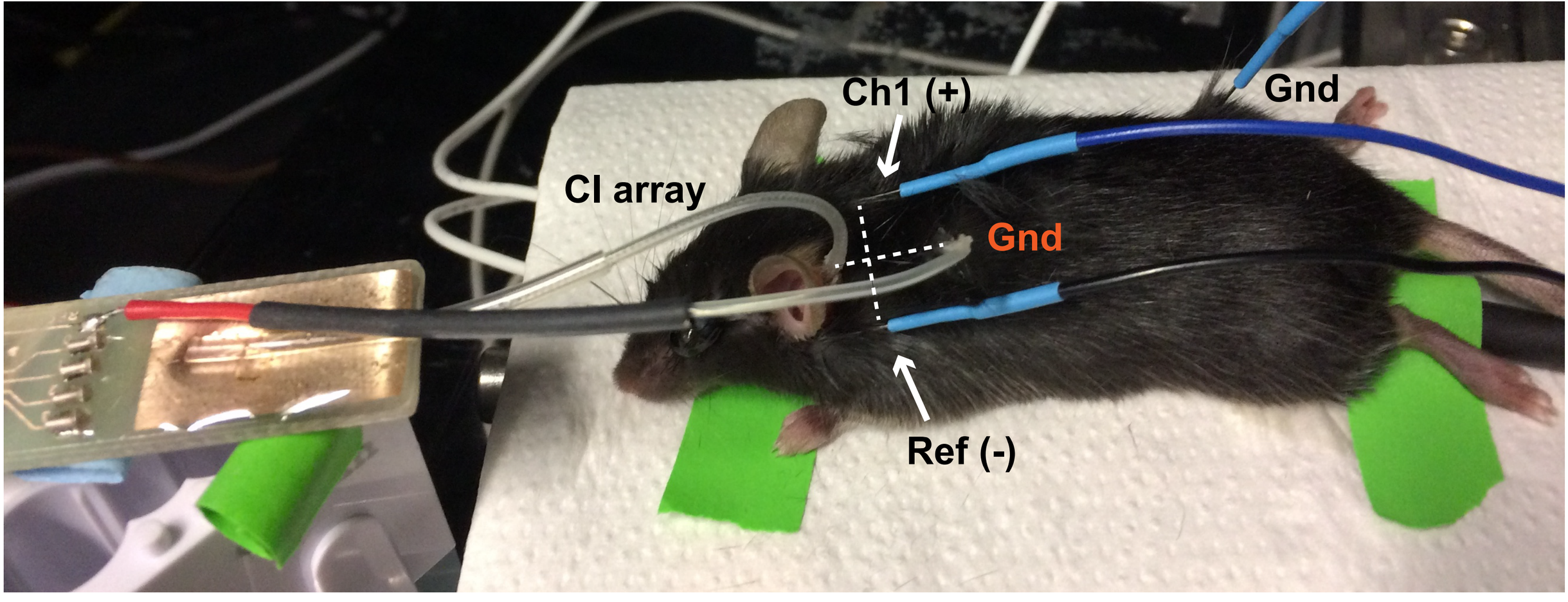
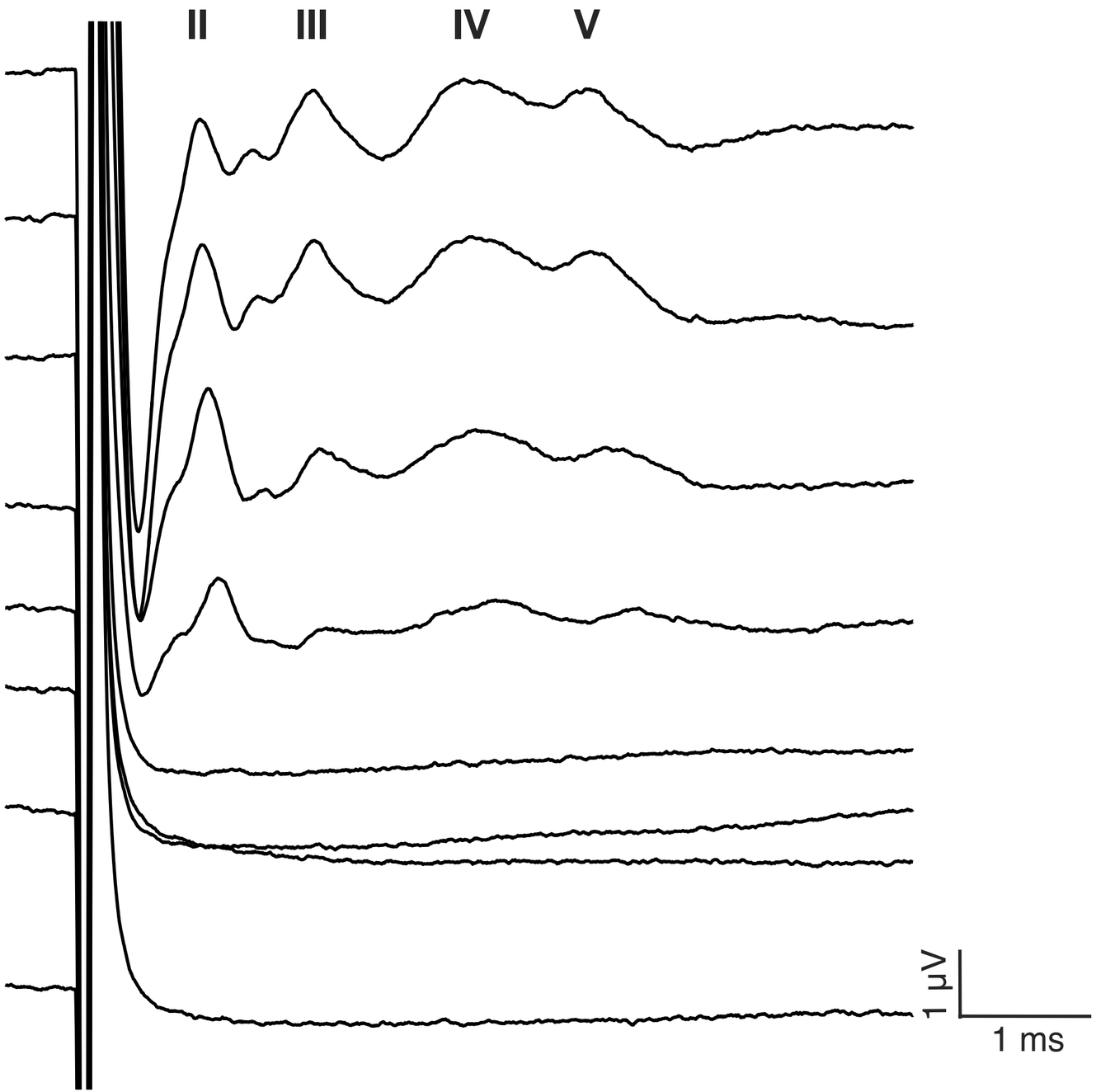
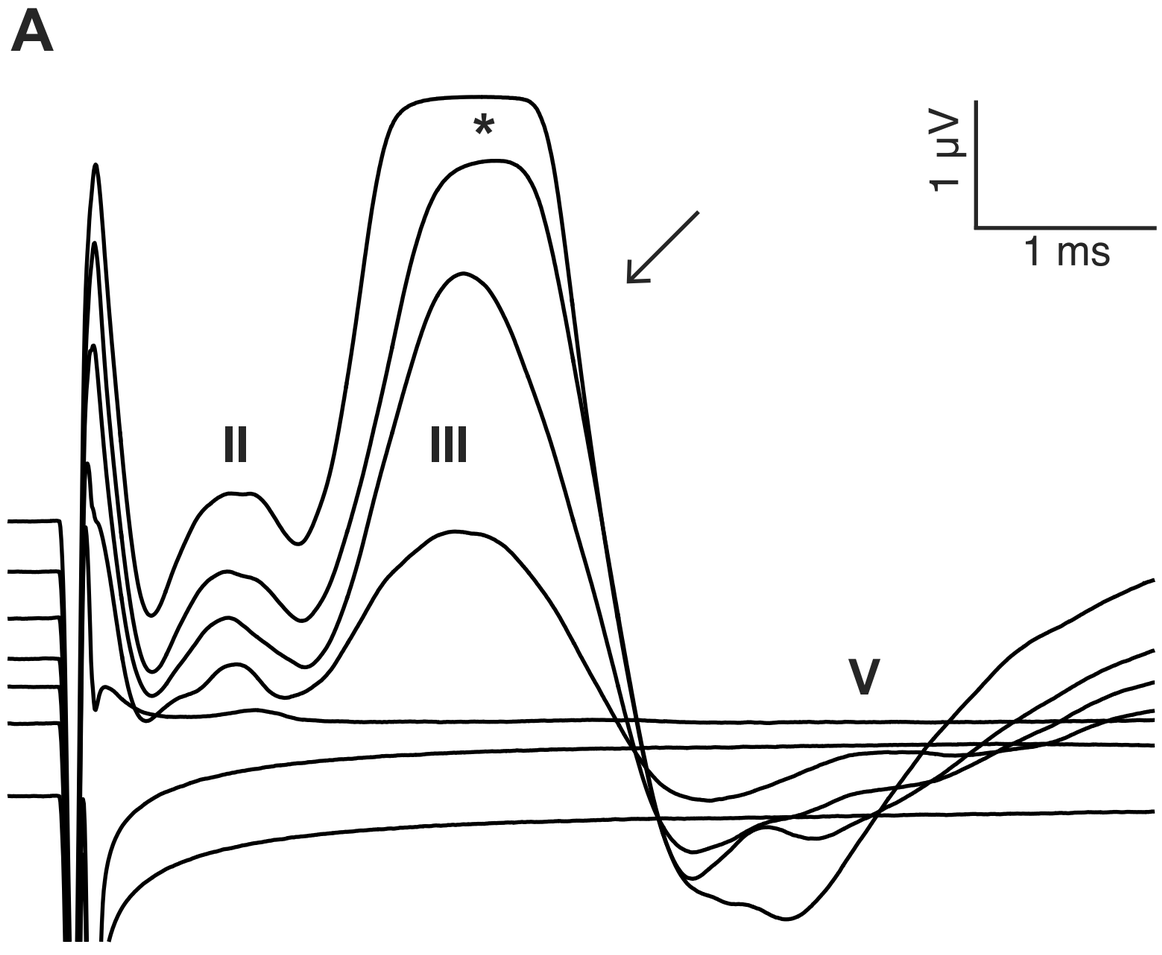
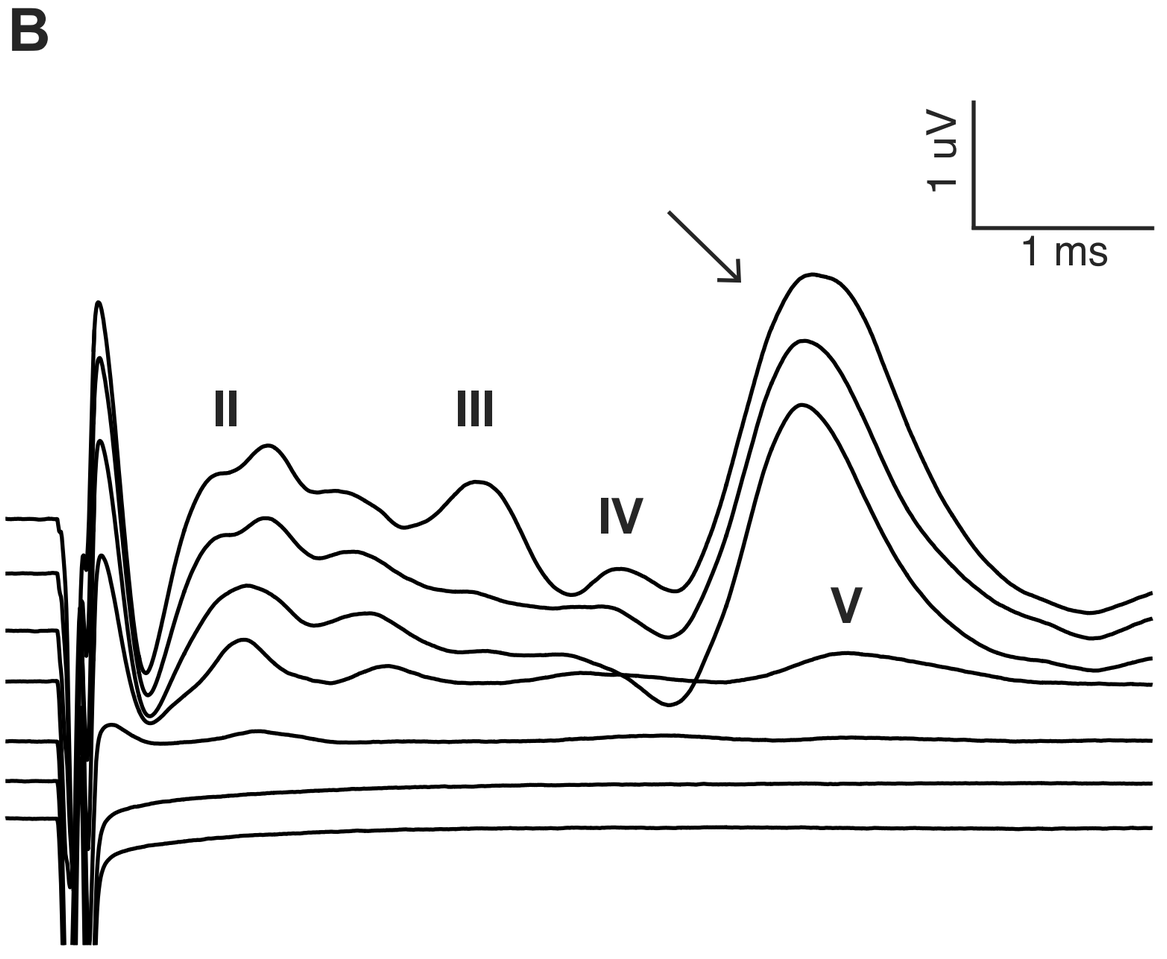


Figure7

Click here to download Figure Figure7.ai







Materials Table

Click here to download Materials Table JoVE_Materials_resubmission.xls

Name of Material/Equipment	Company	Material Number	Comments
Hardware			
Sound-proof booth	IAC Acoustics, Winchester, UK	Mac-2 Enclosure RF Shielded Box 2A	
MF1 Speaker	Tucker Davis Technologies (TDT), FL, USA		
PCB microphone	PCB Piezotronics, Inc, NY, USA	Model 378C01	
Low impedance headstage	TDT, FL, USA	RA4LI	
Medusa pre-amplifier	TDT, FL, USA	RA4PA	
RZ6 auditory processor	TDT, FL, USA		
Animal Stimulator Platform	ASP, Oticon Medical, Nice, France		
Multimeter	Fluks	#115	
Surgical equipment			
Closed-loop heating pad	FHC, Inc. ME, USA		
Eye ointment	Alcon, CH	Lacrinorm Augengel	
Acoustic foam	Otoform Ak, Dreve Otoplastik GmbH	#464	
Disposable subdermal needle electrodes	Horizon, Rochester Electro-Medical Inc.	S83018-R9, 27G	
Self-retaining retractor tool (Mini Collibri Retractor)	Fine Science Tools	#17000-01	
Suction wedges	Agnthos, SE	#42-886-460	
Absorbable paper point (Medium)	WPI, FL, USA	#504182	
Intracochlear electrode array	Bionics Institute, Melbourne, Australia	4 channel	
Spongostan Standard	Ferrosan Medical Devices	#MS0002	
Tissue glue. Loctite 4161 Superbond	Henkel	Part No 19743	
Animal Stimulator Platform (ASP)	Oticon Medical, Nice, France		
Drugs/chemicals			
Ketamine (Narketan)	Provet AG, CH	100mg/mL, #VQ_320265	
Xylazine (Rompun)	Provet AG, CH	Inj Diss 2%, # 1315	

Bupivacaine	Compendium, CH	Bupivacain Sintetica inj Diss 0.5%	
Atropine (Atropinesulfat Amino)	Amino AG, CH	1 mg/ml	
Betadine (Povidone/iodine)	Provedic, CH		
Neomycin (Neomycin trisulfate salt)	Sigma	N1876-25G, Lot#WXBB7516V	
Software			
BioSigRZ	TDT, FL, USA		
Matlab	MathWorks, MA, USA		
ASP software	Oticon Medical, Nice, France		

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Protocol for cochelar implant surgery and electrically-evoked auditory brainstem response recordings in C57BL/6 mice

Author(s): Charlotte Amalie Navntoft, Jeremy Marozeau, Tania Rinaldi Barkat

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/author) via: ☐ Standard Access ☑ Open Access

Item 2 (check one box):

☑ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name:	Charlotte Amalie Navntoft
Department:	Department of Biomedicine, Basel University, Basel, CH
Institution:	Department of Electrical Engineering, Technical University of Denmark, Kgs. Lyngby, DK
Article Title:	Protocol for cochelar implant surgery and electrically-evoked auditory brainstem response recordings in C57BL/6 mice
Signature:	Date:

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

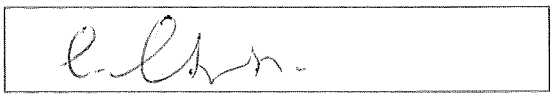
11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name:	Charlotte Amalie Navntoft
Department:	Department of Biomedicine, Basel University, Basel, CH
Institution:	~~Department of Electrical Engineering, Technical University of Denmark, Kgs. Lyngby, DK~~
Article Title:	Protocol for cochelar implant surgery and electrically-evoked auditory brainstem response recordings in C57BL/6 mice
Signature:	 Date: FEB 26 2018

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

DTU Elektro

To Dr. Vineeta Bajaj
Editor of *Jove*

Copenhagen May 2nd 2018

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

CVR-nr. DK 30 06 09 46

Technical University of Denmark
Department of Electrical Engineering
Ørsteds Plads
Building 352
2800 Kgs. Lyngby
Tlf. 45 25 39 30
Dir. +45 45255832
Fax 45 88 05 77
www.elektro.dtu.dk
chamnav@elektro.dtu.dk

<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.

2. *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.

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

Corrected as requested.

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

Corrected as requested.

5. *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 diameter of the electrodes. The denotation of *d* has been added to the figure text of **Figure 5**.

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

Corrected as requested.

7. *Title: Please simplify the title by deleting "Protocol for".*

Corrected as requested.

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

Yes, we did. It is now corrected.

9. *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.

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

Corrected as requested.

11. *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.

12. *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.

13. *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.

14. *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.

15. *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.

16. *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.

17. *2.3? 70% ethanol? Or absolute?*

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

18. *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.

19. *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.

20. *3.5: Blunt dissection with what?*

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

21. *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.

22. *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.

23. *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.

24. *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.

25. *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.

26. *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.

27. *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.

28. *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.

2. *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.

3. *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.

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

We agree and have corrected the sentence.

3. *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.

4. *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.

5. *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.

6. *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.

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

We agree and have corrected the sentence.

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

We agree and have corrected the sentence.

Data Example Recording

Matlab script