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Corresponding Author: Dr. Robert Shepherd,

Corresponding Author's Institution:

First Author: Samuel Irving, PhD

Order of Authors: Samuel Irving, PhD; Matthew I Trotter; James B Fallon; Rodney E Millard; Robert K Shepherd; Andrew K Wise

Abstract: The mouse is becoming an increasingly attractive model for auditory research due to the number of genetic deafness models available. These genetic models offer the researcher an array of congenital causes of hearing impairment, and are therefore of high clinical relevance. To date, the use of mice in cochlear implant research has not been possible due to the lack of an intracochlear electrode array and stimulator small enough for murine use, coupled with the difficulty of the surgery in this species. Here, we present a fully-implantable intracochlear electrode stimulator assembly designed for chronic implantation in the mouse. We describe the surgical approach for implantation, as well as presenting the first functional data obtained from chronic intracochlear electrical stimulation in the mouse.

***Highlights (for review)**

Mouse paper highlights:

- We describe a procedure for cochlear implantation in the mouse
- The first functional data for intracochlear electrical stimulation in the mouse is presented
- Normal-hearing mice are successfully deafened using neomycin
- Chronic cochlear implantation and electrical stimulation can be performed in mice
- Cauterising the stapedial artery has no effect on hearing thresholds in mice

Cochlear implantation for chronic electrical stimulation in the mouse

S. Irving[1,2], M. I. Trotter[1], J. B. Fallon[1,3,4], R. E. Millard[1], R. K. Shepherd[1,3,4], and A. K. Wise[1,3,4]

[1]Bionics Institute, Melbourne, Australia [2]Department of Psychology, [3]Department of Otolaryngology and [4]Department of Medical Bionics, University of Melbourne, Australia

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Corresponding author:

R. K. Shepherd

The Bionics Institute, 384-388 Albert St, East Melbourne 3002, Victoria, Australia. FAX: +613 96677505

rshepherd@bionicsinstitute.org

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Abstract

The mouse is becoming an increasingly attractive model for auditory research due to the number of genetic deafness models available. These genetic models offer the researcher an array of congenital causes of hearing impairment, and are therefore of high clinical relevance. To date, the use of mice in cochlear implant research has not been possible due to the lack of an intracochlear electrode array and stimulator small enough for murine use, coupled with the difficulty of the surgery in this species. Here, we present a fully-implantable intracochlear electrode stimulator assembly designed for chronic implantation in the mouse. We describe the surgical approach for implantation, as well as presenting the first functional data obtained from intracochlear electrical stimulation in the mouse.

1. Introduction

Experimental animals have long been used in auditory research, playing an important role in establishing the safety and efficacy of cochlear implants. With the exception of the congenitally Deaf White Cat (i.e. Hartmann et al., 1997; Ryugo et al., 2010; Klinke et al., 1999), these animals are typically deafened by experimental intervention, such as administration of ototoxic drugs (Shepherd and Martin, 1995; Fallon et al., 2009; Ryugo et al., 2010; Leake et al., 1999), or loud noise exposure (Eggermont and Komiya, 2000; Robertson and Anderson, 1994; Sekiya et al., 2012) to produce a sensorineural hearing loss. Although relevant to clinical populations, these deafness models do not encompass the numerous genetic causes of deafness which are highly prevalent in clinical populations (Morzaria et al., 2004). The etiology of deafness is a significant factor in how deafness-induced changes manifest in the auditory brain (Ryugo et al., 2010;

Klinke et al., 1999; Sekiya et al., 2012), and may therefore influence the efficiency of intracochlear electrical stimulation, as well as synaptic plasticity and cell survival.

Recent research has shown a large increase in the number of genetic mouse models for deafness – at the time of writing, the Jackson Laboratory website alone lists 106 mouse models of human hearing disorders - making the mouse an increasingly attractive model for hearing research (Ahituv and Avraham, 2002; Ohlemiller, 2006). The combination of murine genetic knockouts with intracochlear electrical stimulation would provide critical information on the mechanisms of electrical stimulation in the cochlea. To date, no method has enabled the delivery of chronic electrical stimulation to the mouse cochlea, primarily due to the absence of a reliable electrode-stimulator assembly small enough for chronic murine use, and the difficulty of the implantation surgery in such a comparatively small cochlea. To compound this surgical difficulty, the mouse, like the rat (Lu et al., 2005), has a stapedial artery (SA) that runs inside the middle ear cavity close to the round window (RW) niche, reducing surgical access to the cochlea and increasing surgical risk

We describe the procedure for cochlear implantation of an electrode array in the mouse, with our custom-designed fully-implantable small rodent stimulator (Millard and Shepherd, 2007). We also discuss the need for cauterizing the SA, present functional recordings during intracochlear electrical stimulation, and present histological analysis of cochlear tissue implanted with this device.

2. Methods

All procedures were approved by the Royal Victorian Eye and Ear Hospital animal research Ethics Committee in accordance with the Australian National Health and Medical Research Council's animal experimentation guidelines.

2.1. Subjects

Development of the mouse cochlear implant required a robust animal model that could undergo cochlear implantation in order to optimise the surgical approach and confirm functional activation of the auditory pathway. To this end, C57BL/6 adult mice (n=29) aged 8-10 weeks (weight 20-30 g) were used in this study. Individual animals were divided into experimental groups, undergoing the procedures indicated in Table 1 and described below. Normal hearing was confirmed in all animals by recording auditory brainstem responses (ABRs, see 2.4.1) one week before surgery. The optimum concentration of the aminoglycoside neomycin required for deafening without adverse effects was explored, and administration via the RW permitted the contralateral ear to be used as an intra-animal control.

[TABLE 1 ABOUT HERE]

2.2. Electrode array and stimulator

The fully-implantable stimulator has been described previously (Millard and Shepherd, 2007). Briefly, the stimulator consisted of an electromagnetic coil (Sonion; Passive Telecoil T20 AG 12, 9012-1050184), a bi-directional current regulator, a capacitor and bipolar electrodes (Figure 1). The stimulator was encapsulated in conformal coating and medical grade silicone to prevent

89 ingress of fluid, was compact (3 x 10 mm), and weighed less than 1 g (~3% of the weight of an
90 adult mouse), offering minimal hindrance to the implanted animal.

91

92 [FIGURE 1 ABOUT HERE]

93

94 The stimulator was driven by an external magnetic field that induced current through the
95 current regulator and capacitor to the electrodes situated inside the cochlea. The magnetic field
96 was provided by an excitation coil assembly (not shown; see Millard and Shepherd 2007),
97 composed of three orthogonal coils around an animal enclosure, which allowed the animal to
98 move freely during stimulation. The stimulator produced charge-balanced biphasic current
99 pulses with fixed amplitude. As the current produced by the stimulator circuit was constant, the
100 delivered charge was determined by the width of the magnetic pulse and subsequent current
101 pulse (25-250 μs). To prevent undesirable build-up of charge, the electrodes were shorted
102 between stimuli when the coils were not excited. Pulse width and stimulation rate (50-5000 Hz)
103 were varied using a computer-controlled external control unit.

104 The intracochlear electrode array consisted of four 0.2 mm diameter platinum bands spaced at
105 0.4 mm intervals (Figure 2). The electrode array was designed to have 3 intracochlear
106 electrodes and a platinum marker used as an extracochlear reference during insertion. For the
107 purpose of this study, only the tip and third bands were attached to the stimulator, via helical
108 platinum leadwires embedded in a silicone carrier. This configuration allowed delivery of 'wide'
109 bipolar stimulation. Once assembled, the implant, leadwire, and stimulator assembly was 70
110 mm in length (Figure 2). The leadwire helix was narrowest at the tip (diameter 0.2 mm) and
111 tapered out over 1 cm to its maximal diameter (~0.8 mm) to enable intracochlear insertion and
112 coiling inside the bulla (see Figure 3). After the taper, the leadwire diameter increased to ~1
113 mm to improve the robustness of the device. A Dacron mesh (Invista, USA) flange was placed

30 mm from the tip of the leadwire and another piece of Dacron was also used to cover the stimulator to encourage tissue adhesion and limit movement of the device under the skin (Figure 2).

[FIGURE 2 ABOUT HERE]

2.3. Surgical approach

All surgeries were performed under ketamine (100 mg/kg) and xylazine (6 mg/kg) anaesthesia, delivered in a combined interperitoneal injection. If further anaesthesia was required, a supplement at 1/3 dose was administered intramuscularly.

2.3.1. Accessing the Round Window

In each animal, surgery was carried out on the left ear. After shaving the fur and antiseptic preparation with alcohol and betadine, topical injections (< 0.05 ml) of anaesthetic (lignocaine with adrenaline, 1:200000, 3 mg/kg) were administered into the skin along the intended incision line before making a post-auricular incision. The sternocleidomastoid muscle was then retracted to reveal the facial nerve extending over the bulla. Under a surgical microscope, the tissue overlying the medio-dorsal area of the bulla was removed with a bone-scraper to allow clear visualization of the ridge between the bulla and the mastoid process (Figure 3A). A 0.6 mm diameter diamond burr was then used to expose the middle ear cavity, taking care not to damage the SA (Figure 3B). The bullostomy was extended dorsally towards the mastoid process until the RW niche was clear of overlying bone.

[FIGURE 3 ABOUT HERE]

2.3.2. Cauterizing the Stapedial Artery and Neomycin deafening

The mouse has a relatively large SA within the middle ear, running ventral to the RW niche (Figure 3B), which can cause a fatal bleeding hazard during implantation via the RW. The effects of cauterizing the SA in the mouse have not previously been described, but research in the rat, which also has a prominent SA overlying the RW niche, has shown no adverse effects on hearing thresholds or spiral ganglion neuron survival (SGN; Lu, Xu et al., 2005). In order to determine the effects of cauterizing the SA on hearing thresholds and SGN survival in the mouse, the SA was cauterized in 5 animals. The surgical approach was similar to that described in the rat by Lu and colleagues (2005): the bullostomy was drilled as above, the SA was then cauterized within the bulla using bipolar cautery forceps. The bulla was then closed with sterile polycarboxylate cement (Durelon, ESPE, Norristown, USA), and the wound closed in two layers using an absorbable 6/0 suture. Four weeks following surgery, an ABR was recorded to assess the effects of the procedure on hearing.

As C57BL/6 mice have normal hearing we developed a deafening technique for use in combination with the fully implantable stimulator. In order to quantify the effects of this deafening technique on hearing thresholds and SGN survival, 6 animals were deafened unilaterally using the ototoxic aminoglycoside neomycin. The surgical approach was identical to that presented above, including cauterizing the SA. The RW membrane was then perforated using a probe, and perilymph was slowly aspirated. Concentrations of either 5% or 10% weight/volume neomycin (n = 3/cohort) were then slowly perfused through the RW. Next, a 1 mm^2 piece of gelatine sponge (Gelfoam, Pfizer, New York, USA) soaked in neomycin being placed within the RW niche. The bulla was then closed with sterile polycarboxylate cement, and the

wound closed in two layers using an absorbable 6/0 suture. Four weeks following surgery, an ABR was recorded to evaluate hearing status.

2.3.3. *Acute implantation*

Acute implantation of the intracochlear electrode array was carried out in 4 animals to ensure the dimensions of the electrode arrays were suitable for this species. Two of these animals underwent surgery as above. Following local perfusion of the cochlea with neomycin, an acute intracochlear electrode array was inserted through the RW into scala tympani to a depth where the 4th platinum (marker) ring was located at the RW, giving an insertion depth of approximately 2mm, corresponding to an intracochlear position located at approximately 30 kHz (Muller et al., 2005). The leadwire of the electrode array was connected to a laboratory stimulator to provide electrical stimuli. In the remaining 2 animals, the surgical approach was the same as detailed above, with the exception that the SA was left intact (Figure 3) to evaluate the possibility of cochlear implantation in the mouse without cauterizing the SA. All acute implantation animals had the electrode array secured inside the bulla using polycarboxylate cement. Electrical thresholds were determined by measuring electrically-evoked ABRs (EABRs; see below) directly after surgery to demonstrate the functional efficacy of this procedure.

2.3.4. Chronic implantation

The implantation of the chronic stimulation assembly (Figure 2) followed the same steps detailed above (including cautery of the SA), up to and including slow intra-cochlear perfusion of 5% neomycin. At this stage, the chronic electrode array was inserted as above, and the leadwire was curled inside the bulla (Figure 3D) and fixed in place using polycarboxylate cement. A subcutaneous pocket was blunt-dissected from the scapula down to the area above

188 the hind leg, which was shaped to house the stimulator and leadwire without any tension
189 (Figure 4). The Dacron mesh on the stimulator and leadwire were not sutured to surrounding
190 tissue, but were used to promote tissue adhesion and prevent excess subdermal movement of
191 the stimulator. The wound was then sutured in two layers using 6/0 monocryl.

192

193

194 [FIGURE 4 ABOUT HERE]

195

196 ## 2.4. Functional recordings

197 ### *2.4.1. Auditory brainstem responses (ABR)*

198

199 ABR recordings were used to determine hearing thresholds prior to and after experimental
200 procedures. This procedure has been detailed elsewhere (Shepherd and Clark, 1985; Short et al.,
201 2011). Briefly, ABR recordings were performed in a sound-attenuated and electrically-shielded
202 Faraday room under ketamine (100 mg/kg) and xylazine (6 mg/kg) anaesthesia as detailed
203 above. Click trains (100 reps; 100 μs click; 0-100 dB peak equivalent Sound Pressure Level
204 (SPL)), were generated in a custom IgorPro (Wavemetrics, Lake Oswego, USA) procedure, and
205 presented via a calibrated Richard Allen DT-20 loudspeaker situated at 10 cm from the ear. The
206 contralateral ear was blocked using earmould compression material (Otoform-k, Dreve-
207 Otoplastik, GMBH, Unna, DE). ABRs were recorded using stainless steel electrodes positioned on
208 the snout, vertex and the nape of the neck (ground, positive and negative respectively).
209 Responses were amplified by 10^5 via a World Precision Instruments Bio-80 amplifier and
210 filtered with a Krohn Hite band pass filter (high pass 150 Hz, 24 dB/octave; low pass 3 kHz, 6
211 dB/octave) before digitising with a 10 bit converter sampled at 20 kHz for 12 ms following the

initial click. Stimulus level was varied by 5-10 dB increments. Threshold was determined visually as the lowest amplitude required to produce a clear wave III response (2.5-4 ms post-stimulus). Where thresholds were higher than 100 dB SPL, a threshold of 110 dB SPL was assigned to allow for quantitative analyses.

2.4.2. Electrically-evoked auditory brainstem responses (EABR)

To confirm successful stimulation of the auditory pathway by the intracochlear electrode array, EABRs were recorded using a laboratory stimulator in the 4 animals that received an acute implant. The recording of EABRs has been described elsewhere in guinea pig (Landry et al., 2011). In brief, animals were anaesthetised as for ABRs (see above) and EABRs were recorded in response to biphasic pulse trains (phase: 100 μs, interphase gap: 50 μs; amplitude: 0-1 mA in 20 μA steps) generated by a custom IgorPro procedure and presented via a laboratory stimulator. Recording procedures were the same as for ABRs, with the exception of the inclusion of a sample and hold circuit to minimise stimulus artefact (Black et al., 1983). Threshold was determined visually as for ABRs.

2.4.3. Magnetically-induced, electrically-evoked auditory brainstem responses (mEABR)

To determine electrical threshold in animals implanted with the fully implantable mouse stimulator assembly, magnetically-induced EABRs (mEABRs) were recorded one week after surgery. Additional mEABRs were recorded at the end of the 4-week experimental period to confirm the correct functioning of the fully implanted device. This procedure is detailed in

Millard and Shepherd (2007) for the rat, and is described here briefly. Recordings of mEABRs were the same as EABRs, with the addition that the animal was placed within a small tube surrounded by a single electro-magnetic coil to cover and drive the implanted stimulator (note: this differs from the excitation coil stimulating enclosure used to provide chronic stimulation). The animal's head and the recording electrodes remained outside of the magnetic field to minimise interference. Charge delivery was controlled by varying the duration of the stimulus phase (e.g. 15- 100 μs in 5 μs steps) via a custom IgorPro procedure. Recording procedures were the same as for EABRs. mEABRs were successfully obtained in each of the 3 animals in the chronic stimulation group.

2.5. Chronic Stimulation

Chronic electrical stimulation was carried out in 3 mice (Table 1). Animals were placed, awake, within the excitation coil stimulating enclosure and stimulated at up to 6 dB above their mEABR threshold for 4 hours/day for up to one month.

2.6. Histological analysis

After the final functional recording, each animal was euthanized with an overdose of lethabarb (Virbac, Milperra, Australia; 150mg/kg) and systemically perfused using 20 ml of 10% neutral buffered formalin (NBF). Both cochleae were dissected, the oval window perforated and the cochleae postfixed for 2 hours, followed by 3 washes in 0.1 M phosphate buffered solution (PBS). Cochleae were then decalcified in 4 % ethylenediamine tetraacetic acid (EDTA) in 0.1 M PBS before cryoprotecting in 15% and then 30% sucrose. Cochleae were subsequently

infiltrated with 1:1 30% sucrose and optimum cutting temperature (OCT) compound, followed by 100% OCT under a vacuum to degas (Coleman et al., 2009). The cochleae were oriented and snap frozen with dry ice before sectioning at 12 µm with a cryostat and mounting every third section onto a Superfrost Plus slide (Menzel-Gläser, Braunschweig, Germany). Sections were then stained with 1% Harris haematoxylin and eosin.

2.6.1. SGN counts

SGNs containing an intact soma and visible nucleolus were counted in mid-modiolar sections after delineating and measuring Rosenthal's canal at basal and apical regions in 3 non-sequential sections using ImageJ (National Institute of Mental Health, Maryland, USA). All cell counts were carried out by the same counter who was blind to the subject group, and SGN density was calculated bilaterally and compared between cochleae. Other histological features such as electrode insertion trauma and inflammatory tissue response were also noted, and their extent was determined by measuring the area of the scala tympani and the area of the tissue response within the affected cochlear region using ImageJ. The tissue response was further divided into fibrous tissue and new bone growth.

2.6.2. Electrode array position

In order to determine the position of the electrode array within scala tympani, an additional animal was implanted with an electrode, using the procedure described in section 2.3.4, and perfused with NBF. The cochlea, with the electrode array *in situ*, was then prepared for epoxy embedding, as per Shepherd, Verhoeven et al. (2011). Briefly, the leadwire was secured at the exit of the bulla using cyanoacrylate glue and the stapes footplate was removed. The cochlea,

with the array in place, was then dehydrated by progressively more concentrated ethanol (from 70% to 100%) before being immersed in degassed epoxy resin and subjected to a vacuum to ensure complete infiltration. Images were collected throughout the implanted cochlea along the midmodiolar plane using the 300 µm grinding technique and toluidine blue stain detailed in Shepherd, Verhoeven et al. (2011).

2.7. Statistical analysis

Except where indicated in the text, repeated measures analyses of variance (ANOVAs) were carried out with SPSS 18 (SPSS Inc., Chicago, US) to determine the main effects between ABR thresholds for different groups (Cautery, 5% neomycin, 10% neomycin, implanted unstimulated and chronically stimulated) at different time points (before/after intervention) and for each side (experimental/control; ABR only), as well as SGN counts between groups, by cochlear side (experimental/control) and by cochlear position (base/apex).

3. Results

In order to optimise the surgical approach and functional testing of the fully-implantable intracochlear electrode assembly, experimental procedures were undertaken incrementally, with each result informing the subsequent procedure.

306 ### 3.1. Surgical considerations: Effects of cauterizing the SA

307

308 All animals in the SA cautery group displayed click-evoked ABR waveforms before and 4 weeks
309 after surgery (e.g. Figure 5), and although increases in ABR threshold were observed in the left
310 experimental ear in some animals (up to 40 dB HL), others did not exhibit increased thresholds
311 (mean thresholds shift: 21 dB HL ± 9.5). SA cautery was successful in all cases (Figure 6). There
312 was some evidence of heat/surgical damage to the cochlea in some animals, which may have
313 contributed to the increased ABR thresholds. This could be avoided by further surgical
314 refinement. In order to determine the significance of the shift in ABR threshold pre- and post-SA
315 cauterization with this non-normal distribution, a Wilcoxon test was carried out, which
316 indicated that the threshold shift was non-significant ($p = .102$). There was also no significant
317 difference between ABR thresholds on the left (experimental) and right (control) sides for this
318 group, either pre- ($p = 0.37$) or post-SA cautery ($p = 0.141$). As expected from the lack of
319 significant changes in ABR threshold, cauterizing the SA did not affect SGN densities, compared
320 to the uncauterized control ear, in either the base ($p = 0.68$) or the apex ($p = 0.34$) of the cochlea
321 (Table 2).

322

323 [FIGURE 5 ABOUT HERE]

324

325 [FIGURE 6 ABOUT HERE]

326

327 ### 3.2. Effects of neomycin deafening

328

Animals deafened with 5% and 10% neomycin, combined with SA cauterization, showed significant increases in click-evoked hearing thresholds for the experimental cochlea (pre- vs. post-surgery; 5% neomycin: 41.6 dB ± 3.3 to 76.6 dB ± 4.4, $p = 0.02$; 10% neomycin: 48.3 dB ± 6 to > 100 dB $p = 0.006$ respectively; an average of 35 dB HL for 5% and 58.3 dB HL for 10%). For 5% neomycin, the contralateral ear did not display a significant change in threshold ($p > 0.5$), but 10% neomycin caused significantly increased thresholds in the contralateral, non-experimental ear for the 3 animals in this group ($p = 0.018$). This loss of hearing suggests some transit of the drug at higher concentration to the contralateral cochlea, although the increase in threshold was not as pronounced as that in the treated ear ($p = 0.004$). Interestingly, despite the significant increases in hearing thresholds with the use of neomycin, there was no significant decrease in SGN densities between treated and untreated contralateral control cochleae at either the base (Table 2; 5%: $p = 0.25$; 10%: $p = 0.33$) or the apex (Table 2; 5%: $p = 0.3$; 10%: $p = 0.14$) four weeks post-deafening. Furthermore, no evidence of tissue response was seen in these animals. Due to the bilateral, but asymmetric hearing loss caused by the higher concentration (10%), 5% neomycin was used in all mice detailed below to ensure that ABR thresholds in the contralateral cochlea were unimpaired.

3.3. Cochlear implantation

3.3.1. Acute implantation

EABRs were successfully obtained using a laboratory stimulator in the acutely implanted animals (not shown), and histology confirmed the location of the stimulating array in scala tympani, with excellent positioning adjacent to the SGN cell bodies (Figure 7).

[FIGURE 7 ABOUT HERE]

3.3.2. Chronic stimulation

mEABRs were recorded one week post-surgery in the chronic stimulation group, and at the end of the one-month experimental period (Figure 8) in two of the three chronically-stimulated animals. Behavioural evidence of stimulation was generally seen and manifested as either a halt or an increase in movement after the device was switched on, presumably indicating perception of the stimulus. A final mEABR was not recorded in the third animal due to a technical issue, although chronic stimulation was confirmed by behavioural response at the onset of the daily stimulation regime. Although the mEABR thresholds showed no significant change over the stimulation period in the two mice that completed the stimulation regime (5.5 nC ± 0.6 vs. 6.1 nC ± 6.6; paired t-test; $p = 0.37$) the small sample in this group should be considered when interpreting this result.

[FIGURE 8 ABOUT HERE]

[TABLE 2 ABOUT HERE]

3.3.3. Chronic ES of the mouse cochlea

The response of the mouse cochlea to chronic ES was evaluated by the examination of the tissue response and SGN survival in the 3 chronically treated animals. These results are presented below and in Table 2 for unstimulated controls and chronically stimulated animals.

1) Chronic implantation: unstimulated

Unstimulated implanted control animals exhibited a fibrous tissue response and new bone growth in the implanted cochlea (Figure 9A), which was quantified by determining the area of scala tympani that it occupied. This response was primarily located in the basal turns (encompassing 52% of ST; ± 7.9%), with 40% of ST (±7.3) representing new bone growth and 12% of ST as fibrous tissue, although some tissue response was also seen in the middle turn in 2 animals. SGN densities were comparable to those seen in animals in the 5% neomycin group ($p > 0.05$; Table 2), suggesting that implantation *per se* was not detrimental to SGN survival over a 1 month period. However, more animals are required to confirm that this is the case.

[FIGURE 9 AROUND HERE]

2) Chronic implantation: stimulated

Chronic stimulation did not significantly alter the density of SGNs compared to the unstimulated controls ($p > 0.05$; Table 2 and Figure 9C and D) in the 3 animals that completed the chronic stimulation regime.

Histological analysis of chronically stimulated cochleae showed evidence of a fibrous tissue response in the basal turn (Figure 9B). This tissue response was not significantly different to that seen in unstimulated implanted controls ($p = 0.85$), representing 49 % (± 6.7 %) of the scala tympani in the basal turn, and can be divided into new bone growth (34.6 % of ST; ± 4.6) and fibrous tissue (14.4 % of ST).

4. Discussion

This manuscript presents the first description of the surgical approach for cochlear implantation in the mouse, as well as demonstrating that chronic intracochlear electrical stimulation can be achieved in this species for periods of up to one month. Together, these results enable access to the large range of genetically modified mice models of deafness for cochlear implant studies.

We have also provided evidence that corroborates previous research showing that cauterizing the SA in small rodents with a prominent SA has no significant effect upon hearing thresholds or SGN counts (Lu et al., 2005; Emadi et al., 2004). In a clinical setting there is evidence of hearing loss associated with this procedure (Shuknecht, 2010). Two animals in the SA group displayed elevated thresholds, suggesting that cauterising this structure is not always ~~which were undoubtedly related to mechanical or heat damage to~~ without adverse side effects. ~~the cochlea caused by the cautery forceps.~~ We re-emphasize the need for extreme care during cautery and implantation surgery in order to minimise ~~such damage~~hearing loss. Two animals were successfully acutely implanted and stimulated without cauterizing the SA (not shown). However, due to the increased risk of fatal bleeding with an intact SA, cauterizing is recommended ~~in all animals~~.

In order to develop the chronic electrical stimulation protocol, we used normal hearing cochleae that were deafened ototoxically at the time of implantation. The mouse is a difficult animal to deafen ototoxically (Taylor et al., 2008; Wu et al., 2001), as the concentrations of aminoglycosides required systemically to cause severe hearing loss are typically fatal (Murillo-Cuesta et al., 2010). With this in mind, we investigated the effects of topical application into the round window of two concentrations of neomycin on hearing thresholds and SGN survival. Both concentrations of neomycin elicited significant increases in click-evoked ABR thresholds. However, 10% neomycin caused a large increase in ABR threshold bilaterally, though asymmetrically, suggesting transport to the contralateral cochlea at this higher concentration, presumably via the cochlear aqueduct and the cerebrospinal fluid (Lalwani et al., 2002; Coleman

et al., 2006). The partial loss of hearing associated with the 5% neomycin could be due to the short experimental period (1 month), which may have been sufficient to cause rapid outer hair cell death, but not long enough for inner hair cells to deteriorate to a sufficient level to cause profound hearing loss (Taylor et al., 2008) or deteriorate the supporting cells enough to cause a reduction in SGN numbers (Zilberstein et al., 2012). Furthermore, despite the increases in ABR threshold, there was no significant difference in the SGN densities between the treated and control ears, for either neomycin concentration, one month post-deafening. In light of the above, the topical application of a 5% concentration of neomycin may be appropriate for use as a model for the clinical population who maintain some residual hearing. Cochlear inflammation and tissue response caused by implant insertion has been suggested as a contributor to loss of residual hearing in clinical populations (James et al., 2008). Although not investigated in our study, the pronounced cochlear pathology seen in the mouse after electrode array implantation, combined with the partial hearing loss obtained with the 5% w/v neomycin may provide a new model for the effects of cochlear implantation and chronic stimulation on residual hearing.

The etiology of hearing loss has implications on how deafness-induced changes manifest in the auditory brain (Ryugo, Baker et al. 2010), emphasising the importance of using genetic models of deafness that do not require experimental interventions to produce a clinically-relevant hearing loss and allow investigations into the mechanisms of intracochlear electrical stimulation. It is, however, important to note that due to the surgical approach, genetic mouse models would need to be in good health for chronic cochlear implantation to be viable.

Neural activation via bipolar intracochlear electrical stimulation by the cochlear implant was confirmed by the successful recording of EABRs and mEABRs (acute and chronic electrode arrays respectively). Although the mEABRs recorded in the chronically-stimulated animals were stable over the one-month stimulation period, suggesting a contrast with the increases in EABR threshold previously observed in guinea pig (Landry et al., 2011) and cat (Coco et al., 2007),

more animals need to be tested to confirm this result. As in the cat (Xu et al., 1997) and rat (Lu et al., 2005), non-auditory muscle responses ('myogenic') were initiated by higher stimulation intensities, limiting chronic stimulation to stimulation intensities below the myogenic levels. The latter were notably higher than the functional mEABR thresholds, meaning that chronic stimulation was presented above the electrophysiological threshold without evoking myogenic activity.

We saw no evidence of stimulus-induced SGN rescue in the three chronically-stimulated animals presented here. Although this is a small number of animals, and conclusions may only be drawn cautiously, this corroborates our previous reports of the effects of chronic ES on SGN survival in the guinea pig (Landry et al., 2011; Shepherd et al., 2005) and cat (Wise et al., 2011; Araki et al., 1998; Coco et al., 2007; Shepherd et al., 1994). Furthermore, the tissue response observed in implanted cochleae, both stimulated and unstimulated, is similar to that described elsewhere in cat (Xu et al., 1997; Shepherd et al., 1994), and in clinical populations (Li et al., 2007). It is possible that such tissue response may be exacerbated in the target genetic deafness models which may have compromised immune responses, and should be monitored in future studies using this array in small rodents.

The large and increasing number of mouse genetic models for human deafness makes this species very attractive for auditory research. Furthermore, with appropriate modification of the electrode array, the fully implantable stimulator could have applications in other areas of neuroscience, such as deep brain stimulation.

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Figure 1

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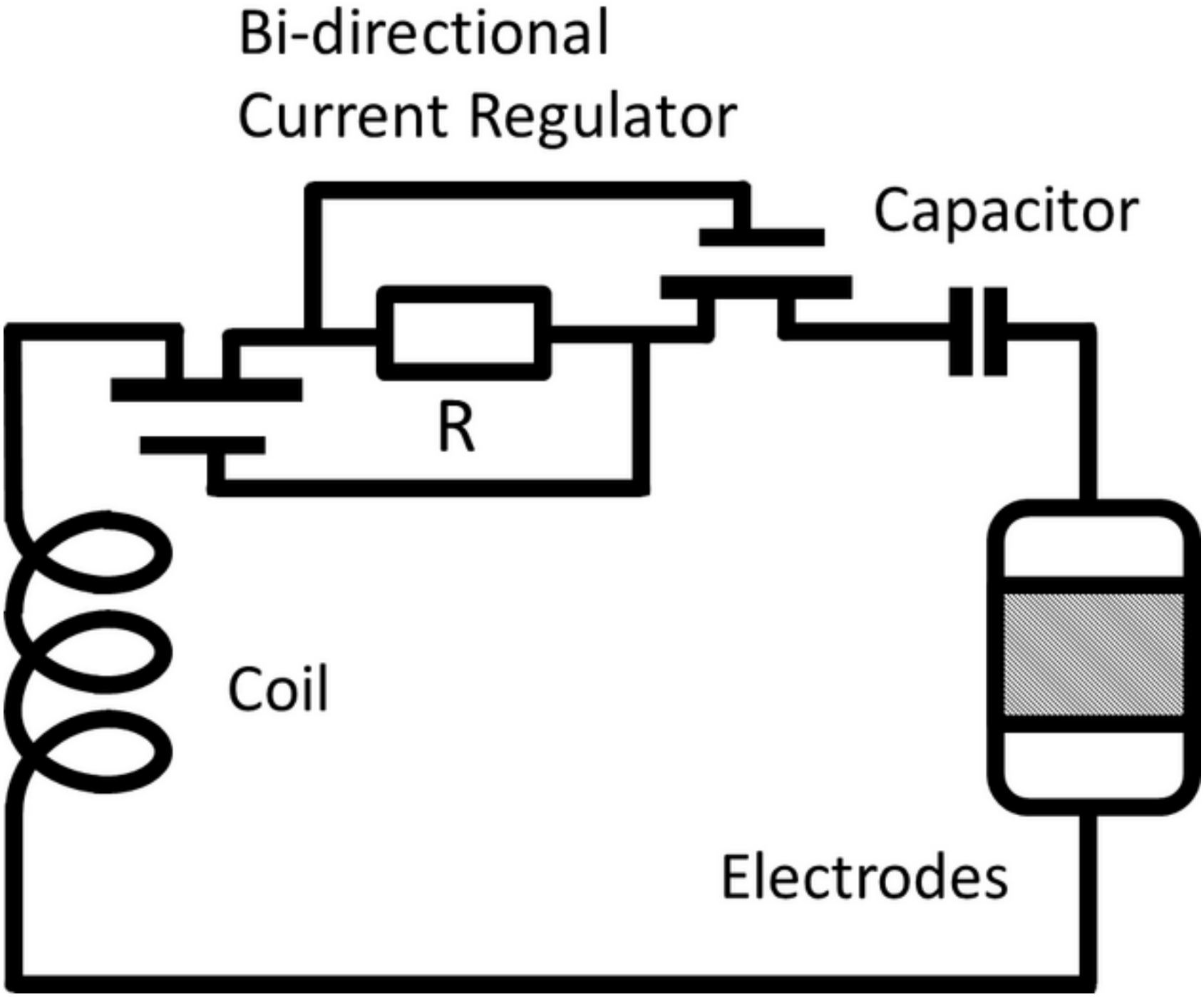

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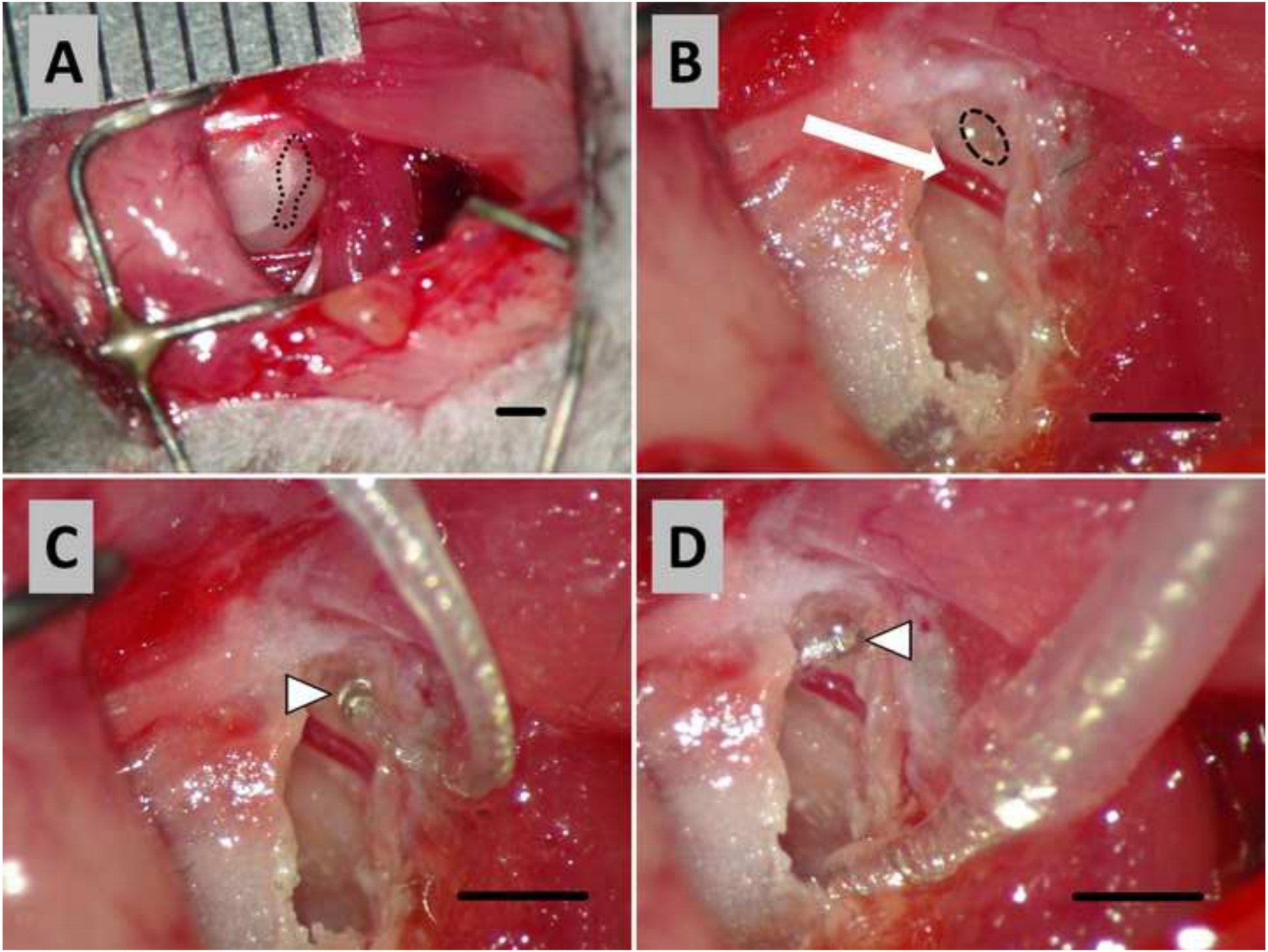

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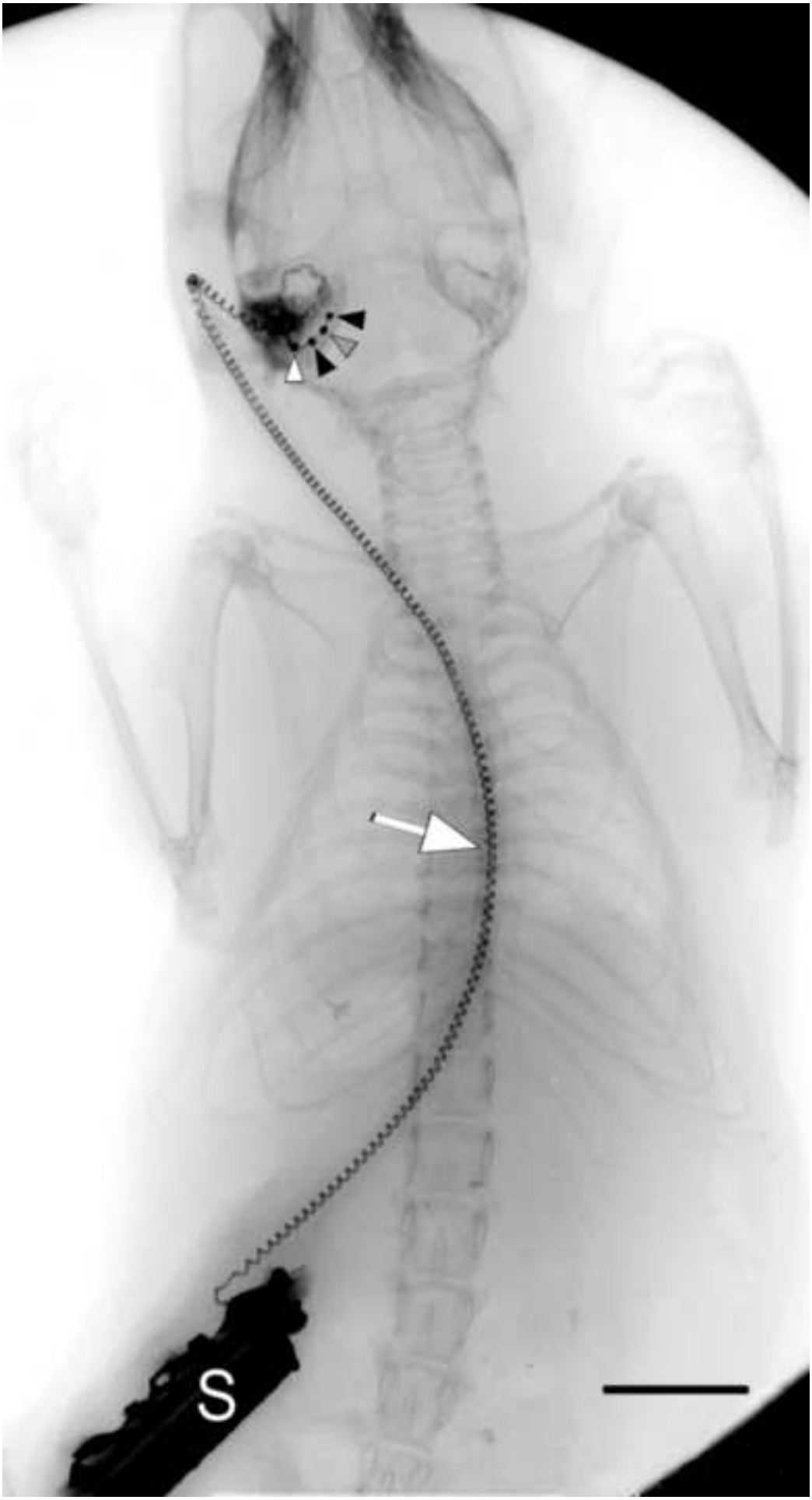

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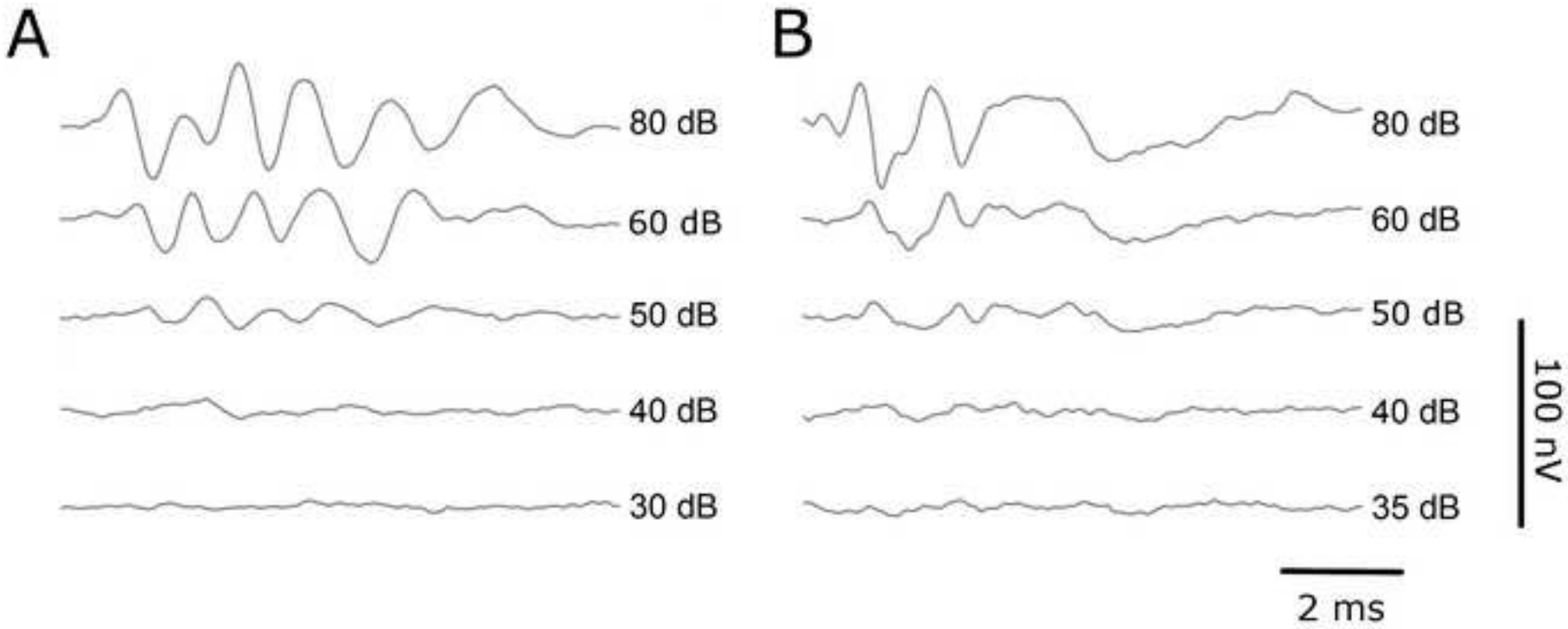

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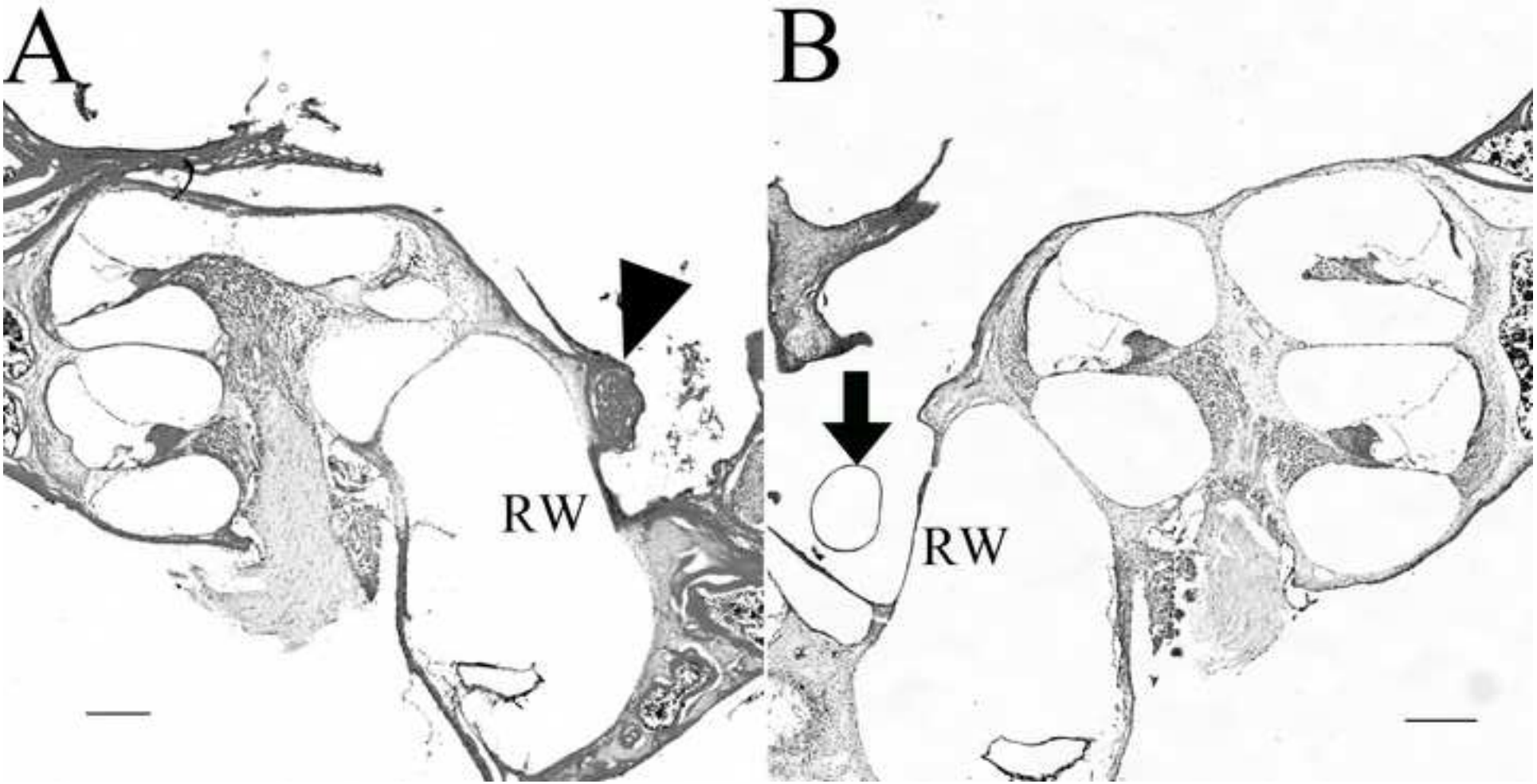

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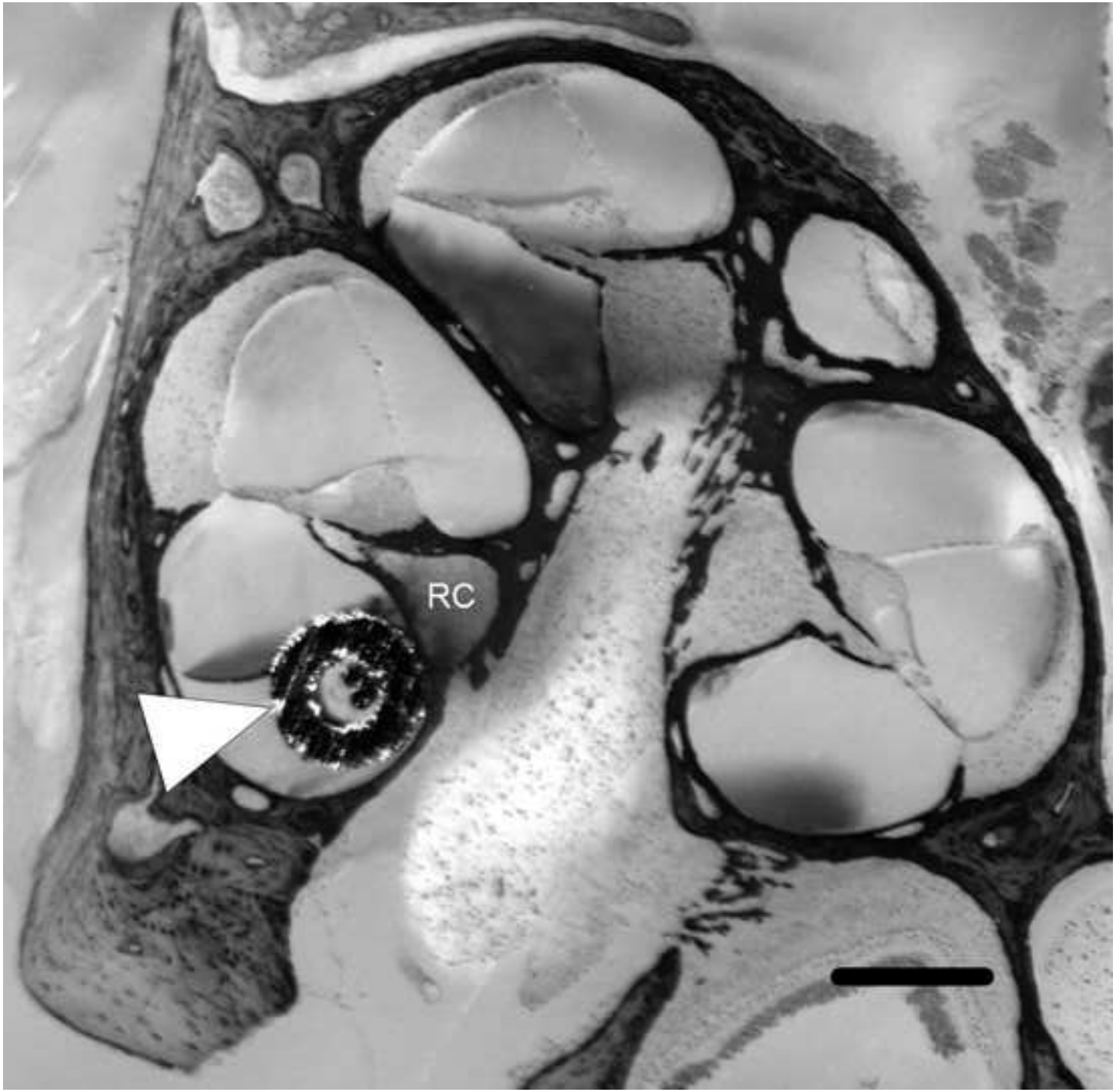

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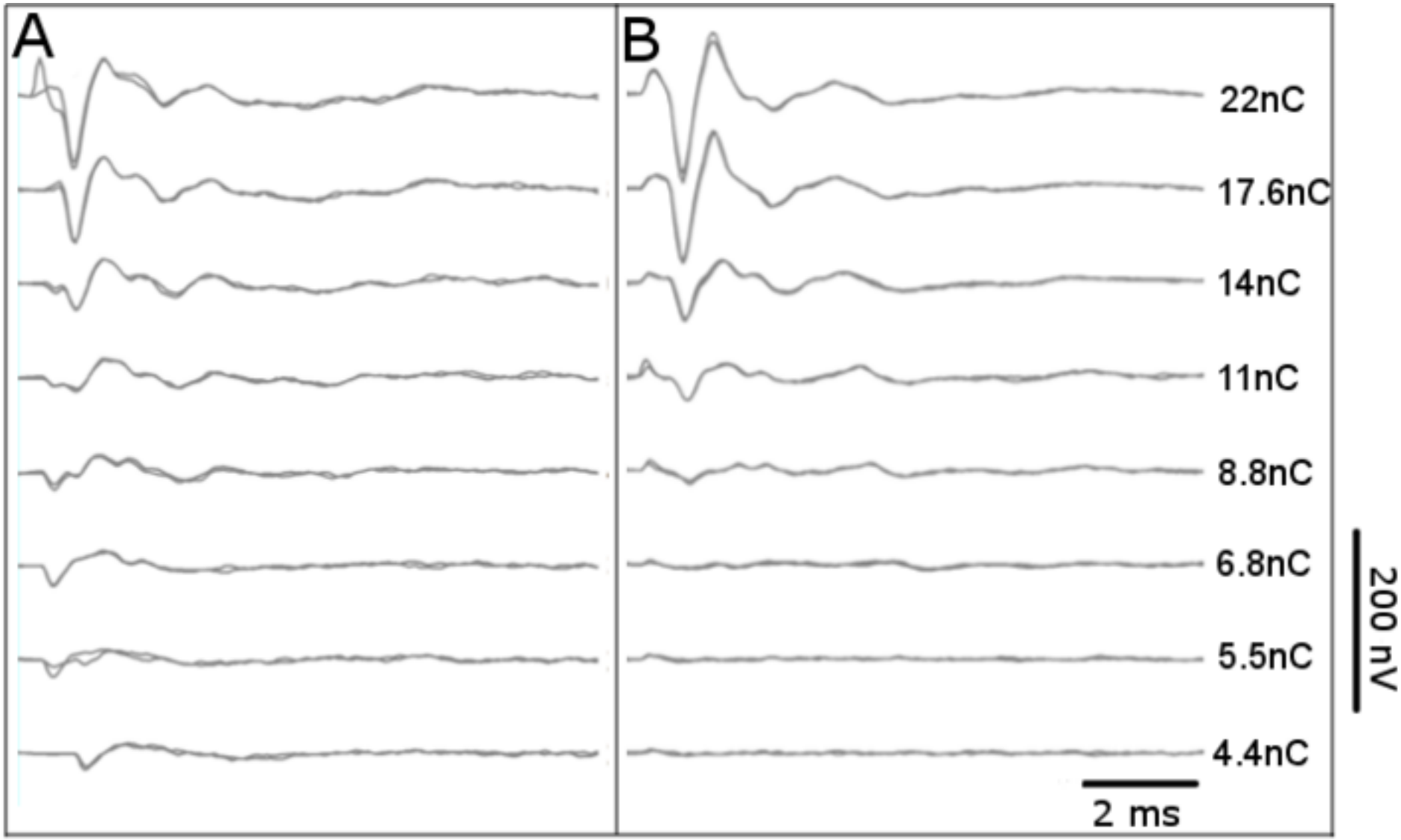

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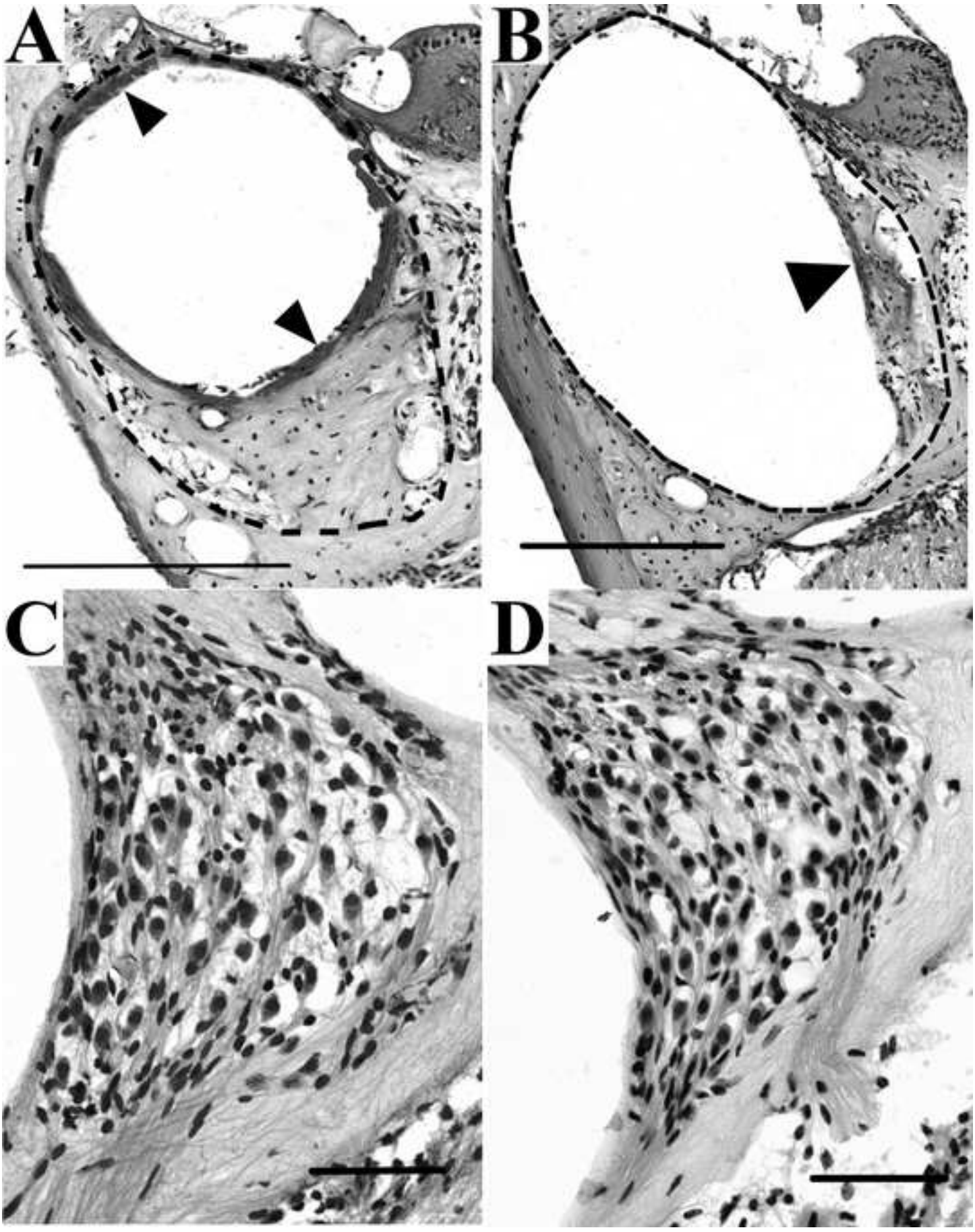

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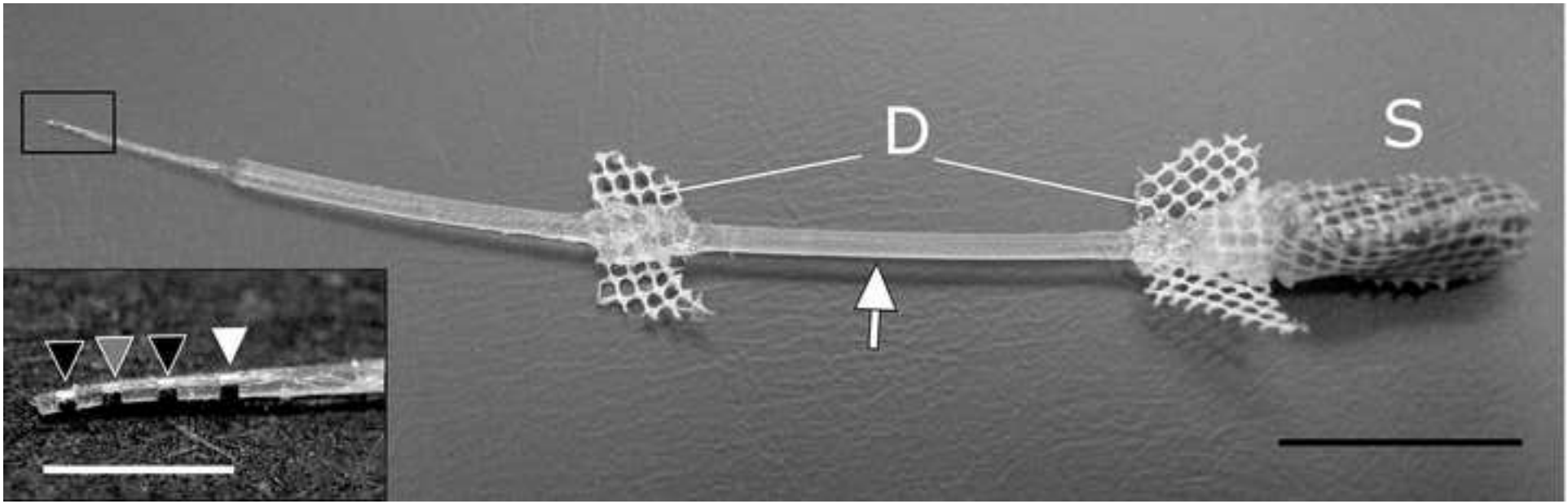

Mouse paper figure and table captions

Table 1. Experimental groups and interventions. SA: Stapedial artery; 5%: 5% w/v of neomycin used for deafening; 10%: 10% w/v of neomycin used for deafening; ES: Electrical stimulation.

Figure 1. Schematic diagram of the stimulator, adapted from Millard and Shepherd (2007). R: Resistor. The implantable stimulator was driven by an external magnetic coil assembly (not shown) which induced charge-balanced biphasic current pulses in response to a pulsed magnetic field, and pulse width and stimulation rates were varied using a computer-controlled external control unit.

Figure 2. Fully implantable mouse stimulator assembly (scale bar = 10mm) and close up of mouse intracochlear electrode array (inset; scale bar = 2mm). D - Dacron mesh; S - stimulator; white arrow - leadwire; black arrowheads - active platinum electrodes; grey arrowhead - unconnected electrode; white arrowhead - extracochlear marker.

Figure 3. Insertion of the electrode array into the scala tympani via the RW in an animal that did not undergo SA cauterisation. (A) exposure of the auditory bulla (dotted line) (B) Bullostomy allowed the visualisation of the stapedial artery (white arrow) and the RW (dotted line). (C) The electrode array was inserted until the marker ring (white arrowhead) sat in the RW niche (D) the narrow leadwire was then coiled inside the bulla for tension relief before application of neomycin and resealing. Scale bars = 1mm.

Figure 4. X-ray of the intracochlear electrode array and stimulating assembly *in situ*. Arrowheads indicate platinum electrodes (Black - active electrodes; grey - intracochlear inactive electrode; white - marker electrode). The white arrow indicates the subcutaneous leadwire connecting the electrode array to the stimulator (S). Scale bar = 5 mm.

Figure 5. Click-evoked ABR recordings pre (A) and 4 weeks post (B) SA cautery. Thresholds were determined visually as the lowest stimulus amplitude to elicit a detectable wave III waveform. In this case, threshold is 40 dB SPL at both time periods. dB denotes dB SPL.

Figure 6. Photomicrographs of (A) experimental cochlea showing cauterised SA (black arrowhead) and (B) contralateral control cochlea showing normal stapedial artery (arrow). RW: Round window. Scale bar = 200µm.

Figure 7. Midmodiolar, epoxy resin-embedded image stained with Toluidine Blue of a left cochlea showing a platinum electrode (white arrowhead), in the basal turn of scala tympani and its proximity to the target SGNs in Rosenthal's Canal (RC). Scale bar = 200 µm.

Figure 8. Typical mEABRs recorded one week post-implantation (A), and at the end of the experimental period (B). Threshold was 6.8 nC at both timepoints.

Table 2. Mean SGN density (cells/mm^2) across groups in basal and apical regions of the experimental and contralateral control ears. Errors are standard errors of the means. ES - Electrical stimulation. See text for details. *note that these were also deafened with 5% neomycin.

Figure 9. (A) Scala tympani (hashed line) in basal turn of a chronically implanted unstimulated cochlea showing pronounced tissue response around the electrode track (arrowheads). (B) Scala tympani (hashed line) of basal turn of a chronically implanted and stimulated cochlea showing a fibrous tissue response (arrowhead). (C) and (D) show magnification of RC in chronically implanted unstimulated and chronically stimulated cochleae respectively. SGN densities were comparable between these groups. Scale bar = 100 µm. (A) and (C) Scale bar: 200 µm.

Table 1

Group	N	SA cautery	Deafening	Implant
Normal Controls	3	-	-	-
SA Cautery	5	Yes	-	-
5% neomycin	3	Yes	5% Neomycin	-
10% neomycin	3	Yes	10% Neomycin	-
Acute implant	2	Yes	5% Neomycin	Acute
Acute implant	2	No	5% Neomycin	Acute
Implant unstimulated	8	Yes	5% Neomycin	Chronic Unstimulated
Chronic ES	3	Yes	5% Neomycin	Chronic Stimulated

Table 2

Group	Left (Experimental) cochlea		Right (Control) cochlea	
	Base (cells/mm²)	**Apex (cells/mm²)**	**Base (cells/mm²)**	**Apex (cells/mm²)**
SA Cautery n = 5	3685 ± 196	3122 ± 118	3942 ± 304	3553 ± 366
5% Neomycin n = 3	3399 ± 230	2832 ± 441	3723 ± 158	3369 ± 114
10% Neomycin n = 3	2913 ± 482	2787 ± 22	3806 ± 245	3559 ± 339
Implant unstimulated* n = 8	3193 ± 399	2713 ± 230	3917 ± 130	3731 ± 153
Chronic ES* n = 3	3046 ± 319	2819 ± 617	3905 ± 161	3807 ± 243