Hearing preservation in cochlear implant surgery

From animal research to clinical application

Sarah Havenith

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Hearing preservation in cochlear implant surgery

From animal research to clinical application

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(met een samenvatting in het Nederlands)

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'Werken en feesten vormt schoone geesten'

- Johanna Westerdijk (100 jaar geleden benoemd tot hoogleraar aan de Universiteit Utrecht, de eerste vrouwelijke hoogleraar in Nederland)

Chapter 1

General introduction

According to the World Health Organization 5% of the worldwide population (360 million people) has disabling hearing loss. Hearing loss is classified from mild to profound (see Table 1). It may result from genetic causes, complications at birth, certain infectious diseases including chronic ear infections, ototoxic drug use, exposure to excessive noise, and ageing. Pathology underlying hearing loss can occur anywhere in the auditory system from the outer ear to the auditory cortex.

Table 1. WHO Grades of hearing impairment (WHO, 2012)

Grade of impairment	Corresponding audiometric ISO value	Performance	Recommendations
0 – No impairment	25 dB or better (better ear)	No or very slight hearing problems. Able to hear whispers.	No
1- Slight impairment	26-40 dB (better ear)	Able to hear and repeat words spoken in normal voice at 1m.	Counseling. Hearing aids may be needed.
2 – Moderate impairment	41-60 dB (better ear)	Able to hear and repeat words spoken in raised voice at 1m.	Hearing aids usually recommended.
3 – Severe impairment	61-80 dB (better ear)	Able to hear some words when shouted into better ear.	Hearing aids needed. If not available lip-reading and signing should be taught.
4 – Profound impairment. Including deafness.	81 dB or greater (better ear)	Unable to hear and understand even a shouted voice.	Hearing aids may help understanding words. Additional rehabilitation needed. Lip-reading and sometimes signing essential.

Grades 2, 3 and 4 are classified as disabling hearing impairment. The audiometric ISO values are averages of values at 500, 1000, 2000, 4000 Hz.

A common form of hearing loss is sensorineural hearing loss (SNHL), which refers to lesions of cochlear and/or retrocochlear structures. Typically, severe or profound SNHL occurs when the sensory cells of the inner ear (the cochlear hair cells) are affected. Hearing aids often help for hearing rehabilitation in cases of moderate impairment, however in most cases of severe to profound hearing loss hearing aids are of little to no benefit. These patients may benefit from a cochlear implant (CI).

On the average, hearing results in patients with a CI continue to improve over the years and with these ongoing advances in performance indication criteria are expanding. Currently, both the influence of the type of surgical technique and the use of otoprotective drugs in CI surgery on preserving cochlear structures and/or residual hearing are discussed. This thesis addresses several topics of this discussion and contributes to further optimize cochlear implantation treatment. Both preclinical studies in guinea pigs and clinical studies are presented.

1. Hearing and the inner ear

First, the basic physiology of hearing and the structure of the inner ear are outlined in this paragraph.
Sound pressure waves reach the outer ear and are transported through the external ear canal to reach the tympanic membrane. Subsequently, the tympanic membrane and the connected middle ear ossicles (malleus, incus and stapes) start to vibrate. The footplate of the stapes connects to the oval window of the cochlea, which causes the fluids in the cochlea, the actual inner ear, to move. The cochlea transduces these mechanical vibrations into electrical signals. These electrical signals are processed in the central auditory pathways of the brain to perceive sound. The cochlea is named after its shape like a snail shell (Greek for snail). It is as a complex bony tube spiraling up around a central core in two and a half turns in humans and accommodates the membranous labyrinth. The membranous labyrinth contains the actual sensory organ, called the organ of Corti.
Figure 1A shows a longitudinal section through the center core (modiolus) of the human cochlea. The cochlear tube contains three fluid-filled compartments: the scala media containing endolymph and both the scalae tympani and vestibuli containing perilymph. Perilymph has a similar ionic composition to cerebro-spinal fluid, rich in sodium and poor in potassium. This in contrast to endolymph, which is rich in potassium and poor in sodium. These unique ionic compositions are essential for their functions in regulating electrochemical signals of cochlear hair cells. The scala tympani and vestibuli meet at the apex through a small opening (the helicotrema). At the base of the cochlea the scala vestibuli terminates at the oval window and the scala tympani at the round window. The scala media is separated from the scala vestibuli by Reissner's membrane and from the scala tympani by the basilar membrane. The basilar membrane supports the sensory cells in the Organ of Corti; the cochlear hair cells (HCs) containing their typical hair-like stereocilia. The single medial row, the inner hair cells (IHCs), has an abundant afferent nerve supply carrying sensory information to the brain. The three outer rows, the outer hair cells (OHCs), mainly receive efferent innervation. Figure 1B shows one of the scalar sections with the organ of Corti, containing the basilar membrane and cochlear HCs, in more detail (Fig. 1C).

Fluid vibrations in the cochlea cause the stereocilia on the HCs to move and release electrochemical signals, after which the IHCs evoke actual action potentials in the spiral ganglion cells (SGCs). These SGCs are located in Rosenthal's canal and their axons, forming the auditory nerve, extend through the central core (the modiolus) to target the brainstem (Fig. 1A). There are two types of SGCs. About 90-95% are type-I SGCs, receiving their sensory input from the IHCs. The remaining type-II SGCs innervate the OHCs. In this way, the IHCs transduce vibrations into nerve impulses.

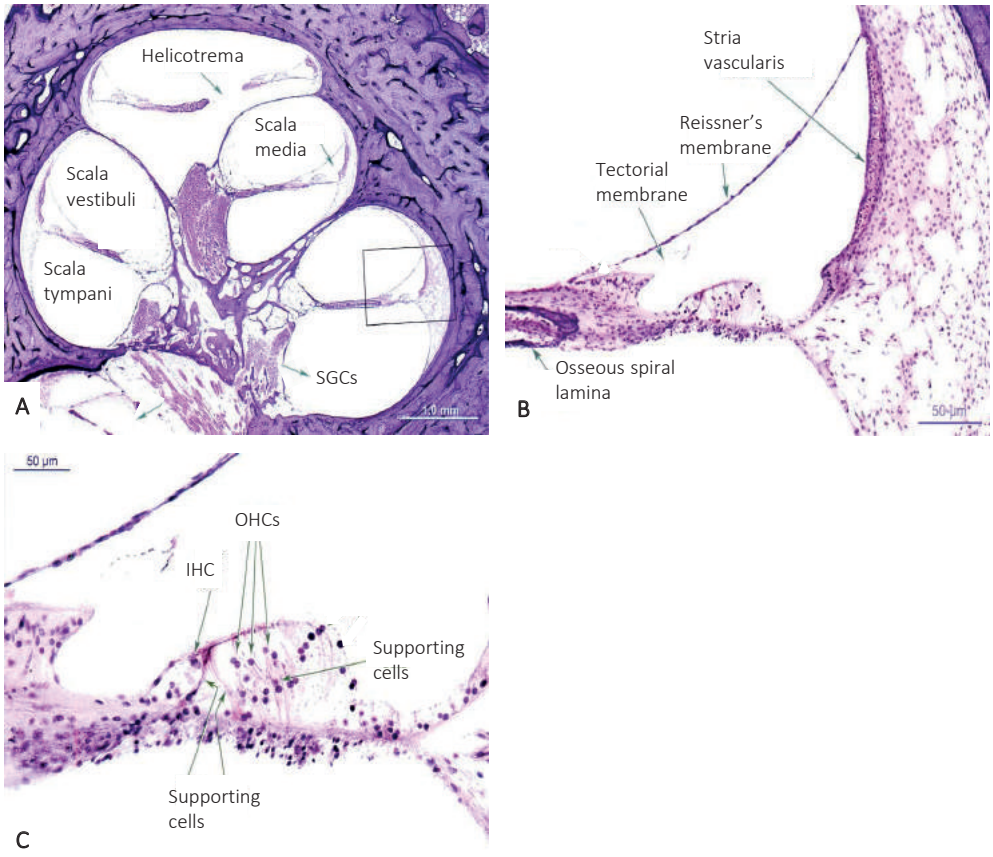

Figure 1. A) Midmodiolar section of a normal human cochlea, B) Scalar transection and C) The organ of Corti in more detail. *Derived and adapted from Schuknechts's pathology of the ear, 3rd edition, 2010. S.N. Merchant and J.B. Nadol Jr.*

As a response to sound and subsequent fluid movements in the cochlea, the basilar membrane produces a travelling wave. This wave reaches a maximum amplitude according to the frequency of the sound: higher frequencies at the base and progressively lower frequencies towards the apex. This frequency specificity is provided by the unique mechanical properties of the basilar membrane, which is most narrow and stiff at the base and most floppy and wide at the apex. The OHCs further increase this sensitivity and frequency selectivity of hearing. The tonotopic organization created in the cochlea extends to the auditory cortex.
(Alberti 1995; Lamore et al. 2000; Davis and Silverman, 1970; Merchant and Nadol, 2010)

2. Specific aspects relevant to the guinea pig in otologic research

As mentioned, in the text above, this thesis contains preclinical studies in guinea pigs. Guinea pigs (***Cavia Porcellus***) have often been used in basic otologic research for several reasons. The anatomy of the ear makes the cochlea relatively easily accessible (Albuquerque et al., 2009; Wysocki, 2005). The otic capsule of the guinea pig is not embedded in the temporal bone of the skull as it is in humans, but it protrudes into the middle ear space as an air cell with a thin bony capsula surrounding it, the bulla. Inner ear structures are therefore easily accessible through a postauricular surgical approach. The tympanic bulla, which gives direct access to the cochlea, can be opened using minimally invasive surgical techniques. Figure 2 shows a lateral (A) and a lower (B) view of the guinea pig skull containing the tympanic bulla.

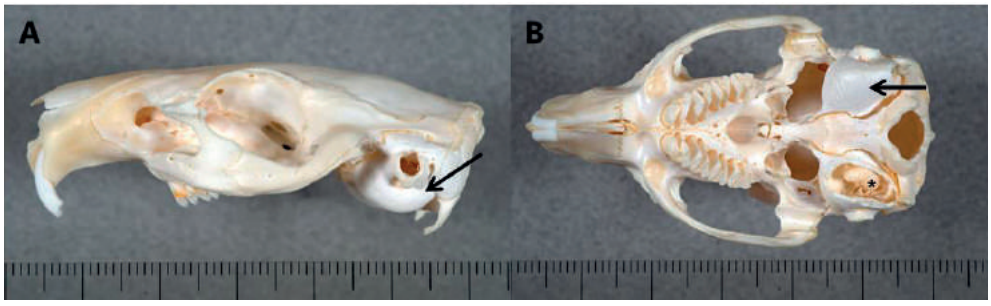

Figure 2. A) lateral and B) lower view of the guinea pig skull with both left and right tympanic bullae (arrows). In B) the right tympanic bulla is opened, in which the asterisk indicates the cochlea.

The cochlea itself is, compared to other rodents such as the rat or mouse, of reasonable size in the guinea pig (Thorne et al., 1999) and is easy to remove and prepare for histological analysis without damaging essential structures (Albuquerque et al., 2009). The internal skeleton of the cochlea is composed of the spiral lamina and the centrally situated modiolus (as in humans). The spiral lamina is fixed to the modiolus and with the spiral canal forms 3.5 to 3.75 turns, in contrast to 2.5 turns in humans. It is surrounded by a thin bony lamina only, which is a typical feature in most rodents. Besides these differences, other intracochlear structures are similar to those in humans. Also, the guinea pig's hearing range is, though shifted by 1-1.5 octave, similar to that of humans (~50-50.000 Hz for guinea pigs and ~20-20.000 Hz for humans). Therefore, as stated above, many basic anatomical and physiological data in published otologic research are on guinea pigs. Thus, we can compare and relate our data obtained in the guinea pig to a wealth of data in literature.

3. Cochlear implantation

A CI consists of an external part with a microphone that captures sound waves and the speech processor that converts these sounds into digital signals and sends them to a magnetic headpiece. This headpiece subsequently sends the signals transcutaneously to the internal implant. The internal implant (the receiver) converts these digital signals into electrical energy which is transferred to the electrode array inside the cochlea (Figure 3). The electrodes directly stimulate the SGCs of the auditory nerve and thereby restore the perception of sound. Therefore, for optimal hearing performance with a CI the number of excitable SGCs is an important factor (Richardson et al. ,2006; Pettingill et al., 2007). Post-mortem histological analysis of the cochleas of CI users have confirmed this, reporting a positive correlation between the number of surviving SGCs with an individal's hearing performance (speech recognition scores) (Seyyedi et al., 2014; Kamakura and Nadol, 2016).

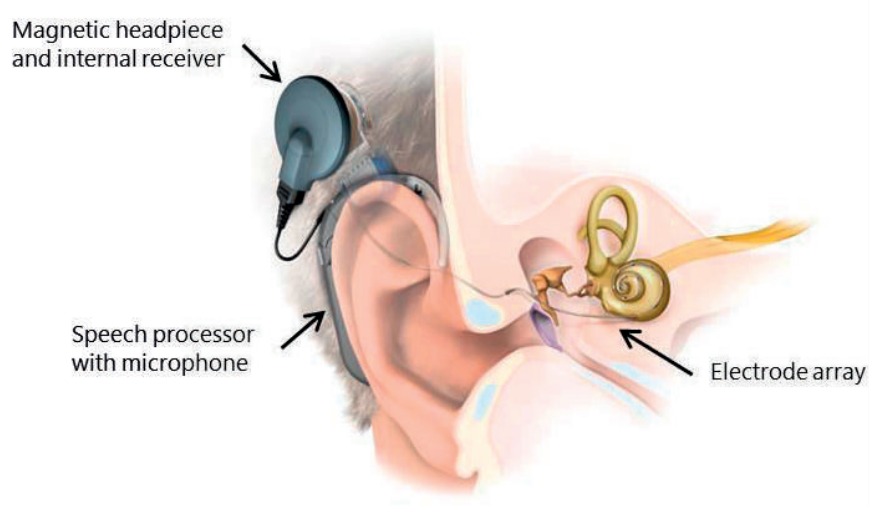

Figure 3. The cochlear implant. *Image courtesy of MED-EL, Innsbruck, Austria.*

4. Hearing preservation in cochlear implant surgery

In the Netherlands, a CI is traditionally provided when the speech perception score in silence, with the most optimally fitted hearing aids, is 50% or less at the normal conversational speech level of 65 dB sound pressure level (SPL). Indications and criteria for cochlear implantation are continuously expanding because of the (slow) ever-improving postoperative hearing results, for example for patients with moderate speech recognition with hearing aids, single-sided deafness and geriatric patients (Arnoldner et al., 2013; Leigh et al., 2016). Also, patients with substantial residual low-frequency hearing are fitted with a CI nowadays. Especially for

them, a novel strategy of combined electric and acoustic stimulation (EAS) was developed by von Ilberg et al. (1999); acoustic stimulation of the low frequencies with a hearing aid in combination with electric stimulation of the high frequencies with the CI. This poses a problem: on the one hand acoustic stimulation with hearing aids is insufficient in these patients, but on the other hand cochlear implantation may lead to loss of residual hearing. In the last decade, much attention has been given to minimize CI insertion trauma in these patients by optimizing the electrode design; developing shorter, thinner and more flexible electrode arrays (i.e. Gantz et al., 2009; Gstoettner et al., 2006; Lenarz et al., 2009; Skarzynski et al., 2009). Minimal traumatic insertion techniques and protection of cochlear structures are in general considered of importance, also for CI recipients with minimal or no residual hearing. Improving surgical techniques and applying otoprotective drugs may enhance CI hearing performance.

4.1. Development of atraumatic surgical techniques

Besides the attention that has been given to minimize trauma by optimizing the electrode design, also several aspects regarding the surgical techniques used to preserve residual hearing are frequently being argued without much scientific substrate. A minimal traumatic opening of the cochlea and insertion of the electrode is thought to be essential for hearing preservation. Two major 'atraumatic' surgical techniques have been promoted: the round window approach and the 'soft surgery' cochleostomy technique. In the former the CI electrode array is introduced through a small opening in the round window, in the latter technique a small hole is drilled in the basal turn of the cochlea. The 'soft surgery' part also refers to several other principles used during CI surgery and was originally described by Lehnhardt (1993). Both approaches or insertion techniques have shown to cause relatively little damage to intracochlear structures such as the modiolus, the bony spiral lamina or the basilar membrane if executed correctly (Briggs et al., 2006). However, it is as yet not known which type of insertion technique is superior on postoperative residual hearing outcome.

4.2. The use of otoprotective drugs

In addition to atraumatic surgical techniques during cochlear implantation, also much research is being performed on identifying drugs that may further prevent structural and/or functional insertion damage. The most important drugs and also the ones used in the present research are neurotrophic factors and corticosteroids. Several application methods for these compounds in the cochlea have been described; systemic delivery, local extracochlear delivery (in the middle ear), direct intracochlear application or a combination of these methods (Plontke et al., 2017). Local drug delivery strategies are thought to have several advantages over systemic delivery methods (i.e., higher concentrations in the inner ear, reduction of possible systemic side effects and lower doses required) (Rivera et al., 2012; Plontke et al., 2017). Local drug delivery in the middle ear (also called intratympanic delivery) is minimally invasive

and uses the permeability characteristics of the round window membrane for substances to enter the cochlea. However, the exact dose of the substances reaching the cochlea is not known. This can be partly overcome by direct intracochlear delivery, which is a more invasive technique requiring surgery (Rivera et al., 2012). However, since in CI surgery the cochlea is already opened, intracochlear delivery seems an attractive method. Combining these techniques might enhance the possible otoprotective effects. Animal research is performed in order to investigate the most optimal delivery strategy for several otoprotective drugs and assess their safety and effectivity. Below we will discuss some aspects of both categories of drugs in relation to their application in the cochlea.

4.2.1. Neurotrophic factors

The organ of Corti and its HCs release neurotrophins, which play a role in cochlear development and survival and maintenance of SGCs. Especially brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) play an important role in the auditory system (Pirvola et al., 1994; Davis, 2003). These neurotrophins bind to specific receptors in the SGC membrane (tropomyosin-related kinase [Trk] B and TrkC, for BDNF and NT-3, respectively) and thereby affect a multitude of intracellular pathways (Ramekers et al., 2012). Severe SNHL is caused by loss of the HCs, which results in SGC degeneration. As mentioned above, recent evidence has shown that speech performance of CI users is positively correlated with the number of surviving SGCs (Seyyedi et al., 2014; Kamakura and Nadol, 2016). Therefore, clinically applicable methods, involving treatment with exogenous neurotrophic factors, to protect the auditory nerve from degeneration have become pertinent.

It is well known from animal studies that cochlear delivery of neurotrophic factors prevents secondary loss of SGCs in hair cell-deprived cochleas. In most of these studies the neurotrophins were delivered intracochlearly by means of a so called mini-osmotic pump device (e.g. Agterberg et al., 2008, 2009; Ramekers et al., 2015). This has shown to be highly effective but not considered clinically applicable due to its unacceptable risk of infection to the inner ear. Other intracochlear delivery methods (coated electrode array and gene therapy) were less effective (e.g. Staecker et al., 1998; Richardson et al., 2009). Therefore, less invasive extracochlear local delivery methods were investigated as well (e.g. Noushi et al., 2005; Endo et al., 2005; Ito et al., 2005). Polymer beads (Noushi et al., 2005) and a biodegradable hydrogel infiltrated with neurotrophic factors (Endo et al., 2005; Ito et al., 2005) have been successfully applied in the middle ear as sustained-release carriers in preclinical studies in guinea pigs. Local delivery to the middle ear seems an attractive and clinically applicable delivery method of neurotrophic factors.

4.2.2. Glucocorticoids

Corticosteroids are hormones that are released in the body as a reaction to stress and trauma in order to maintain homeostasis. Both the glucocorticoid and mineralocorticoid receptors,

the latter to a lesser extent, have been located in inner ear structures (i.e., at cochlear HCs, SGCs, spiral ligament). They are thought to have immunosuppressive and anti-inflammatory functions due to glucocorticoid receptor activation, and may influence ion transport functions in the inner ear due to mineralocorticoid receptor activation. However, the exact molecular mechanisms underlying glucocorticoid actions in the cochlea are as yet unknown. Synthetic glucocorticoids are commonly clinically applied for sudden sensorineural hearing loss, Meniere's disease and auto-immune hearing disorders. (Meltser et al., 2011; Trune et al., 2009)

It is thought that mechanical trauma due to insertion of an electrode array may lead to oxidative stress and inflammation, which may induce loss of residual hair cells due to necrosis and/or programmed cell death (Bas et al., 2012). Several inflammatory cytokines, which may be associated with IHC loss, are found to be elevated after cochlear implantation (i.e., TNFα). Dexamethasone (a synthetic glucocorticoid) has shown to upregulate anti-apoptotic genes (i.e., Bcl-2, Bcl-xl) and downregulate a pro-apoptotic gene (i.e., Bax) in organ of Corti explants in vitro (Dinh et al., 2008). Also, preclinical studies have indicated that intracochlear dexamethasone treatment had an effect on the normalization of homeostasis in the cochlea of guinea pigs implanted with a dexamethasone-eluting electrode array (e.g. Takumi et al., 2014).

Recently, several research groups have explored various factors that may influence the degree of preserving residual hearing in CI surgery (e.g., a meta-analysis by Causon et al. 2015). Dexamethasone treatment is already routinely applied per(i)-operatively in CI patients with low-frequency residual hearing. However, the benefit of dexamethasone on postoperative residual hearing is under debate. Studies describe different routes of administration (locally in the middle ear and/or systemic delivery), differences in the timing of administration (before, during and/or after surgery) and differences in dosages used (Rajan et al. 2012, Santa Maria et al. 2014, Causon et al. 2015). Clinically there is great interest in delivery by means of a coated implant device. Advantages compared to other delivery methods are direct delivery at the target site, less systemic side effects and a continuous delivery over time due to slow release. Animal studies in which dexamethasone is delivered by means of a coated implant electrode array show somewhat contradictive results regarding the protection of the cochlea against structural and/or functional insertion trauma (e.g. Stathopoulos et al., 2014; Bas et al., 2016; Wilk et al., 2016).

In conclusion, there are reports in literature that synthetic glucocorticoids (dexamethasone) have an otoprotective effect on residual hearing preservation after cochlear implantation. Hence, they are already routinely applied per(i)operatively in CI patients in some clinics. However, results on their effectiveness are not consistent. Also, there is no consensus on the optimal delivery method, dosage and/or timing.

5. Main objective and outline of this thesis

The main objective of this thesis is to contribute to the knowledge that is necessary for clinical application of potential otoprotective strategies resulting in better hearing preservation for CI recipients. Developments in optimizing surgical techniques and identifying potential otoprotective drugs may also be important for improving hearing with a CI. This thesis presents four main sections in relation to this topic.

First, the protective effect on the auditory nerve using potentially clinically applicable local delivery strategies of a known otoprotective agent, the exogenous neurotrophic factor BDNF, is presented. In **chapter 2** the protective effect of the local delivery of BDNF on the intact round window membrane using absorbable gelatine sponge as a carrier in deafened guinea pigs was investigated. In **chapter 3** a similar experiment was conducted applying BDNF on the perforated round window membrane. The perforation may possibly enhance its effects.

Second, the protective effect of dexamethasone on preserving residual hearing and preventing foreign body reactions to the implanted electrode array is investigated in a potentially clinically applicable intracochlear delivery method. **Chapter 4** presents the effect of intracochlear delivery of dexamethasone using a coated CI device in normal-hearing guinea pigs.

Third, **chapter 5** describes an animal model of partial deafness, resembling the specific group of CI recipients with low-frequency residual hearing for whom hearing preservation is thought to be essential for combined electroacoustic CI hearing performance. Research in guinea pigs with a partially damaged auditory nerve as opposed to deafened or normal-hearing guinea pigs more accurately represents this group of patients, and may show different results.

Fourth, in view of the ongoing debate about the optimal surgical technique for opening the cochlea during cochlear implantation, **chapter 6** reviews the evidence on the round window and the cochleostomy insertion techniques, respectively, and compares their effects on postoperative residual hearing preservation. Finally, in **chapter 7** a descriptive overview of hearing preservation results in a group of CI patients with low-frequency residual hearing are presented and discussed.

The main findings from this thesis are summarized and discussed in **chapter 8**. In this concluding chapter both the scientific and clinical implications as well as possible future directions of research are outlined.

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Chapter 2

Spiral ganglion cell survival after round window membrane application of brain-derived neurotrophic factor using gelfoam as carrier

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Abstract

Several studies have shown that treatment with various neurotrophins protects spiral ganglion cells (SGCs) from degeneration in hair-cell deprived cochleas. In most of these studies the neurotrophins are delivered by means of intracochlear delivery methods. Recently, other application methods that might be more suited in cochlear implant patients have been developed. We have examined if round window membrane application of gelfoam infiltrated with a neurotrophin resulted in SGC survival in deafened guinea pigs. Two weeks after deafening, gelfoam cubes infiltrated with 6 mg of brain-derived neuro- trophic factor (BDNF) were deposited onto the round window membrane of the right cochleas. Electric pulses were delivered through an electrode positioned within the round window niche to electrically evoke auditory brainstem responses (eABRs). Two or four weeks after deposition of the gelfoam all cochleas were histologically examined. We found that local BDNF treatment enhances the survival of SGCs in the basal cochlear turn after two and four weeks. The treatment had no effect on SGC size or shape. In animals treated with BDNF, eABR amplitudes were smaller than in normal-hearing control animals and similar to those in deafened controls. We conclude that BDNF delivered by means of local gelfoam application provides a protective effect, which is limited compared to intracochlear delivery methods.

1. Introduction

In sensorineural hearing loss, cochlear hair cells are lost or damaged, which is followed by degeneration of spiral ganglion cells (SGCs) caused by a loss of neurotrophic support. Cochlear hair cells release neurotrophic factors, which play a role in cochlear development and in survival and maintenance of SGCs. An example of a neurotrophic factor is brain-derived neurotrophic factor (BDNF), which has been identified in the ears of rodents along with its high-affinity receptor TrkB (Fritzsch et al., 2004). It has been demonstrated that an absence of BDNF leads to a loss of cochlear and vestibular sensory neurons (Ernfors et al., 1995; Fritzsch et al., 1999, 2004). Furthermore, cochlear delivery of exogenous BDNF and/or other neurotrophins prevents secondary loss of SGCs in hair- cell deprived cochleas. The majority of studies on the latter topic have used an intracochlear delivery method, in which neurotrophins are directly infused into the cochlea by means of a mini- osmotic pump delivery system (Ernfors et al., 1996; Staecker et al., 1996; Miller et al., 1997; Gillespie et al., 2003, 2004; Shinohara et al., 2002; Shepherd et al., 2005; Wise et al., 2005; Agterberg et al., 2008, 2009; Scheper et al., 2009). Although intracochlear delivery is effective in preserving SGCs in experimental animals, the clinical applicability of such an approach is strongly disputed. The first objection is that the surgery involved is traumatic and complex and it is presumed that inserting a cannula into the human inner ear presents an unacceptable risk of infection (Richardson et al., 2006; Pettingill et al., 2007). Another disadvantage of the mini-osmotic pump is posed by its limited delivery period. Although the long-term effect on the maintenance of SGC survival is not known as yet (Agterberg et al., 2009), it is likely that repeated replacement including surgery would be needed, which is clinically undesirable.

In order to avoid these drawbacks, a variety of alternative intracochlear delivery systems have been developed and tested during the past few years. Richardson et al. (2005) studied if a single infusion of neurotrophin-3 (NT-3) via a cannula into the cochlea would be an effective alternative, but although this approach indeed resulted in an increase in SGC perikaryal size, a protective effect on SGC survival was not observed. Recently, it was investigated if neurotrophin-eluting polymer electrode coatings could be used as an intracochlear delivery system. Richardson et al. (2009) inserted an electrode coated with NT-3 into cochleas of deafened guinea pigs and found a slight improvement in SGC survival, but only in animals that had simultaneously received electrical stimulation. A disadvantage of polymer electrode coatings is that they have a limited drug reservoir capacity and will only be useful during the first few weeks after cochlear implantation. Another option could be gene therapy, leading to expression of BDNF after inoculating the cochlea with a viral vector containing the BDNF gene (Staecker et al., 1998; Lalwani et al., 2002; Nakaizumi et al., 2004; Rejali et al., 2007). Transfer of the BDNF gene into the cochlea results in higher SGC survival from the basal up to the lower middle turn (Rejali et al., 2007). A possible problem with gene transfection, however, is that especially adenovirus vectors can cause severe immune responses and loss

of viral expression (Staecker et al., 1998) as well as induction of cell toxicity and variability in the duration of expression of neurotrophin transgenes (cf., Richardson et al., 2006).
An interesting advance is the development of extracochlear application methods that are based on the diffusion of neurotrophins through the round window membrane. Neurotrophin- soaked alginate polymer beads (Noushi et al., 2005) and a biodegradable hydrogel infiltrated with BDNF (Endo et al., 2005; Ito et al., 2005) have been successfully applied as sustained-release carriers. Both strategies led to SGC preservation from the basal up to the apical cochlear turns. Furthermore, Ito et al. (2005) provided factual evidence for the diffusion of BDNF through the guinea pig round window as they demonstrated by means of ELISA that BDNF can be detected in the perilymph up to 14 days after hydrogel placement. Advantages are that the carriers used are biodegradable and, therefore, do not pose the risk of cell toxicity. The use of absorbable gelatin sponge (gelfoam) provides another model of slow BDNF release to the cochlea. Diffusion-based delivery of substances to the cochlea by means of gelfoam placed onto the round window membrane has frequently been used in both animal and clinical studies. Guitton et al. (2003) have demonstrated the diffusion of an NMDA-antagonist in the perilymphatic fluids of the rat cochlea after placement of gelfoam cubes on the round window membrane, and Silverstein et al. (1999) have shown the beneficial effect of round window membrane application of gentamicin in treating patients with Ménière's disease.
In the present study we examined if gelfoam containing BDNF, when placed on the round window membrane, results in enhanced SGC survival in deafened guinea pigs. SGC survival was measured in terms of packing density. In addition, we assessed two other morphological parameters of SGCs, cell size (i.e. perikaryal area) and cell shape (i.e. circularity), especially since these have been demonstrated to be affected by BDNF treatment (Shepherd et al., 2005; Richardson et al., 2005; Agterberg et al., 2008). Also, we recorded electrically evoked auditory brainstem responses (eABRs) to monitor the excitability of the SGCs (Hall, 1990; Mitchell et al., 1997; Shepherd et al., 2005; Agterberg et al., 2009).

2. Materials and methods

2.1. Animals and experimental design

Twenty-four albino female guinea pigs (strain: Dunkin Hartley; weighing 340e800 g) were purchased from Harlan Laboratories (Horst, The Netherlands) and housed in the animal care facility of the Rudolf Magnus Institute of Neuroscience (Utrecht, The Netherlands). All animals had free access to both food and water and were kept under standard laboratory conditions. Lights were on between 7:00 am and 7:00 pm. Temperature and humidity were kept constant at 21^{o}C and 60%, respectively.

Twelve guinea pigs were deafened (see Section 2.2) and allotted to two experimental groups, each containing six deafened animals (Fig. 1A,B). Two weeks after deafening, a gelfoam cube soaked in a BDNF solution was positioned onto the round window membrane of the right cochlea. The left cochleas were not treated. The animals were sacrificed and both right and left cochleas were processed for histological analysis, either two weeks (Fig. 1A) or four weeks (Fig. 1B) after gelfoam placement.

Another six deafened guinea pigs (Fig. 1C) were not treated with BDNF and served as a control group. A second control group consisted of six normal-hearing guinea pigs (Fig. 1D). In both control groups, a gelfoam cube soaked in phosphate-buffered saline was placed upon the round window membrane of the right cochleas and left in situ for four weeks. Next, the animals were sacrificed and the right and left cochleas were processed for histological analysis. For histological analyses a comparison was made between right BDNF-treated cochleas and left untreated cochleas. For electrophysiological analyses a comparison was made between deafened BDNF-treated animals and deafened controls.

All experimental procedures were approved by the University's Committee on Animal Research (DEC-UMC # 03.04.036).

2.2. Deafening procedure

Animals were anesthetized with xylazine (Sedamun®; 10 mg/kg im) and (S)-ketamine (Ketanest-S®; 40 mg/kg im). Before the deafening procedure, click-evoked acoustic auditory brainstem responses (aABRs) were recorded to check hearing thresholds. When normal thresholds were confirmed (20e30 dB peSPL), kanamycin sulphate (SigmaeAldrich, St. Louis, USA; 400 mg/kg) in isotonic saline was administered as a single subcutaneous injection followed (w30 min later) by cannulation of the right jugular vein and slow intravenous infusion of furosemide (Centrafarm, Etten- Leur, The Netherlands; 100 mg/kg). This procedure, originally reported by West et al. (1973), has been shown to eliminate almost all cochlear hair cells (Gillespie et al., 2003; Versnel et al., 2007). Control animals received isotonic saline instead of kanamycin and furosemide. One week and two weeks after the deafening procedure, aABRs were recorded to assess the extent of hearing loss. Animals with a threshold shift of >50 dB were included in this study.

2.3. Gelfoam placement and BDNF treatment

Two weeks after the deafening procedure the animals were anesthetized with xylazine and ketamine. The right cochlear bulla was exposed through a retro-auricular approach followed by creating a small hole in the bulla to expose the round window. Small cubes (1 mm3) of gelfoam were soaked in 6 ml of a 1 mg/ml solution of BDNF (PeproTech Inc., Rocky Hill, NJ, USA) in physiological saline and positioned onto the round window membrane. The dose of 6 mg is in the range with that applied in a majority of studies successfully using a mini-osmotic pump system for the delivery of BDNF into the guinea pig cochlea (e.g., 10 mg: Wise et al.,

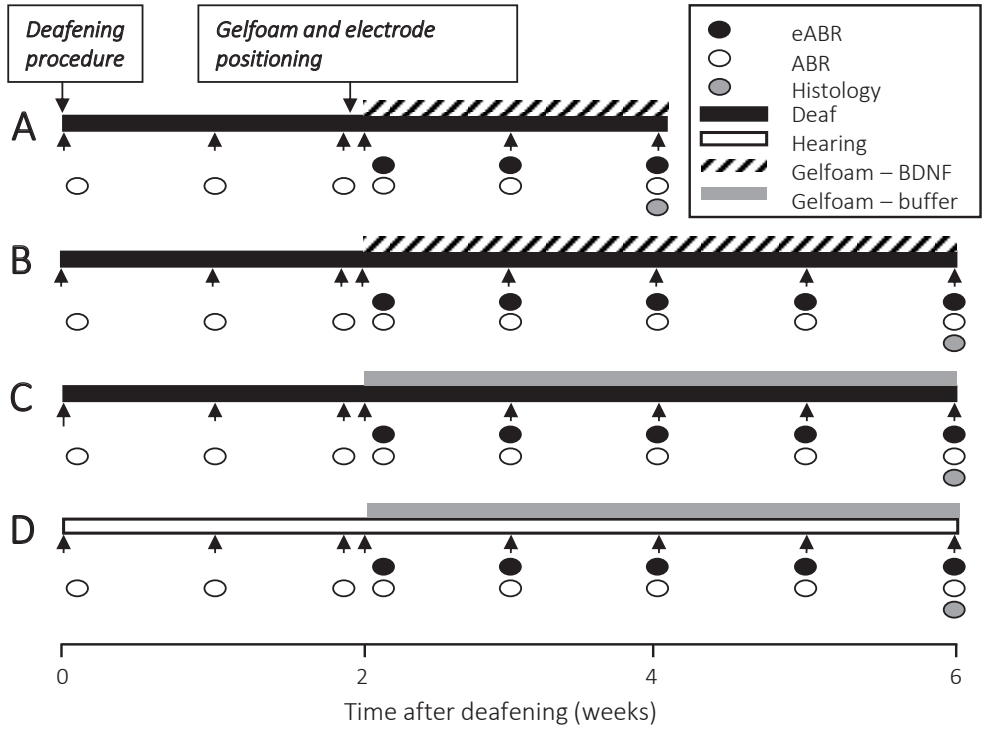

Fig. 1. Treatment schedule for the four different animal cohorts (A-D). Each group contained six animals. (A) First experimental group consisting of deafened guinea pigs, treated with BNDF for two weeks (2-weeks BDNF-treated). (B) Second experimental group consisting of deafened guinea pigs, treated with BDNF for four weeks (4-weeks BDNF-treated). (C) Control group consisting of deafened guinea pigs, treated with phosphate-buffered saline for four weeks (deafened controls). (D) Control group consisting of normal-hearing guinea pigs, treated with phosphate-buffered saline for four weeks (normal-hearing controls). Deafening was performed systemically and, hence, affected both ears. Gelfoam was applied to the right ears. Following gelfoam placement and electrode positioning, eABRs were measured in each group by electrically stimulating the right ear. For electrophysiological analysis, eABRs of the BDNF-treated animals (4-weeks BDNF-treated; B) were compared to those of normal-hearing controls (D) and deafened controls (C).

2005, 20 mg: Miller et al., 2007; Agterberg et al., 2008). However, the actual amount of BDNF entering the perilymph is unknown. Note that much lower doses have been shown to be effective as well (e.g., 10 ng, guinea pig: Miller et al., 1997; 1 mg, rat: McGuinness and Shepherd, 2005).

An electrode, consisting of a stainless steel wire with a gold-ball tip, was used to manipulate the gelfoam cubes onto the round window membrane. Correct positioning of the gelfoam was checked directly during surgery. While the primary purpose of the electrode was to deliver electric pulses to the cochlea to evoke eABRs, it was also used to keep the gelfoam in place. The gold-ball tip was positioned in the round window niche (for details, see Klis et al., 2000). The stainless steel wire was fixed onto the bulla with dental cement (Ketac-Cem

Aplicap; ESPE Dental Supplies, Utrecht, The Netherlands). The wire contact was placed in a connector and fixed with dental cement onto the skull.

2.4. Electrophysiological recordings

aABRs and eABRs were recorded in awake guinea pigs in a sound-attenuated chamber (Agterberg et al., 2009). Three stain- less steel screws (8.0 1.2 mm) were inserted into the skull bone to record ABRs. The screws were inserted 1 cm posterior to bregma, 2 cm anterior to bregma, and 1 cm lateral from bregma (Mitchell et al., 1997). Stimulus generation and signal acquisition were controlled with custom-written software and a personal computer. The stimuli were synthesized and attenuated using a TDT3 system (modules RP2, PA5 (2x), and SA1; Tucker-Davis Technologies Inc., Alachua, FL, USA). The responses were amplified differentially using a PAR113 pre-amplifier (amplification x5,000; band pass filter 0.1e10 kHz; Princeton Applied Research, Oak Ridge, TN, USA) with the posterior and anterior screws as active and reference electrodes, respectively, and the lateral screw as ground electrode. The amplified signal was digitized by the TDT3 system (module RP2) for off-line analysis.

aABRs were measured once a week during the entire experi- ment, with the first recording starting just before deafening and the last recording ending just before euthanasia (see Fig. 1). The aABRs were evoked with broadband click stimuli consisting of monophasic rectangular pulses (width 20 ms; interstimulus interval 99 ms) presented in free field. The stimuli were delivered via a speaker (Blaupunkt PCxb352) positioned 10 cm above the awake guinea pig. The click stimuli were presented from 75 dB above the average threshold of normal-hearing animals (w110 dB peSPL) down to threshold in 10-dB steps. Threshold was defined as the sound level at which the aABR was just visible.

After implantation of the stimulation electrode on the round window membrane in the right ear, i.e. two weeks after deafening, the eABRs were recorded immediately after electrode positioning and, then, once a week during a period of either two or four weeks (see Fig. 1). Electrical stimuli were monophasic rectangular pulses of 20 ms with amplitudes descending from 400 mA to below threshold with 2-dB steps. The pulses were converted to current pulses by a linear stimulus isolator (type A395; World Precision Instruments, Sarasota, FL, USA). Stimuli were presented with alternating polarity in order to reduce the stimulus artifact. The lateral screw in the skull was used as return electrode. The main parameter was the peak-to-peak amplitude of the eABR wave N1- P2, which occurs around 1 ms after stimulus onset (for details, see Agterberg et al., 2009). The N1-P2 threshold and N1 latency were also determined. Thresholds were defined as the stimulus level that evoked a 2.0-mV N1-P2 waveform and determined by interpolation. Due to failure of the stimulation electrode, eABRs could not be adequately recorded in 5 out of 18 guinea pigs.

2.5. Histology

Immediately after the final ABR measurements, the cochleas were fixed by intralabyrinthine perfusion with a fixative consisting of 3% glutaraldehyde, 2% formaldehyde, 1% acrolein, and 2.5% DMSO in 0.08 M sodium cacodylate buffer (pH 7.4) followed by immersion in the same fixative for 3 h at room temperature. Further histo- logical processing of the cochleas was performed according to a standard protocol (for details, see De Groot et al., 1987). Semithin midmodiolar sections (1 mm) were stained with methylene blue and azur II in sodium tetraborate and used for light microscopical evaluation and quantitative analyses.

To verify the effectiveness of the deafening procedure, in addition to aABR thresholds, the number of remaining inner hair cells (IHCs) and outer hair cells (OHCs) were counted at seven cochlear locations at a half-turn spacing (see Fig. 2A).

To determine SGC packing densities, digitized light microscopic images of Rosenthal's canal at four cochlear locations (B1, B2, M1, and M2; Fig. 2A) were analyzed using Image J (version 1.39t; http:// rsbweb.nih.gov/ij) image-processing software. SGC packing densities could not always be determined for the apical locations, due to tangential sectioning. The bony boundaries of Rosenthal's canal were outlined using a pressure-sensitive stylus on a Wacom CTE-650 digitizer interfaced to a Macintosh computer, and its cross-sectional area was calculated. The number of all (i.e. type-I and type-II) SGC perikarya, with and without a nucleus, was counted. SGC packing densities were calculated by dividing the number of SGCs by the cross-sectional area of Rosenthal's canal at each cochlear location, and expressed as the number of SGCs per mm2.

For quantitative analysis of cell size (i.e. perikaryal area) and cell shape (i.e. circularity) only myelinated (type-I) SGCs with an obvious nucleus were subjected to measurements, especially since the nucleus of type-I SGCs can be easily identified in semithin sections. For determination of perikaryal area and circularity it is important that the cross-sectional area is as close to the center of the cell body as possible. Since the nucleus is situated in the center of the cell body, it was used to define a fixed plane of transection. These measurements were performed at locations B1 and B2, i.e. the basal turn. Both locations were chosen, because an effect of BDNF was more likely to occur in the basal region of the cochlea than more apically, due to the proximity of the round window.

Both perikaryal area and cell circularity were measured directly in the Image J program after outlining the innermost boundary of the myelin sheath enveloping the perikaryon. Circularity is calculated as follows: 4pA/L2, where A is area and L the perimeter.

All quantitative analyses (SGC packing densities, perikaryal area and cell circularity) were performed in a single-blind fashion by two investigators, independently of one another, and both sets of data were subsequently averaged.

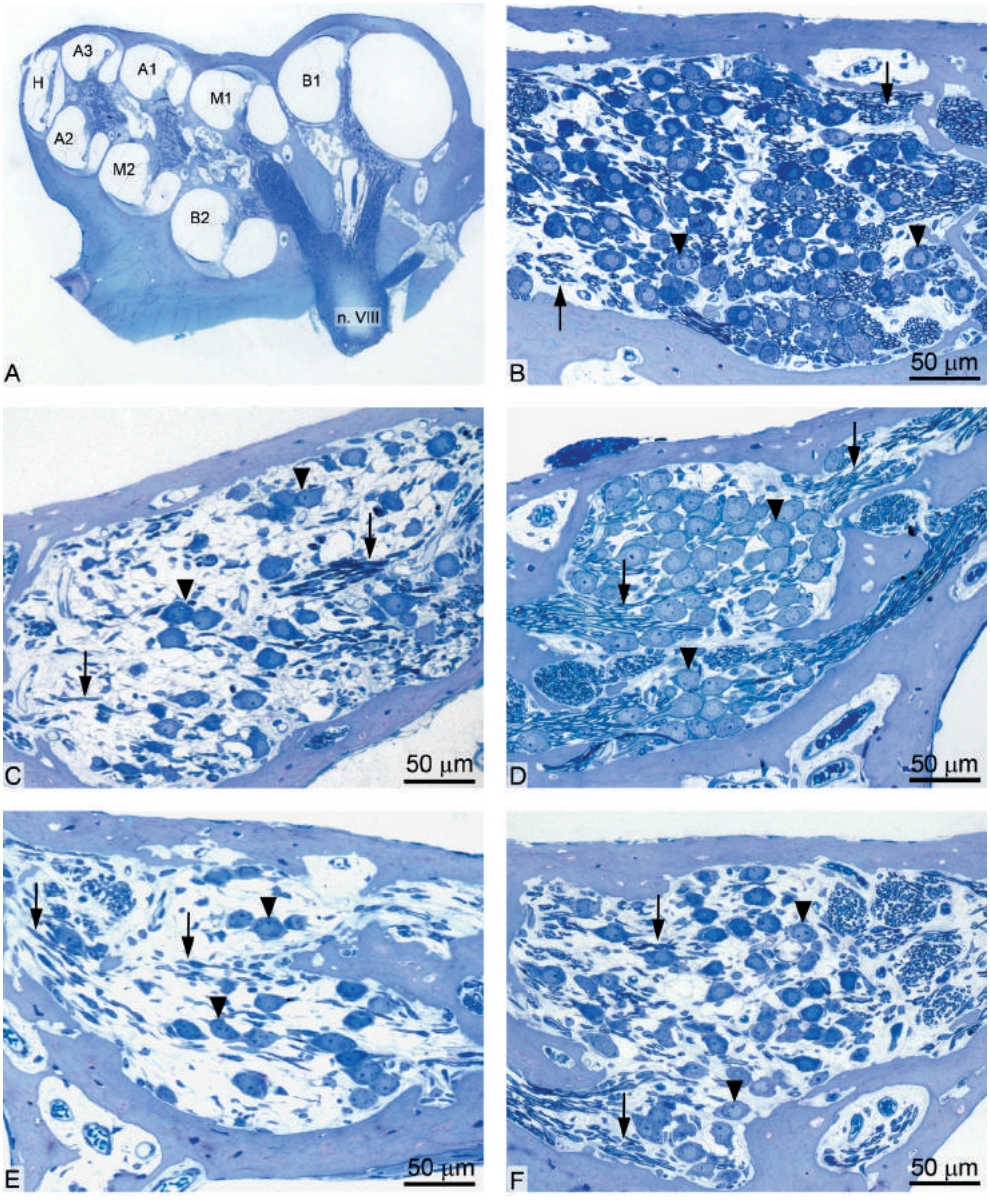

Fig. 2. (A) Low-magnification micrograph of a normal guinea pig cochlea showing the different locations at which SGCs were examined (N. VIII: cochlear nerve). The high magnification micrographs (B-F) are from the transected Rosenthal's canal at cochlear location B1 illustrating the distribution of SGCs (arrowheads) and nerve fibers (arrows) in: (B) a normal cochlea; (C) the left cochlea of an animal, four weeks after deafening; (D) the right BDNF-treated cochlea from the same animal as in C, four weeks after deafening and two weeks after gelfoam placement; (E) the left cochlea of an animal, six weeks after deafening; and (F) the right BDNF-treated cochlea from the same animal as in E, six weeks after deafening and four weeks after gelfoam placement.

2.6. Statistical analyses

The statistical analyses were performed by means of repeated- measures analysis of variance (rm ANOVA), and paired and unpaired t tests, using SPSS for Windows (version 15.0).

3. Results

3.1. Effects of the deafening procedure

A total of eighteen guinea pigs (Fig. 1A,B,C) were deafened by the method described in section 2.2. All deafened animals showed a threshold shift of 55 dB or higher for click-evoked aABRs. In two animals, both in the deafened control group, some OHCs and IHCs were still present in the basal turn of the cochlea. In the other
animals, OHCs and sporadically an IHC could be observed in the apical turn.

3.2. Effect of gelfoam deposition

Comparing the right placebo-treated cochleas (gelfoam containing physiological saline) with the contralateral untreated cochleas in both deafened and normal-hearing controls, there were no significant differences in hair cell counts (paired t test, remaining OHCs and IHCs, $p > 0.1$). The SGC packing densities were also comparable for both groups (paired t test, for each cochlear location separately, $p > 0.1$).

3.3. SGC packing densities

Fig. 2 shows light micrographs of Rosenthal's canal at the lower basal cochlear turn (location B1). These images provide typical examples of SGCs in the cochleas from a normal-hearing control and deafened animals. Rosenthal's canal in the normal cochlea (Fig. 2B) contains densely packed SGCs and nerve fibers embedded in a matrix consisting of vascularised connective tissue. Four weeks after deafening there is a clear loss of SGCs (Fig. 2C), which is even greater after six weeks (Fig. 2E). Two weeks after BDNF treatment (Fig. 2D) the amount of SGCs is similar to that in the normal cochlea (Fig. 2B), whereas after four weeks a minimal loss of SGCs is observed (Fig. 2F). Several of the remaining SGCs, particularly six weeks after deafening (Fig. 2E,F), demonstrate a more elongated and dendritic appearance as compared to the characteristic ovoid shape of normal SGCs (Fig. 2B).

In Fig. 3 packing density data of all individual animals from both experimental groups (2- and 4-weeks treated) are shown. BDNF treatment seems to result in SGC survival mainly in the basal turn of the cochlea. This effect (at least 33% more SGC survival than in the contralateral untreated cochlea) is seen in five animals from the 2-weeks treated group and four animals from the 4-weeks treated group at location B1. At location B2, four animals from the 2-weeks treated group and two animals from the 4-weeks treated group showed this effect. Fig. 4 shows the mean SGC packing densities at cochlear locations B1 to M2. First, an

rm ANOVA with location (B1-M2) and treatment (right vs. left cochlea) as within factors and experimental group (2- and 4-weeks treated) as between factor was performed. Because of an interaction between both within factors the analyses were performed for each location separately. SGC packing densities in BDNF-treated cochleas at location B1 were near-normal and significantly higher than in the untreated cochleas (rm ANOVA, p 0.001) and show a trend to be higher than those in the untreated cochleas at locations B2 and M1 (rm ANOVA, B2: p 0.06; M1: p 0.07). In the 2-weeks treated group, the protective effect of BDNF was virtually complete at location B1 (unpaired t test, 2-weeks BDNF-treated vs. normal, p 0.4). At all other locations, packing densities in both the BDNF- treated and untreated cochleas were lower than normal (unpaired t tests, $p < 0.05$). In the 4-weeks treated group, packing densities in both the BDNF-treated and untreated cochleas were significantly lower than normal, at all locations (unpaired t test, $p < 0.05$).

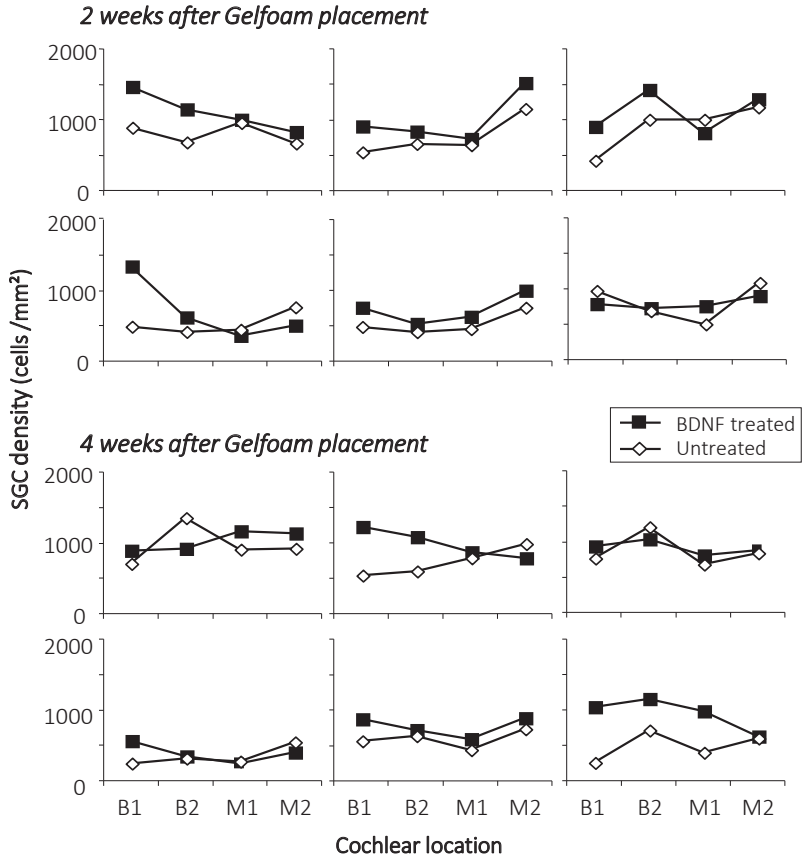

Fig. 3. SGC packing densities in all individual deafened animals from both experimental groups (2-and 4-weeks treated) at cochlear locations B1 to M2. The right cochleas of the animals were treated with BDNF, while the left cochleas remained untreated.

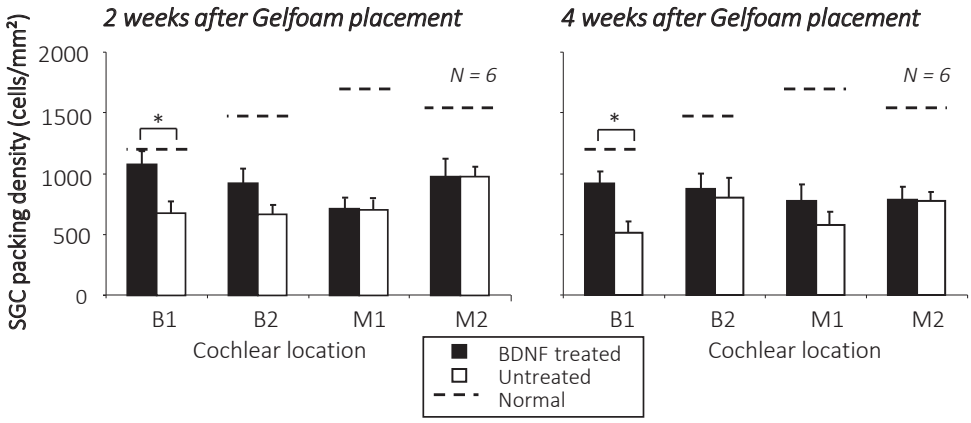

Fig. 4. Mean SGC packing densities at cochlear locations B1 to M2 in both experimental groups (2- and 4-weeks treated) compared to normal-hearing controls (dashed line), with n= 6 for all three groups and for each location. **: $p < 0.01$. Error bars: SEM.

3.4. SGC perikaryal area and cell circularity

Fig. 5 shows the mean values of the perikaryal area (A) and cell circularity (B) of SGCs at cochlear locations B1 and B2. An rm ANOVA with location (B1 and B2) and treatment (right vs. left) as within factors and experimental group (2- and 4-weeks treated) as between factor was performed. BDNF treatment did not show any difference with respect to cell size or shape, as both perikaryal area and cell circularity of BDNF-treated SGCs were comparable to those of SGCs in the untreated cochleas in both experimental groups ($p > 0.5$).

Comparison of the experimental groups with normal-hearing controls showed no difference on SGC size and shape at cochlear locations B1 and B2 for the 2-weeks treated group (post-hoc Tukey's HSD, $p > 0.1$). However, in the 4-weeks treated group both BDNF-treated and untreated SGCs were significantly smaller and less circular than those in normal-hearing controls at both locations B1 and B2 (post-hoc Tukey's HSD, perikaryal area: $p < 0.01$; circularity: $p < 0.001$).

In addition, we observed an effect on SGC size in relation to the cochlear location. We found that SGCs at the most basal location B1 were significantly larger than those in B2 for all groups (rm ANOVA, $p < 0.001$).

3.5. Electrically evoked auditory brainstem responses

In Fig. 6 examples of eABR waveforms at various moments (one to four weeks) after implantation of the stimulation electrode are given. The eABRs of a 4-weeks BDNF-treated deafened animal (Fig. 6C) and a deafened control (Fig. 6B) were smaller than in a normal-hearing control (Fig. 6A). The late peaks (after 2 ms) were less well-defined in the deafened animals than in the normal- hearing control animal. The latency of the early peak, N1, appeared somewhat longer in deafened animals compared to the normal- hearing control animal. In

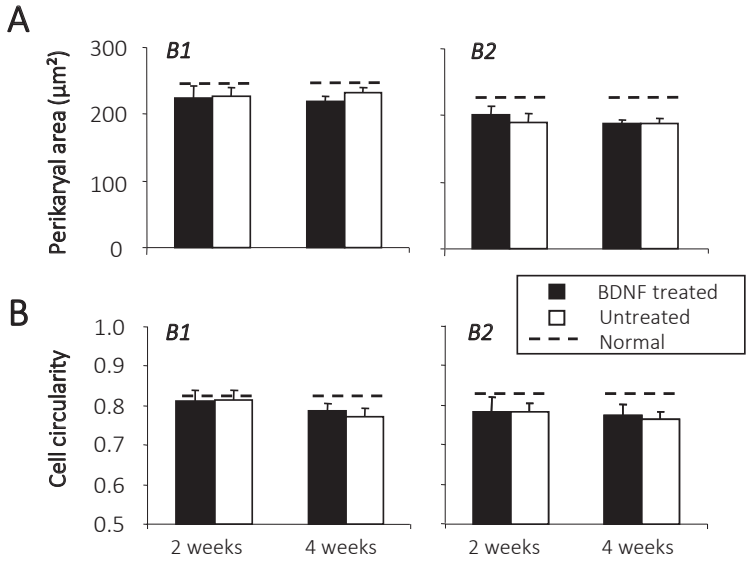

Fig. 5. Mean perikaryal area (A) and cell circularity (B) at cochlear locations B1 and B2 in both experimental groups (2- and 4-weeks treated) compared to normal-hearing controls (dashed line). Data shown were obtained by averaging all individual cell measurements within the same spiral ganglion transection, followed by averaging over the cochleas. Error bars: SEM.

all three animals, the eABRs and, in particular, their early N1-P2 complexes were fairly robust over time. There were no differences in eABR waveforms in the first two weeks between the 2- and 4-weeks treated groups (not shown).

The early complex of the eABR was further analyzed by deriving the peak-to-peak amplitude of N1-P2, the threshold of N1-P2, and the N1 latency. The average outcomes of these parameters are shown in Fig. 7 for normal-hearing controls, 4-weeks BDNF-treated deafened animals, and deafened controls. The eABR parameters amplitude, threshold and latency were analyzed using rm ANOVA with time as within factor and group as between factor. The amplitudes (Fig. 7A) were significantly smaller in both groups of deafened animals than in the normal-hearing controls ($p < 0.01$), while there was no difference in amplitude between the 4-weeks BDNF-treated animals and the deafened controls. There was a small amplitude growth with time, which was significant ($p < 0.05$). The thresholds and latencies showed similar trends as the amplitude data. A comparison between both deafened groups (4-weeks BDNF-treated animals and deafened controls) did not show significant differences in latency or threshold ($p > 0.2$), and the threshold and latency decreased slightly but significantly with time ($p < 0.05$). Also, all deafened animals tended to have higher thresholds and longer latencies than normal-hearing animals (threshold: $p = 0.07$; latency: $p = 0.05$).

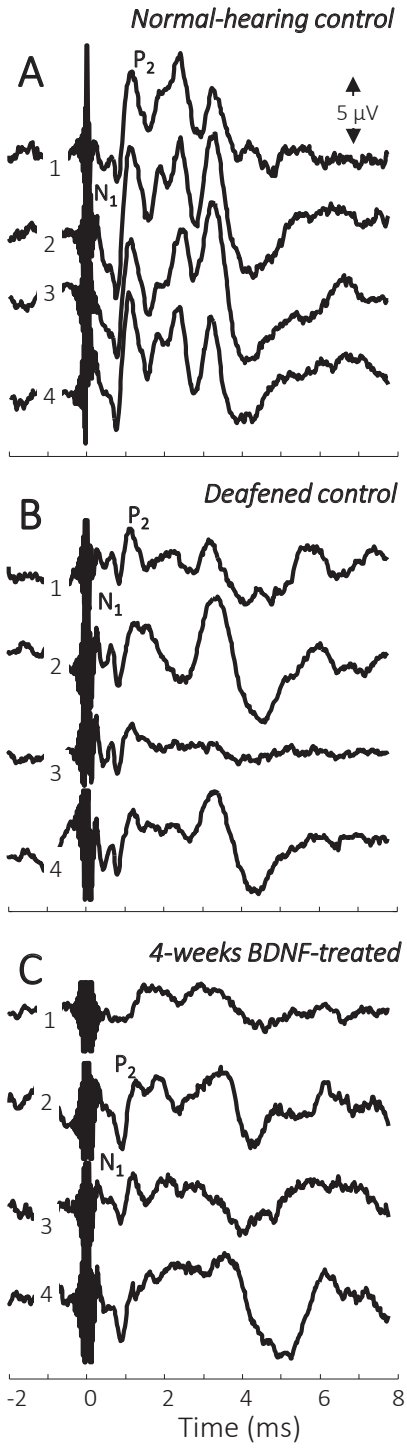

Fig. 6. eABR waveforms at various moments after implantation of the stimulation electrode in: (A) a normal-hearing control animal; (B) a deafened control animal; and (C) a 4-weeks BDNF-treated deafened animal. Weeks after implantation are indicated in the boxes. The N1 and P2 peaks were used for analysis. The electric stimulus was a monophasic pulse with a width of 20 ms and a current of 250 mA (4 dB below 400 mA).

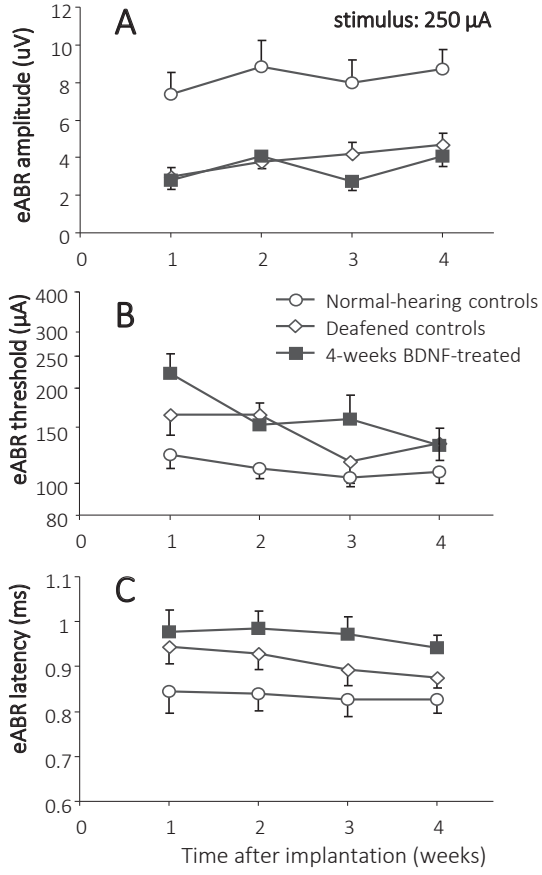

Fig. 7. Mean amplitudes (A), thresholds (B) and latencies (C) of eABRs in normal- hearing controls (n =4), deafened controls (n =4), and 4-weeks BDNF-treated deaf- ened animals (n = 5). The amplitude is based on the N1-P2 complex and the latency on the N1 peak. The stimulus applied in the amplitude and latency data had a width of 20 ms and a current of 250 mA. In B, the vertical axis is scaled logarithmically. Error bars: SEM.

4. Discussion

This study examined a local delivery method for protecting SGCs in hair-cell deprived cochleas that is thought to be relatively safe for clinical application. Gelfoam infiltrated with BDNF was placed onto the round window membrane and its effect was studied using several structural and functional measures. BDNF treatment was started two weeks after deafening and its effect was evaluated two and four weeks after gelfoam placement. The present study showed enhanced survival of SGCs two and four weeks after placement of gelfoam, infiltrated with 6 mg of BDNF, in the basal region of the cochlea (Figs. 3 and 4). The perikaryal area and cell circularity of SGCs treated with BDNF showed no significant differences compared to the SGCs in contralateral untreated cochleas (Fig. 5). Finally, in animals treated with BDNF, eABR amplitudes were smaller than in normal-hearing controls, and similar to those in deafened controls.

4.1. Spiral ganglion cell survival

Two weeks after gelfoam placement the protective effect of BDNF was complete at location B1, as evidenced by the (near-) normal SGC packing densities in the BDNF-treated cochleas (Fig. 4). These results are in accordance with a tracer study showing that trimethylphenylammonium placed upon the round window membrane barely reaches the apical turn, while high concentrations of this low molecular-weight marker were found in the basal and middle turns of the cochlea (Salt and Ma, 2001). This basal- apical concentration gradient following drug application onto the round window membrane has also been reported for gentamicin and dexamethasone in guinea pigs (Salt and Plontke, 2009). The survival effect is smaller than after intracochlear delivery of neurotrophins using mini-osmotic pumps (Table 1). In those studies SGC survival rates are reported to be (near-)normal, at least up to the lower middle turn (Miller et al., 1997; Shepherd et al., 2005; Wise et al., 2005; Agterberg et al., 2008), and in several studies the effect was observed even throughout the entire cochlea (Yamagata et al., 2004; Maruyama et al., 2008; Agterberg et al., 2009).

This is the first study that demonstrates a significant survival of SGCs after round window membrane application of BDNF-containing gelfoam, two and four weeks after treatment. In contrast to our findings, Richardson et al. (2006) reported that NT-3-containing gelfoam placed onto the round window membrane did not show a significant protective effect on SGC survival in guinea pigs after four weeks of treatment, although the SGC packing density in the treated cochleas was higher than in the contralateral untreated cochleas (their Fig. 3).

Noushi et al. (2005) placed alginate beads (0.5e1 mm) containing 6-8.5 mg NT-3 onto the round window membrane. They did find a protective effect after four weeks throughout the entire cochlea, but SGC survival was less than in normal-hearing animals. Endo et al. (2005) placed 2 ml of a hydrogel solution containing 84 mg of BDNF onto the round window membrane and observed a beneficial effect from the basal up to the apical region after seven days of treatment. The higher concentrations of the neurotrophins and/or the alternative delivery methods might explain why both studies found a larger effect. We used 1-mm3 gelfoam cubes which absorbed 6 mg of BDNF. Noushi et al. (2005) used comparable concentrations, but the neurotrophin applied was NT-3. Endo et al. (2005), however, used a more than 10-fold higher concentration of BDNF (84 mg).

Table 1. Comparison of the intracochlear mini-osmotic pump system and the extracochlear gelfoam method.

	Intracochlear cannula (Agterberg et al., 2009)	**Extracochlear Gelfoam (current study)**
SGC packing density	> Location B1 – A1	> Location B1
	> Untreated	= Untreated
SGC size	= Normal-hearing controls	< Normal-hearing controls
eABR amplitudes	> Deafened controls	= Deafened controls

4.2. SGC perikaryal area

We found a decrease in SGC perikaryal area, i.e. SGC size, after deafening which is consistent with earlier observations (Staecker et al., 1996; Leake et al., 1999; Shepherd et al., 2005; Richardson et al., 2005; Agterberg et al., 2008; Glueckert et al., 2008). However, the lack of an effect of BDNF on SGC size in the basal turn is in contrast with the substantial increase in SGC size that is found after BDNF treatment using mini-osmotic pumps (Shepherd et al., 2005; Agterberg et al., 2008; Glueckert et al., 2008).

4.3. Electrically evoked auditory brainstem responses

Six weeks after deafening the eABRs had smaller amplitudes, longer latencies and higher thresholds than normal. These obser- vations are in line with other findings. The amplitude findings agree with studies that similarly measured the N1-P2 amplitude (Hall, 1990; Agterberg et al., 2009) and with studies that based amplitude measurements on late peaks (Smith and Simmons, 1983; Sly et al., 2007) as well as with the results of Maruyama et al. (2007), who measured the P1 amplitude. The latency findings agree with the result of Agterberg et al. (2009) who recorded eABRs before and after deafening in the same animals using an intra- cochlear stimulation electrode. In most studies only the threshold data are given and not the amplitude and latency at suprathreshold stimulation levels. In general, four to six weeks after deafening threshold increases of 1e5 dB have been found (Shinohara et al., 2002; Yamagata et al., 2004; Shepherd et al., 2005; Chikar et al., 2008; Maruyama et al., 2007, 2008; Agterberg et al., 2009), which is similar to our findings (2e3 dB).

When neurotrophic treatment delivered by means of a mini- osmotic pump resulted in enhanced and near-normal SGC survival throughout the cochlea, eABR amplitudes were found to be greater than in deafened controls (Maruyama et al., 2008; Agterberg et al., 2009). Although the effect on SGC survival was smaller than in those studies and limited to the basal part of the cochlea, some effect on the eABRs was expected. However, no effect, or even a trend of an effect, was found. This implies that the electrically evoked response of the rescued cells was weaker or contained more jitter (and thus less synchrony) than normal. An alternative explanation would be that the SGCs in the basal turn of the cochlea contribute less to the N1-P2 complex than the more apically located SGCs. The latter is not unlikely since response thresholds for SGCs in the middle cochlear turn are lower than those for SGCs in the basal cochlear turn, as has been demonstrated in single-fiber recording experiments using the same electrical stimulation mode (monopolar, round window) as in our study (Van den Honert and Stypulkowski, 1987).

4.4. Clinical applicability

In order to treat cochlear implant candidates with neurotrophin-based therapy it is necessary to develop a safe, effective and minimally invasive delivery method. The present study applied a potentially safer technique compared to intracochlear delivery methods and

showed a protective effect in the basal cochlear turn. Clinical application of BDNF by means of extracochlear delivery requires the ability of BDNF to diffuse across the human round window membrane and subsequently enter the intracochlear fluids in sufficient amounts. In general, diffusion of molecules through the round window membrane depends on a variety of physicochemical factors, but is also influenced by the membrane's thickness. In humans the round window membrane is 70 mm thick, whereas in the guinea pig it measures 12 mm (Goycoolea and Lundman, 1997; Saber et al., 2009). Nevertheless, it has been demonstrated that a variety of molecules can diffuse through the round window membrane in humans. Two examples that are currently used in clinical practice are intratympanic injection of gentamicin in patients suffering from Ménière's disease (Sajjadi and Paparella, 2008; McCall et al., 2010) and the intratympanic perfusion of corticosteroids in patients with idiopathic sudden sensorineural deafness and Ménière's disease (Lefebvre and Staecker, 2002; Slattery et al., 2005; Garduno-Anaya et al., 2005; Boleas-Aguirre et al., 2008; Sajjadi and Paparella, 2008).

Several clinical studies have placed gelfoam near the round window membrane and used it as carrier to deliver drugs to the inner ear. Silverstein et al. (1996, 1999) have demonstrated that corticosteroid-containing or gentamicin-containing gelfoam is safe and effective in treating patients with Ménière's disease.

In developing a new technique, the experimental animal model should be representative for the situation in humans. In both studies of Silverstein et al. (1996, 1999) the tympanic cavity was (partially) filled up with medication. Mikulec et al. (2009) state that an intratympanic drug delivery system, in which a great part of the tympanic bulla is filled with solution, does not provide a representative animal model. They have found high drug concentrations in the apical regions of the cochlea (via diffusion through the very thin otic capsule in rodents) in this application method. It can be assumed that the thicker bone of the human otic capsule will represent an effective boundary, resulting in different distribution patterns following intratympanic drug application (Mikulec et al., 2009; Salt and Plontke, 2009). Therefore, a representative animal model would be a delivery system in which the drug is placed only onto the round window membrane. Taking into account that the average surface area of the round window in the guinea pig is 1.2 mm2 (Ghiz et al., 2001), we applied gelfoam cubes of 1 mm3 and used the tip of the electrode to keep it in place. Noushi et al. (2005) placed 4-5 alginate beads sized 0.5-1 mm, covering an area greater than the round window surface area. Endo et al. (2005) placed a hydrogel solution (2 ml) on the round window, which could migrate through the tympanic bulla and subsequently diffuse through the otic capsule. Perhaps this explains the SGC survival found in the apical region of the cochlea in these two studies.

We conclude that the gelfoam delivery method minimizes migration of fluids through the tympanic bulla and therefore might be representative for the situation in humans. The permeability of the human round window membrane for neurotrophins (i.e. BDNF) is yet unknown and therefore this should be investigated prior to clinical application.

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Chapter 3

Local Delivery of Brain-Derived Neurotrophic Factor on the Perforated Round Window Membrane in Guinea Pigs: A Possible Clinical Application

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Abstract

Hypothesis/Background: Local delivery of neurotrophic factors on the intact round window membrane (RWM) of hair cell-deprived cochleas reduces degeneration of the cochlear nerve. In an animal model of profound hearing loss, we investigated whether this otoprotective effect could be enhanced by perforation of the RWM. Such method could be highly relevant for future clinical applications.

Methods: Guinea pigs were deafened by coadministration of kanamycin and furosemide. Two weeks after deafening, Gelfoam cubes infiltrated with brain-derived neurotrophic factor (BDNF) were deposited onto the RWM of the right cochlea. In the ex- perimental condition, the RWM was perforated. Electrically evoked auditory brainstem responses (eABRs) were recorded weekly. Two or four weeks after Gelfoam placement, both left (untreated) and right (BDNF-treated) cochleas were processed for histology.

Results: In BDNF-treated cochleas, both with and without perforation, neural survival in the basal turn of the cochlea was significantly larger than in untreated cochleas. Amplitudes of electrically evoked auditory brainstem responses were larger in BDNF-treated cochleas with an RWM perforation than in those without a perforation and comparable to those of normal-hearing controls. Perforation did not lead to collateral cochlear damage.

Conclusion: When considering clinical applications of neuroprotective agents such as BDNF, delivery on a perforated RWM seems to be a safe and effective option.

1. Introduction

Recently, evidence has been provided that speech reception performance of cochlear implant users is positively correlated with the number of surviving spiral ganglion cells (SGCs) (Seyyedi et al., 2014). Therefore, clinically applicable methods to protect the auditory nerve from degeneration have become pertinent. It is well known from animal studies that cochlear delivery of neurotrophic factors prevents secondary loss of SGCs in hair cell-deprived cochleas (Ramekers et al., 2012). The majority of studies on neurotrophic treatment have used an intracochlear delivery method, in which neurotrophins are directly infused into the cochlea by means of a miniosmotic pump delivery system (e.g., Ernfors et al., 1996; Staecker et al., 1996; Miller et al., 1997; Gillespie et al., 2004; Wise et al., 2005; Agterberg et al., 2008; Agterberg et al., 2009). Although intracochlear delivery is effective in preserving SGCs, the clinical applicability of such an approach is strongly disputed mainly because of an un- acceptable risk of infection of this traumatic and complex delivery system.

To avoid these drawbacks, we previously applied an alternative delivery method in which brain-derived neurotrophic factor (BDNF) soaked in absorbable gelatin sponge (Gelfoam) was placed on the round window membrane (RWM) (Havenith et al., 2011). This extracochlear delivery method resulted in enhanced SGC survival in the most basal region of the cochlea in deafened guinea pigs. Function- ally, the effect seemed to be absent because electrically evoked auditory brainstem response (eABR) amplitudes were smaller in BDNF-treated animals compared with normal-hearing controls and similar to those in deafened controls (Havenith et al., 2011).

The use of Gelfoam is an indirect delivery method of BDNF to the cochlea in which the substance must first diffuse through the RWM before reaching the perilymphatic fluid. Diffusion-based delivery of substances to the cochlea by means of Gelfoam placed onto the RWM has frequently been used in both animal and clinical studies. Guitton et al. (2003) have demonstrated the diffusion of an NMDA-antagonist in the perilymphatic fluids of the rat cochlea after placement of Gelfoam cubes on the RWM, and Silverstein et al. (1999) have shown the beneficial effect of RWM application of gentamicin in treating patients with Ménière's disease. Furthermore, Eastwood et al. (2010) demonstrated that RWM delivery of dexamethasone can ameliorate both low- and high-frequency hearing loss associated with the trauma of cochlear implantation. Wimmer et al. (2004) applied several substances on the RWM, which can be used as an effective treatment in the prevention of cisplatin toxicity.

In the present study, we examined if Gelfoam containing BDNF, when placed on a perforated RWM, results in more SGC survival and/or functional otoprotective effect com- pared with BDNF treatment on the intact RWM (Havenith et al., 2011). In our approach, SGC survival was measured in terms of packing density. Also, we recorded eABRs to monitor the excitability of the SGCs (Hall, 1990; Mitchell et al., 1997; Shepherd et al., 2005; Agterberg et al., 2008).

2. Materials and Methods

2.1. Animals and Experimental Design

Twenty-eight albino female guinea pigs (Dunkin Hartley strain, weighing 250-350 g) were purchased from Harlan Laboratories (Horst, The Netherlands) and housed in the central animal care facility of the University of Utrecht, The Netherlands. All animals had free access to both food and water and were kept under standard laboratory conditions. Lights were on between 7:00 AM and 7:00 PM. Temperature and humidity were kept constant at 21ºC and 60%, respectively.

Seventeen guinea pigs were deafened and allotted to three experimental groups (2 weeks' survival after BDNF treatment + perforated RWM and 4 weeks' survival after BDNF treatment with or without a perforated RWM; Table 1). Two weeks after deafening, a Gelfoam cube soaked in a BDNF solution was positioned onto the intact or perforated RWM of the right cochlea. The left cochleas were not treated. The animals were sacrificed, and both right and left cochleas were processed for histologic analysis either 2 or 4 weeks after Gelfoam placement. Another four deafened guinea pigs were not treated with BDNF and served as a control group. A second control group consisted of seven normal-hearing guinea pigs. In both control groups, a Gelfoam cube soaked in phosphate-buffered saline was placed on the perforated or intact RWM of the right cochleas and left in situ for 4 weeks. Also, we compared the new data with data from the previous study in which Gelfoam was placed on an intact RWM (Havenith et al., 2011). See Table 1 for a complete overview of the experimental groups.

For histologic analyses, the primary comparison was made between right BDNF-treated cochleas and left untreated cochleas. For electrophysiologic analyses, a comparison was made between deafened BDNF-treated animals and deafened controls.

All experimental procedures were approved by the University's Committee on Animal Research (DEC-UMC 2011.I.01.006).

Table 1.

	Perforation round window membrane		
	YES	*NO*	*Havenith et al., 2011*
Normal-hearing controls	5	2	4
Deafened controls	4		4
2 weeks BDNF treated	6		
4 weeks BDNF treated	6	5	5

2.2. Deafening Procedure

Animals were anesthetized with xylazine (10 mg/kg intra- muscularly; Sedamun) and (S)-ketamine (40 mg/kg intramuscularly; Ketanest-S). Before the deafening procedure, click-evoked acoustic auditory brainstem responses (aABRs) were recorded to check hearing thresholds.

When normal thresholds were confirmed (20-30 dB peSPL), kanamycin sulfate (400 mg/kg; Sigma- Aldrich, St. Louis, MO, USA) in isotonic saline was administered as a single subcutaneous injection followed (~30 min later) by cannulation of the right jugular vein and slow intravenous infusion of furosemide (100 mg/kg; Centrafarm, Etten- Leur, The Netherlands). This procedure, originally reported by West et al. (1973), has been shown to eliminate almost all cochlear hair cells in guinea pigs (Gillespie et al., 2004; Versnel et al., 2007). Control animals received isotonic saline instead of kanamycin and furosemide. One week and 2 weeks after the deafening procedure, aABRs were recorded to assess the extent of hearing loss. Animals with a threshold shift of more than 50 dB after 2 weeks were included in this study.

2.3. Perforating the RWM, Gelfoam Placement, and BDNF Treatment

Two weeks after the deafening procedure, the animals were again anesthetized with xylazine and ketamine. The right cochlear bulla was exposed through a retroauricular approach followed by creating a small hole in the bulla to expose the RWM. Subsequently, the RWM was perforated in 21 animals (Table 1) using a surgical micropick instrument (Fig. 1). In 12 of those animals, small cubes (1 mm3) of Gelfoam were soaked in 6 µL of a 1-mg/mL solution of BDNF (PeproTech Inc., Rocky Hill, NJ, USA) in physiologic saline and positioned onto the RWM. The dose of 6 Kg is in the range of doses applied in a majority of studies successfully using a mini-osmotic pump system for the delivery of BDNF into the guinea pig cochlea (e.g., 10 µg [Gillespie et al., 2004; Wise et al., 2005] and 20 µg [Agterberg et al., 2008]). However, the actual amount of BDNF entering the perilymph is unknown. Note that much lower doses have been shown to be effective as well (e.g., 10 ng, guinea pig [Miller et al., 1997]; 1 µg, rat [McGuiness and Shepherd, 2005]).

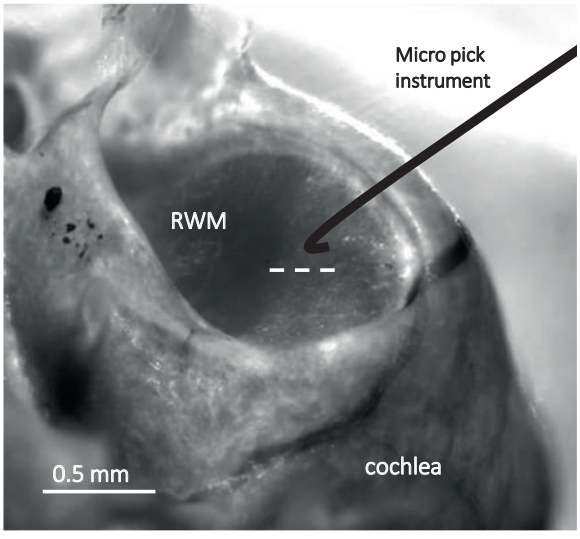

FIG. 1. Low-magnification micrograph of the guinea pig cochlea, illustrating the technique used to perforate the round window. Round window perforation marked as the dashed line. The surgical micropick has a slightly bent tip with a diameter of approximately 0.15 mm. The actual perforation had a diameter of approximately 0.3 mm.

An electrode, consisting of a stainless steel wire with a goldball tip, was used to manipulate the Gelfoam cubes onto the RWM. Correct positioning of the Gelfoam was checked directly during surgery. The Gelfoam covered the entire surface area of the RWM, preventing the risk of perilymph leakage. Overt vestibular trauma after the perforation of the RWM was not observed postoperatively.
Although the primary purpose of the electrode was to deliver electric pulses to the cochlea to evoke eABRs, it was also used to keep the Gelfoam in place. The gold-ball tip was positioned in the RWM niche (for details, see [Klis et al., 2000]). The stainless steel wire was fixed onto the bulla with dental cement (Ketac-Cem Aplicap; ESPE Dental Supplies, Utrecht, The Netherlands). The wire contact was placed in a connector and fixed with dental cement onto the skull.

2.4. Electrophysiologic Recordings

The aABRs and eABRs were recorded in awake guinea pigs in a sound-attenuated chamber (Agterberg et al., 2009; Havenith et al., 2011). Three stainless steel screws (8.0 x 1.2 mm) were inserted into the skull bone to record ABRs. The screws were inserted 1 cm posterior to the bregma, 2 cm anterior to the bregma, and 1 cm lateral from the bregma (Mitchell et al., 1997).
The aABRs were measured once a week during the entire experiment, with the first recording starting just before deafening and the last recording ending just before euthanasia (Fig. 2). The aABRs were evoked with broadband click stimuli consisting of monophasic rectangular pulses (width, 20 μs; interstimulus interval, 99 ms) presented with alternating polarity in free field. The stimuli were delivered via a speaker (Blaupunkt PCxb352) positioned 10 cm above the awake guinea pig. The click stimuli were presented from 75 dB above the average threshold of normal-hearing animals (~110 dB peSPL) down to threshold in 10-dB steps. Threshold was defined as the sound level at which the aABR was just visible. The ABR thresholds were used to assess global hearing loss.
After implantation of the stimulation electrode on the RWM in the right ear, that is, 2 weeks after deafening, the eABRs were recorded immediately after electrode positioning and, then, once a week during a period of either 2 or 4 weeks (Fig. 2). Electrical stimuli were monophasic rectangular pulses of 20 microseconds, with amplitudes descending from 400 μA to below threshold with 2-dB steps. The current pulses, de- livered by a linear stimulus isolator (Type A395; World Precision Instruments, Sarasota, FL, USA), were presented with alternating polarity to reduce the stimulus artifact and to avoid buildup of charge. Stimuli were presented with alternating polarity to reduce the stimulus artifact. The main parameter was the peak-to-peak amplitude of the eABR wave N1-P2, which occurs approximately 1 millisecond after stimulus onset (for details, see Agterberg et al., 2009). The N1-P2 threshold and N1 latency were also determined. Thresholds were defined as the stimulus level that evoked a 2.0-μV N1-P2 waveform and determined by interpolation. Note that electrophonic nerve activity

that may be evoked in normal-hearing guinea pigs cannot contribute to the early N1-P2 wave because the electrophonic activity is relatively small and follows about 1 millisecond after the directly evoked nerve activity (Stronks et al., 2010).

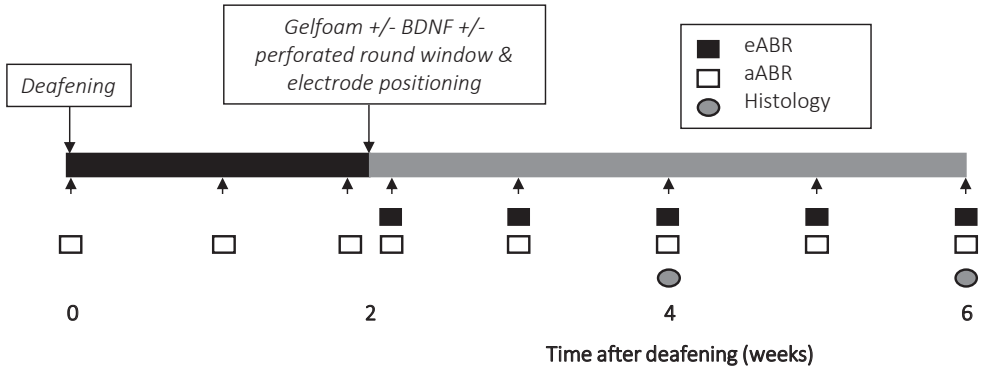

FIG. 2. Treatment schedule. For the number of animals in each group, see Table 1. Deafening was performed systemically and, hence, affected both ears. Gelfoam was applied to the right ears. After perforation of the round window membrane with a surgical micropick, Gelfoam placement, and electrode positioning, eABRs were measured in each group by electrically stimulating the right ear. Animals were sacrificed for histologic analyses 2 or 4 weeks after BDNF treatment.

2.5. Histology

Immediately after the final ABR measurements, the cochleas were fixed by intralabyrinthine perfusion with a fixative consisting of 3% glutaraldehyde, 2% formaldehyde, 1% acrolein, and 2.5% dimethyl sulfoxide in 0.08 mol/L sodium cacodylate buffer (pH 7.4), followed by immersion in the same fixative for 3 hours at room temperature. Further histologic processing of the cochleas was performed according to a standard protocol (for details, see De Groot et al., 1987). Semithin midmodiolar sections (1 µm) were stained with methylene blue and Azur II in sodium tetraborate and used for light microscopic evaluation and quantitative analyses.

To verify the effectiveness of the deafening procedure, in addition to aABR thresholds, the number of remaining inner hair cells and outer hair cells (OHCs) were counted at seven cochlear locations at a half-turn spacing.

To determine SGC packing densities, digitized light microscopic images of Rosenthal's canal at four cochlear locations (B1, B2, M1, and M2) were analyzed using ImageJ (version 1.39 t; http://rsbweb.nih.gov/ij) image processing software. SGC packing densities could not always be determined for the apical locations because of tangential sectioning. The bony boundaries of Rosenthal's canal were outlined using a pressure-sensitive stylus on a Wacom CTE-650 digitizer interfaced to a Macintosh computer, and its cross-sectional area was calculated. The number of all (i.e., Type I and Type II) SGC perikarya, with and without a nucleus, was counted. SGC packing densities were calculated by dividing the number of SGCs by the cross-sectional

area of Rosenthal's canal at each cochlear location and expressed as the number of SGCs per square millimeter.

For quantitative analysis of cell size (i.e., perikaryal area), only myelinated (Type I) SGCs with an obvious nucleus were subjected to measurements, especially because the nucleus of Type I SGCs can be easily identified in semithin sections. Because the nucleus is situated in the center of the cell body, it was used to define a fixed plane of transection. These measurements were performed at location B1, that is, the most basal turn of the cochlea (Fig. 4A) because an effect of BDNF was most likely to occur in the most basal region of the cochlea because of the proximity of the RWM. Perikaryal area was measured directly in the ImageJ program after outlining the innermost boundary of the myelin sheath enveloping the perikaryon.

2.6. Statistical Analyses

The statistical analyses were performed by means of repeated- measures analysis of variance (rm ANOVA), and paired and unpaired t tests, using SPSS for Windows (version 15.0).

3. Results

3.1. Effects of the Deafening Procedure

A total of 21 guinea pigs (Table 1) were deafened as described in the Materials and Methods section. All deafened animals showed a threshold shift of 55 dB or higher for click-evoked aABRs. In general, consistent with this hearing loss, hair cell loss was profound; only in some animals were hair cells still present. In one animal in the deafened control group, some OHCs were still present in the basal turn of the cochlea. In some other animals, some OHCs and sporadically an inner hair cell could be observed in the apical turn. The hearing losses did not differ significantly among the experimental groups (ANOVA, $p = 0.3$).

3.2. Effect of Round Window Perforation

To assess possible damage caused by creating a perforation in the RWM, we compared ABR thresholds, SGC packing densities, and hair cell counts between normal-hearing controls with and those without a perforation (including animals from the previous study [Havenith et al., 2011]; Table 1). Figure 3A shows that aABR thresholds were comparable for both groups until 4 weeks after surgery, both having a minimal hearing loss (~10 dB). Correspondingly, no hair cell loss was seen in either of the normal-hearing animals (data not shown). Figure 3B shows mean SGC packing densities of the right cochleas for both normal-hearing controls with and without a perforated RWM, which were comparable (paired t test, for each cochlear location separately, $p > 0.1$). Also, in both normal-hearing controls, SGC packing densities in the right placebo-treated cochleas (Gelfoam containing physiologic saline) were comparable to those in the contralateral untreated cochleas (paired t test, for each cochlear location separately,

p > 0.1; figure not shown). In the deafened control animals we found, like for the normal-hearing control group, no significant differences in aABR thresholds and SGC packing densities between perforated and intact RWMs (data not shown). Because there was no functional or histologic damage after perforation of the RWM, we analyzed all normal- hearing controls (n = 11) as one group and all deafened controls (n = 8) as one group. Note that we included data of the previous study using intact RWMs (Havenith et al., 2011).

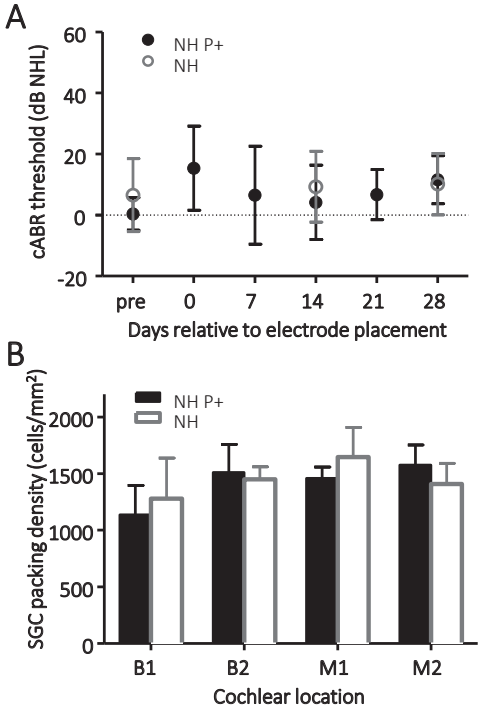

FIG. 3. A, The aABR thresholds for normal-hearing controls with a perforated round window (NH P+, n = 5) or intact round window (NH, n = 6). B, Mean SGC packing densities at cochlear locations B1 to M2 in normal-hearing controls with (NH P+, n = 5) or without (NH, n = 6) a perforated round window. Error bars show standard deviation in both plots.

3.3. SGC Packing Densities and Perikaryal Area

Figure 4 shows light micrographs of Rosenthal's canal at the lower basal cochlear turn (location B1). These images provide typical examples of SGCs in the cochleas from one normal-hearing control and two deafened animals. Rosenthal's canal in the normal cochlea (Fig. 4B) contains densely packed SGCs and nerve fibers embedded in a matrix consisting of vascularized connective tissue. Four weeks after deafening, there is a clear loss of SGCs (Fig. 4C), which is even greater after 6 weeks (Fig. 4E). Two weeks after BDNF treatment (Fig. 4D), the amount of SGCs is similar to that in the normal cochlea (Fig. 4B), whereas, after 4 weeks, only a minimal loss of SGCs is observed (Fig. 4F).

BDNF treatment results in SGC survival mainly in the basal turn of the cochlea. This effect (at least 25% more SGC survival than in the contralateral untreated cochlea) is seen in five

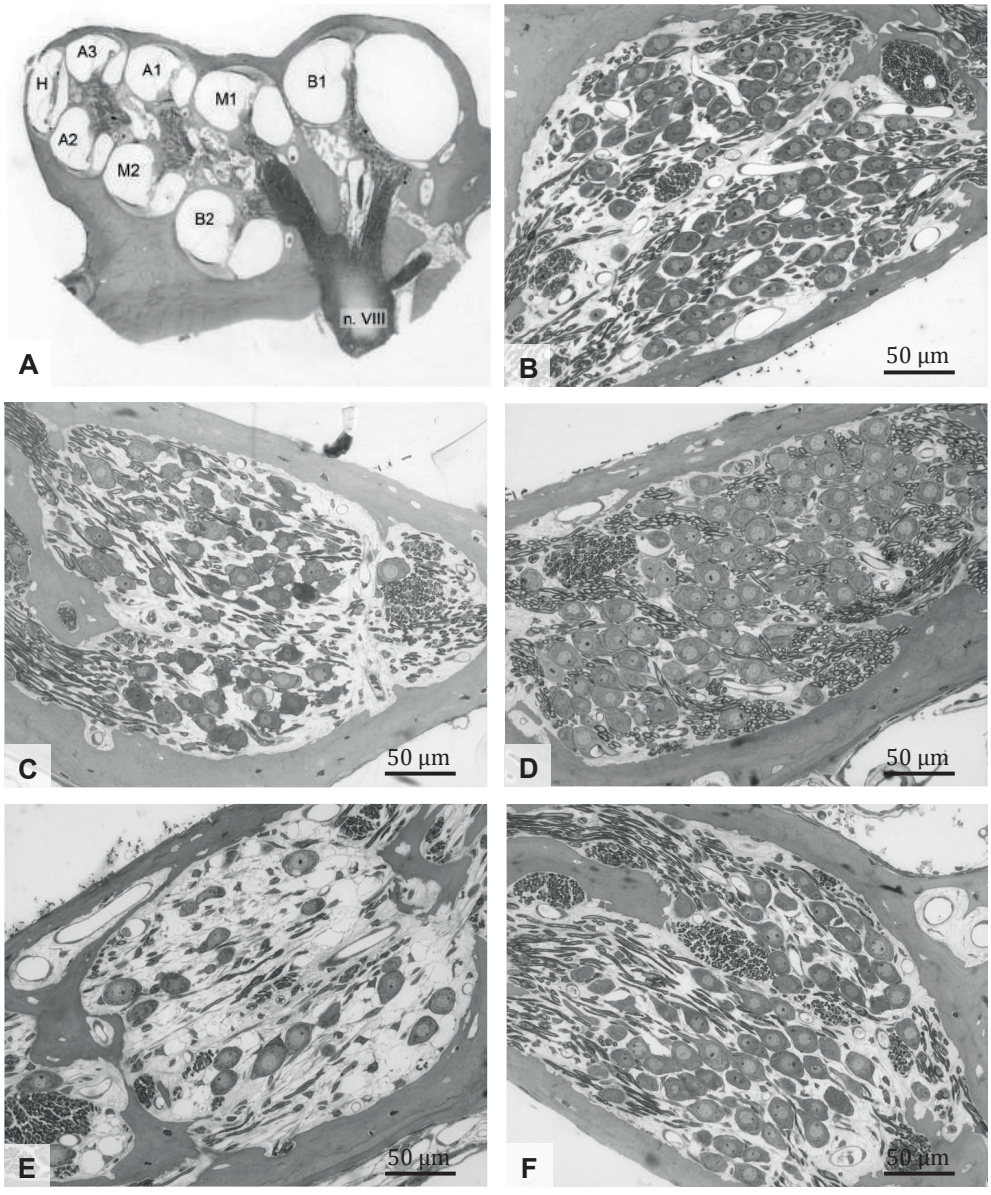

FIG. 4. A, Low-magnification micrograph of a normal guinea pig cochlea showing the different locations at which SGCs were examined (N. VIII: cochlear nerve). The high-magnification micrographs (B-F) are from the transected Rosenthal's canal at cochlear location B1 illustrating the distribution of SGCs (arrowheads) and nerve fibers (arrows) in (B) a normal cochlea; (C) the left cochlea of an animal, 4 weeks after deafening; (D) the right BDNF-treated cochlea on a perforated RWM from the same animal as in (C), 4 weeks after deafening and 2 weeks after Gelfoam placement; (E) the left cochlea of an animal, 6 weeks after deafening; and (F) the right BDNF-treated cochlea on a perforated RWM from the same animal as in (E), 6 weeks after deafening and 4 weeks after Gelfoam placement.

animals from the 2-week-treated group, and all six animals from the 4-week-treated group at location B1. Figure 5 shows the mean SGC packing densities at cochlear locations B1 to M2. First, an rm ANOVA with location (B1-M2) and treatment (right versus left cochlea) as within factors and experimental group (2- and 4-week-treated) as between factor was performed. Because of an interaction between both within factors, the analyses were performed for each location separately. SGC packing densities in BDNF-treated cochleas at location B1 were significantly higher than in the untreated cochleas (rm ANOVA, $p < 0.0001$), whereas SGC packing densities were similar at the other three cochlear locations. SGC survival 4 weeks after BDNF treatment did not significantly differ between the perforated and non- perforated RWM groups (Fig. 5B, C; unpaired t tests B1-M2, $p = 0.4$).

In the 2-week-treated group, the protective effect of BDNF was virtually complete at location B1, with a 90% SGC survival relative to normal (not significantly lower than normal, unpaired

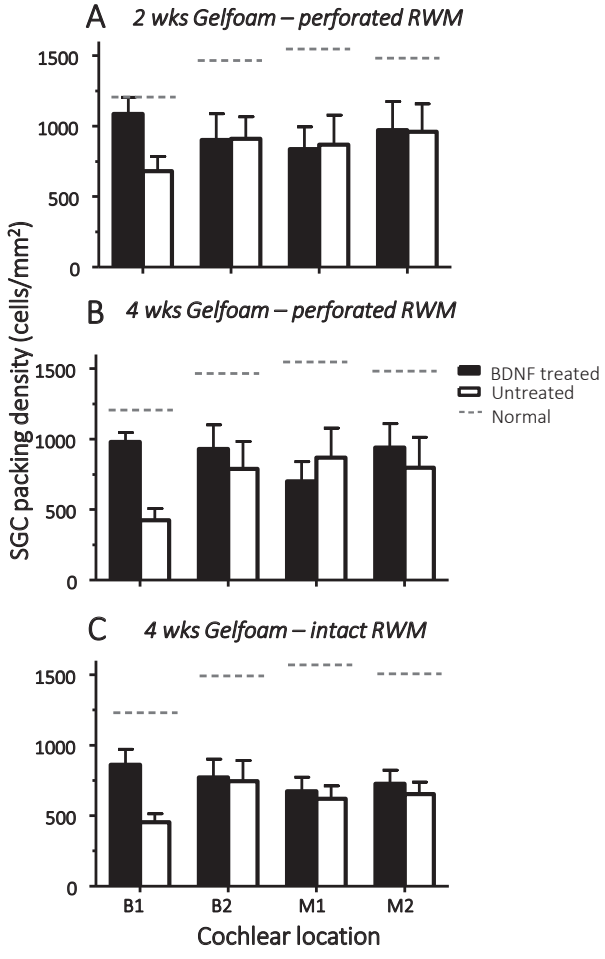

FIG. 5. Mean SGC packing densities at cochlear locations B1 to M2 in both experimental groups with a perforated RWM compared with normal-hearing controls (dashed line, n = 11) and those treated for 2 (A) and 4 weeks (B) (n = 6). C, Mean SGC at cochlear locations B1 to M2 in the 4-week-BDNF-treated group with an intact RWM (n = 10). Error bars: SEM.

t test, p = 0.4). Note that the treatment started 2 weeks after deafening when survival is expected to be down to indeed about 90% in B1 (24). At all other locations, packing densities in both the BDNF-treated and untreated cochleas were lower than normal ($p < 0.05$). In the 4-week-treated group, packing densities in both the BDNF-treated and untreated cochleas were lower than normal at all locations (unpaired t test: B1, p = 0.06; other locations, $p < 0.05$).

Comparison of the perikaryal area between the experimental groups and normal-hearing and deafened controls showed no differences in SGC size at cochlear location B1 (data not shown). It is however well known that SGCs shrink after deafening (e.g., Agterberg et al., 2008, 2009; Ramekers et al., 2012). We probably did not find a difference in SGC size between deafened and normal-hearing animals at location B1 because SGC shrinkage is less prominent at location B1 and is visible from 6 weeks after deafening (van Loon et al., 2013).

3.4. Electrically Evoked ABRs

In Figure 6, examples of eABR waveforms at various time points (1-4 weeks) after implantation of the stimulation electrode are given. The early complex of the eABR was analyzed by deriving the peak-to-peak amplitude of N1-P2, the threshold of N1-P2, and the N1 latency. The average outcomes of these parameters are shown in Figure 7 for normal-hearing controls, 4-week- BDNF-treated deafened animals with and without a perforated RWM, and deafened controls. As previously mentioned, we analyzed all normal-hearing controls (perforated and intact RWM) as one group and all deafened controls as one group. Also, we analyzed all 4-week- BDNF-treated deafened animals without a perforation as one group (n = 10; Table 1) because they received the same treatment and were comparable (rm ANOVA, p 9 0.1).

The eABR parameter amplitude, threshold, and latency were analyzed using rm ANOVA with time as within factor and group as between factor. In all groups, there was a small amplitude growth with time, which was significant ($p < 0.05$). This is probably caused by scar tissue formation around the stimulation electrode, which leads to less current shunting and recovery from surgery-induced trauma, as we have observed previously (4). The amplitudes (Fig. 7A) of the 4-week-BDNF-treated animals with a perforated round window were comparable to those of normal-hearing controls (ANOVA, post hoc Tukey, p = 0.99), while the amplitudes of both 4-weeks BDNF-treated animals with an intact RWM and the deafened controls were significantly lower (ANOVA, post hoc Tukey, 4 weeks after Gelfoam placement, $p < 0.01$). There were no significant differences between the groups regarding threshold (Fig. 7B) and latency (Fig. 7C). Furthermore, the threshold and latency decreased slightly but significantly with time ($p < 0.05$), probably related to the amplitude increase with time.

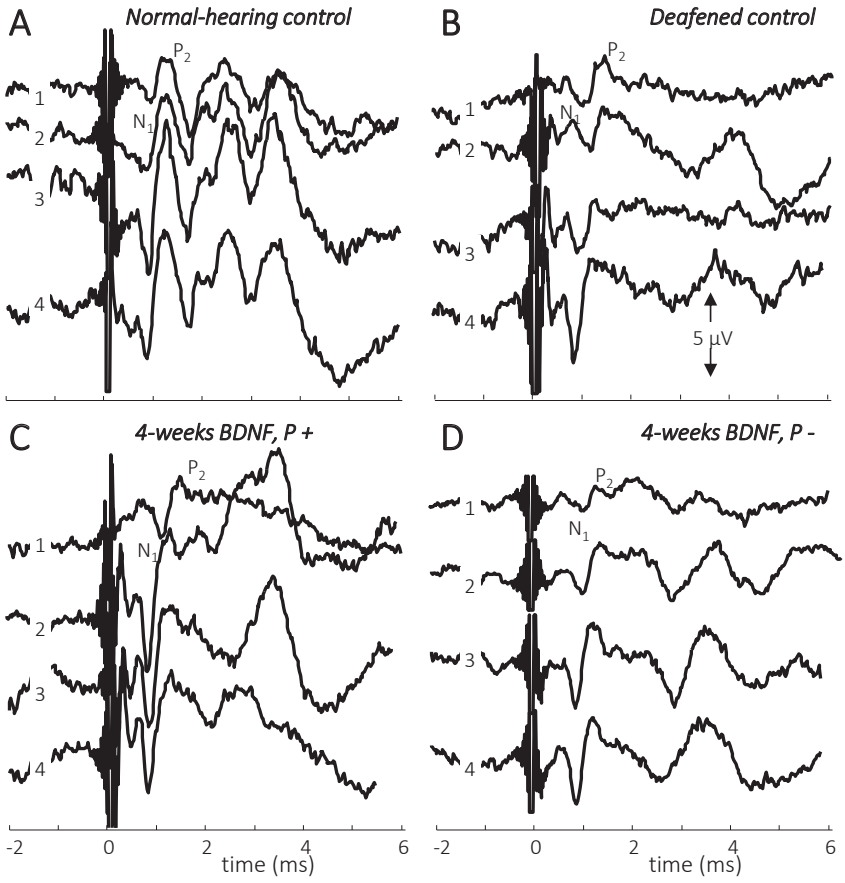

FIG. 6. The eABR waveforms at various moments after implantation of the stimulation electrode in (A) a normal-hearing control animal; (B) a deafened control animal; (C) a 4-week-BDNF-treated deafened animal with a perforated RWM; and (D) a 4-week-BDNF-treated deafened animal with an intact RWM. Weeks after implantation are indicated in the traces. The N1 and P2 peaks were used for analysis. The electric stimulus was a monophasic pulse with a width of 20 µs and a current of 250 µA.

4. Discussion

The present study showed enhanced survival of SGCs in guinea pigs 2 and 4 weeks after placement of Gelfoam, infiltrated with 6 µg of BDNF, on the perforated RWM (Fig. 5). This enhanced survival was restricted to the most basal region of the cochlea. Also, in animals treated with BDNF on a perforated RWM, eABR amplitudes were similar to those of normal-hearing controls and larger than those in deafened controls and BDNF-treated animals with an intact RWM (Fig. 7), as investigated in our previous study (Havenith et al., 2011).

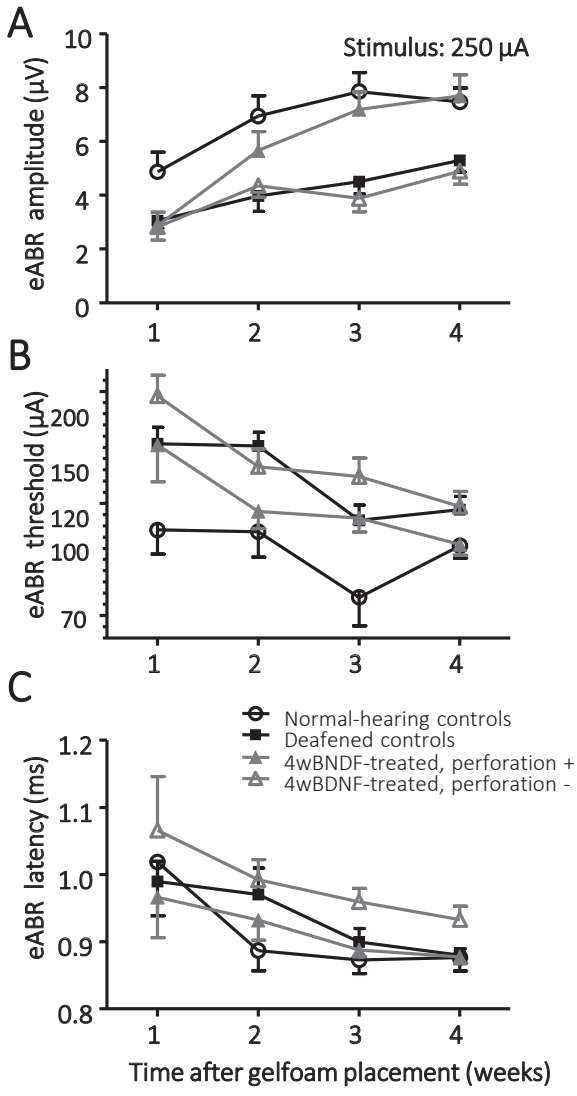

FIG. 7. Mean amplitudes (A), thresholds (B), and latencies (C) of eABRs in normal-hearing controls (n = 11), deafened controls (n = 8), and 4-week-BDNF-treated deafened animals with (n = 6) and without (n = 10) a perforated RWM. The amplitude is based on the N1-P2 complex and the latency on the N1 peak. The stimulus applied in the amplitude and latency data had a width of 20 µs and a current of 250 µA. In (B), the vertical axis is scaled logarithmically. Error bars: SEM.

4.1. Electrically Evoked ABRs

Surprisingly, although SGC counts were similar (Fig. 5B, C), eABR amplitudes were larger in the perforated group than in the nonperforated group (Fig. 7). Therefore, the explanation for the difference in eABR amplitude seems not to depend on the SGC quantity. Because eABR thresholds were comparable in both normal-hearing and deafened animals with and without a perforated round window, possible round window electrode stimulation properties (i.e., electrode impedance) seem not to be an explanation for this difference. It might be that the SGCs at basal location B1 in the perforated group treated with BDNF were more responsive.

Alternatively, the perforation allowed the BDNF to increase the responsiveness of the SGCs in more apical regions (B2, M1; Fig. 5) without an effect on survival in those regions.

4.2. Perforating the RWM

Perforating the RWM enhances the effectiveness and bypasses the unknown permeability of the human RWM for neurotrophins, which is an important limiting factor in RWM delivery. Although the human RWM has a two times larger surface compared with the guinea pig, which, in theory, favors diffusion; on the other hand, its four times thicker RWM will strongly impair its diffusion characteristics (Ghiz et al., 2001; Saber et al., 2010). To avoid this possible limiting factor, a perforation was performed.

We analyzed the macroscopic aspect of the RWM in random samples of animals with and without a perforated RWM. In both groups, scar tissue formation was found probably because of the presence of the stimulation electrode. There were no obvious differences between the two groups. The aABR data (Fig. 3A) also indicate that perforation did not cause additional trauma. Our findings agree with Lamm et al. (1986) who perforated the RWM with a platinum wire. They observed spontaneous healing of the RWM 1 week after perforation and found no difference in aABRs between the animals with repaired and unrepaired membrane damage.

In humans, a perforation of the oval window is performed on a regular basis as part of surgical procedures, such as a stapedotomy (e.g., Bittermann et al., 2013). In current hybrid cochlear implantation surgery, RWM perforation is an established technique and has shown to have a relatively low complication rate in patients with residual hearing (Havenith et al., 2013). Our study supports that these perforation procedures can be performed safely because no changes in aABR thresholds, SGC, or hair cell counts were found in normal- hearing animals with a perforated RWM (Fig. 3).

4.3. Clinical Applicability

Numbers of surviving SGCs are thought to be clinically relevant (Seyyedi et al., 2014), but the functionality of the rescued SGCs is important as well. Therefore, the increased excitability in addition to the enhanced survival in the basal cochlear region is a relevant finding toward clinical application of this BDNF delivery method. To treat patients, such as cochlear implant candidates, with neurotrophin-based therapy, it is necessary to develop a safe, effective, and minimally invasive delivery method. Table 2 shows an overview of the otoprotective effects of BDNF, comparing several delivery methods. Because it is frequent practice in humans to safely perforate the oval window or RWM during several surgical procedures, it may be a future drug application route. McCall et al. (2010) however discuss some potential drawbacks to local drug delivery onto the RWM. First, several processes involved in inner ear pharmacokinetics, including the rate of transfer across the membrane, the inner ear distribution, and clearance of the drug from the inner ear, may influence the effectiveness. In line with this discussion, King et al. (2013) compared hearing loss and vestibulotoxicity in gentamicin

administration on the stapes footplate or the RWM in guinea pigs, which resulted in greater hearing loss and hair cell loss in the lower basal turn of the cochlea when applied on the stapes footplate. They discuss that access of gentamicin to the inner ear may have been via the annular ligament, which may have a higher permeability than the RWM. Also, they suggest diffusion between both the scala vestibuli and scala tympani, which is thought to be through the interstitial spaces of the spiral ligament (King et al., 2013; see their Introduction). Because in approximately 20% to 25% of humans the RWM is difficult to reach surgically because of a partial or complete obstruction (bony overhang, pseudomembrane, or fibrous plug; e.g., Silverstein et al., 1997; Alzamil and Linthicum, 2000) and the possible greater diffusion through the oval window, one could consider applying drugs on the (perforated) oval window. In the case of round window insertion, when the RWM is no longer accessible, one could consider to place the Gelfoam with BDNF on the oval window and/or the Gelfoam can be sealed around the hub of the electrode array in which case the BDNF diffuses through the round window alongside the electrode. Because studies show a pattern of sustained preservation of SGC survival after treatment cessation at least up to 4 weeks, repeated application would not be necessary (Maruyama et al., 2007; Agterberg et al., 2009; Ramekers et al., 2012).

Besides BDNF, this delivery method could potentially be used for several other substances regarding protection of the auditory nerve in cisplatin ototoxicity or noise- induced hearing loss, in stapedotomy surgery, or regarding minimizing trauma caused by cochlear implantation surgery where systemic drug use is proven moderately effective at most.

Table 2.

	Intracochlear cannula (Agterberg et al., 2009)	**Extracochlear Gelfoam Intact RWM**	**Extracochlear Gelfoam Perforated RWM**
SGC packing density	> Location B1 – A1	> Location B1	> Location B1
SGC size	> Untreated	= Untreated	= Untreated
eABR amplitudes	= Normal-hearing controls	< Normal-hearing controls	= Normal-hearing controls
	> Deafened controls	= Deafened controls	> Deafened controls

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Chapter 4

Residual hearing preservation in guinea pigs with and without intracochlear corticosteroid treatment

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Abstract

Background: For cochlear implantation it is regarded important to preserve cochlear structures and residual hearing. We investigated whether intracochlear corticosteroid treatment by means of a coated implant device reduced hearing loss and cochlear fibrosis after cochlear implantation.

Methods: The right ears of 14 normal-hearing guinea pigs were implanted with a custom-made intracochlear electrode lead, of which six were coated with 10% dexamethasone (60µg). Both acoustically and electrically evoked ABRs and acoustically evoked CAPs were recorded weekly. Four weeks after implantation all animals were sacrificed and the cochleas were processed for histological analysis (assessing structural cochlear damage and fibrosis in the cochlea).

Results: Functionally, greatest CAP and ABR threshold shifts were found mainly in the 8 to 16 kHz region. Recovery of hearing, if any, occurred in the first week after implantation. Strikingly, the electrophysiological results showed either distinct recovery or a complete absence of recovery, regardless of treatment. Overall there was only little protective effect of dexamethasone on hearing thresholds. The degree of insertion trauma ranged from mild trauma with an elevation of the basilar membrane to severe damage with rupture of the osseous spiral lamina. A reduction of fibrosis was observed in the most basal region of the cochlea in the dexamethasone-treated animals, however not statistically significant. At last, the degree of structural damage and fibrosis in the cochlea did not correlate to hearing thresholds.

Conclusion: There was no greater risk of infection or worse hearing recovery in the guinea pigs with severe damage to cochlear structures. Dexamethasone treatment was mildly effective at best. Importantly, dexamethasone seemed safe when administered directly into the cochlea.

1. Introduction

For cochlear implantation, it is regarded important to preserve cochlear structures, in particular when the patient has residual hearing. Often, corticosteroids are applied to the cochlea per(i)-operatively to enhance hearing preservation. Several research groups have explored various factors, including corticosteroid treatment, that may influence the degree of preserving residual hearing following cochlear implant surgery (recent meta-analyses by Santa Maria et al. 2014 and Causon et al. 2015). The benefit of dexamethasone on postoperative residual hearing is under debate, in which the preferred route of administration is a point of discussion. Santa Maria et al. (2014) performed a meta-analysis, including a number of 24 studies and data of 500 individual patients. Besides several surgery related factors they found that the use of postoperative systemically administered steroids were associated with better hearing preservation. However, intra-operative topical or systemically administrated steroids showed minimal to no advantages in the degree of hearing preservation. Causon et al. (2015) performed a meta-analysis, including a number of 12 studies, with individual data of 200 patients. They reported that 72% of the patients receiving intraoperative steroids performed better than the median for when no steroids were used. Further, they did not find postoperative steroid treatment to be of influence on the level of residual hearing. Concluding, for per(i)-operative steroid treatment conflicting results were described applying different routes of administration.

Several animal studies showed that direct intracochlear delivery of dexamethasone with a mini-osmotic pump can prevent implantation related shifts in hearing thresholds in normal-hearing guinea pigs (Eshraghi et al., 2007; Vivero et al., 2008). Since this is not considered a clinically applicable and safe method, there is a great interest in intracochlear delivery by means of a coated implant device. Advantages compared to the other clinically applied delivery methods mentioned above are direct delivery at the target site, less systemic side effects and a continuous delivery over time due to slow release.

In several animal studies intracochlear corticosteroid delivery was investigated by means of a coated implant device (i.e. Douchement et al. 2014; Stathopoulos et al. 2014; Liu et al. 2015; Astolfi et al. 2016; Bas et al. 2016; Wilk et al. 2016). Dexamethasone-coated electrode arrays were implanted in normal-hearing guinea pigs or gerbils and both their functional and histological effects regarding insertional trauma and hearing preservation were assessed in these studies. The results regarding functional hearing preservation were conflicting, ranging from significant hearing protection (Douchement et al., 2014; Liu et al., 2015; Astolfi et al., 2016; Bas et al., 2016), no effect on hearing thresholds (Stathopoulos et al., 2014) to inducing more hearing loss (Wilk et al., 2016) in dexamethasone treated animals. Further, only two studies mentioned the actual degree of structural damage in the cochlea caused by implantation. Stathopoulos et al. (2014) described structural damage to the organ of Corti in only 1 out of 36 animals and Liu et al. (2015) only mentioned that the general architecture of the organ of

Corti was destroyed to various extents in most animals. However, in the case of more severe damage to cochlear structures dexamethasone may have a higher risk of infection or other adverse effects due to its immunosuppressive effects, and therefore may be less effective. We were especially interested in the effects of dexamethasone on hearing preservation and to relate this to the degree of trauma to cochlear structures.
We quantified hearing loss with both auditory brainstem responses (ABRs) and acoustically evoked compound action potentials (CAPs). Additionally, we assessed the degree of structural damage to the cochlea. Also, the possible protective effect of dexamethasone on the degree of cochlear fibrosis following cochlear implantation was investigated.

2. Materials and methods

2.1. Animals and experimental design

Fourteen female guinea pigs (strain: Dunkin Hartley) were purchased from Harlan Laboratories (Horst, the Netherlands) and housed in the animal care facility of Utrecht University (Utrecht, the Netherlands). All animals had free access to both food and water and were kept under standard laboratory conditions. Lights were on between 7:00 am and 7:00 pm. Temperature and humidity were kept constant at 21°C and 60%, respectively.
Normal-hearing animals were implanted with an intracochlear electrode loaded with or without dexamethasone (see Fig. 1). Weekly electrophysiological recordings were performed, after which the animals were sacrificed and both right and left cochleas were processed for histological analysis. All experimental procedures were approved by the University's Committee on Animal Research (DEC-UMC # 2011.I.11.109).

2.2. Electrodes and cochlear implantation surgery

The custom-made cochlear implant devices were manufactured by MEDEL, Innsbruck, Austria. The silicone-based cochlear implant lead had a total length of 6 cm, including a single electrode contact at the distal end (positioned 1 mm from the tip). The lead tip had a diameter of 0.3 mm, progressively increasing towards the base of the electrode lead. The distal 4 mm of the device contained a silicone coating with 10%-dexamethasone (60 µg). Control devices contained silicone only.
Animals were anesthetized with dexmedetomidine (Dexdomitor®; 0.25 mg/kg im) and ketamine (Narketan®; 40 mg/kg im). Prior to the surgical procedure, click- and tone-evoked ABRs were recorded. Surgery was performed on the animals' right ears.
First a retroauricular incision was made and a hole was created in the bulla. The basal turn of the cochlea and the round window membrane were visualized. The implant was inserted through a cochleostomy just anteroinferiorly of the round window niche, created with a 0.7-mm diameter hand drill. Before actual electrode implantation a rigid silver wire was inserted

and subsequently removed in order to cause substantial trauma to cochlear structures. This method was performed in 13 out of 14 animals. This extra manipulation was added since in a pilot experiment the electrode implantation itself did not cause the severe trauma that we aimed for. Then the cochlear implant was inserted up to the 5 mm point where it sealed off the cochleostomy without leakage of perilymph, therefore no added cochlear seal was used. In addition, a gold-ball electrode was placed onto the round window membrane in the round window niche (for details, see Klis et al., 2000). The stainless steel electrode lead was fixed onto the bulla with dental cement (ProBase Cold; Ivoclar Vivadent AG, Schaan, Liechtenstein). Both the intracochlear and gold-ball electrodes were inserted into a connector and fixed with dental cement onto the skull. The round window electrode was used to record acoustically evoked CAPs. The electrically evoked ABRs (eABRs) were recorded using the round window electrode as the stimulation electrode.

2.3. Electrophysiological recordings

Electrophysiological recording were performed in awake guinea pigs in a sound-attenuated chamber (Agterberg et al., 2009). Stimulus generation and signal acquisition were controlled with custom-written software and a personal computer. The stimuli were synthesized and attenuated using a TDT3 system (modules RP2, PA5 (2x), and SA1; Tucker-Davis Technologies Inc., Alachua, FL, USA). The responses were amplified differentially using a PAR113 pre-amplifier (amplification x5,000; band pass filter 0.1-10 kHz; Princeton Applied Research, Oak Ridge, TN, USA). The amplified signal was digitized by the TDT3 system (module RP2) for off-line analysi*s.*

2.3.1. Acoustically evoked auditory brainstem responses

The first acoustically evoked ABR measurements (aABRs) were performed prior to implantation surgery, using three subcutaneous needle electrodes. After implantation, weekly recordings were performed in awake guinea pigs in a sound-attenuated chamber (Agterberg et al., 2009). Three stainless steel screws (8.0x1.2 mm) were inserted into the skull bone to record ABRs. The screws were inserted 1 cm posterior to bregma, 2 cm anterior to bregma, and 1 cm lateral from bregma (Mitchell et al., 1997), with the posterior and anterior screws as active and reference electrodes, respectively, and the lateral screw as ground electrode.

aABRs were measured using both clicks and tone pips as stimuli (Fig. 1). Click stimuli consisted of monophasic rectangular pulses (width 20 µs) with alternating polarity. Tone pips were presented with alternating polarity. Frequencies were 0.5, 1, 2, 4, 8, 11.3 and 16 kHz, durations were 8 ms (1-16 kHz) or 12 ms (0.5 kHz) and rise/fall times were 1 ms (4-16 kHz), 1.5 ms (2 kHz), 2 ms (1 kHz) or 4 ms (0.5 kHz). Click and tone stimuli were presented in a free-field configuration with a Blaupunkt speaker (PCxb352; 4 Ohm; 30 W) positioned at 10 cm from the pinna. Using a pair of attenuators (TDT3 system; module PA5), we varied sound levels from about 100 dB SPL (frequency dependent) down to below threshold in 10-dB steps.

The click stimuli were presented from 75 dB above the average threshold of normal-hearing animals (~110 dB peSPL) down to threshold in 10-dB steps. The interstimulus interval was 99 ms. The ABR was obtained by adding the responses evoked by stimuli of opposite polarity. The ABR amplitude was defined as the difference between the largest negative peak and the most pronounced subsequent positive peak. Thresholds were defined as the stimulus level that evoked a 0.30 or 0.35 µV reproducible waveform for respectively click- and tone pip evoked ABRs (determined by interpolation).

2.3.2 Electrically evoked auditory brainstem responses

After implantation of the electrode lead and stimulation electrode on the round window membrane in the right ear, eABRs were recorded weekly during the whole experiment (Fig. 1). Electrical stimuli were monophasic rectangular pulses of 20 µs with amplitudes descending from 400 µA to below threshold with 2-dB steps. The pulses were converted to current pulses by a linear stimulus isolator (type A395; World Precision Instruments, Sarasota, FL, USA). Stimuli were presented with alternating polarity in order to reduce the stimulus artifact. The lateral screw in the skull was used as return electrode. The main parameter was the peak-to-peak amplitude of the eABR wave N_1-P_2, which occurs around 1 ms after stimulus onset (for details, see Agterberg et al., 2009). The N_1-P_2 threshold and N_1 latency were also determined. Thresholds were defined as the stimulus level that evoked a 2.0-µV N_1-P_2 waveform and determined by interpolation.

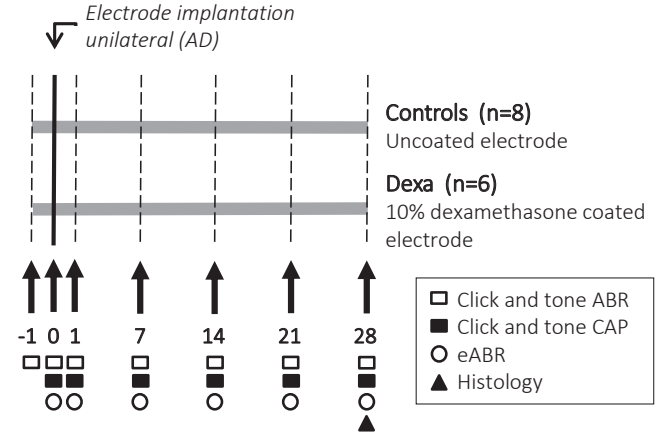

Figure 1. Treatment schedule (AD: right ear, ABR: auditory brainstem response, CAP: compound action potential, eABR: electrically evoked ABR)

2.3.3 Compound action potentials

After implantation of the cochlear implant and round-window electrodes in the right ear, acoustic stimuli were presented as described for the aABRs above. Thresholds for the click

evoked responses were defined as the stimulus level that evoked a 3 µV reproducible waveform (determined by interpolation). Thresholds at the higher frequencies (4-16 kHz) were defined as the stimulus level that evoked a 3 µV reproducible waveform (determined by interpolation). At the lower frequencies where CAPs were still clearly visible below 3 µV we used lower criteria (0.5 and 1 kHz: 1.5 µV, 2 kHz: 2 µV).

2.4. Histology

Immediately after the final measurements, the animals were euthanized and the cochleas were fixed by whole-body perfusion with a fixative consisting of 3% glutaraldehyde, 2% formaldehyde, 1% acrolein, and 2.5% DMSO in 0.08 M sodium cacodylate buffer (pH 7.4) followed by immersion in the same fixative for 3 h at room temperature. Further histological processing of the cochleas was performed according to a standard protocol (for details, see De Groot et al., 1987). Semithin midmodiolar sections (1 µm) were stained with methylene blue and azur II in sodium tetraborate and used for light microscopical evaluation and quantitative analyses.

2.4.1. Spiral ganglion cell and hair cell counts

To determine the degree of damage the number of inner hair cells (IHCs) and outer hair cells (OHCs) were counted at seven cochlear locations at a half-turn spacing (Figure 2). To determine SGC packing densities, digitized light microscopic images of Rosenthal's canal at five cochlear locations (B1, B2, M1, M2 and A1; Fig. 2A) were analyzed using ImageJ (version 1.39t; http://rsbweb.nih.gov/ij) image-processing software. SGC packing densities could not always be determined for the apical locations, due to tangential sectioning. The bony boundaries of Rosenthal's canal were outlined using a pressure-sensitive stylus on a Wacom CTE-650 digitizer interfaced to a Macintosh computer, and its cross-sectional area was calculated. The number of all (i.e., both type-I and type-II) SGC perikarya, with and without a nucleus, was determined. SGC packing densities were calculated by dividing the number of SGCs by the cross-sectional area of Rosenthal's canal at each cochlear location, and expressed as the number of SGCs per mm^2.

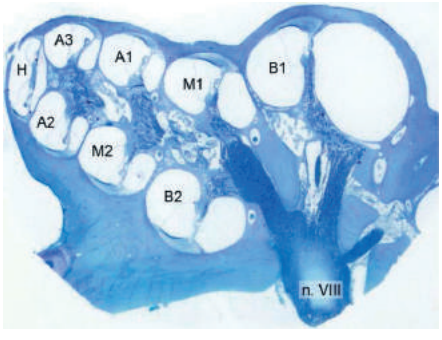

Figure 2. Low-magnification micrograph of a normal guinea pig cochlea (midmodiolar section with B1-2: basal, M1-2: middle, A1-3: apical turns, H:helicotrema, N. VIII: cochlear nerve).

2.4.2. Degree of mechanical trauma

The degree of insertion trauma caused by implantation of the electrode array was examined analyzing microscopic midmodiolar section images of the cochleas, which included the cochleostomy site. We graded the trauma as described by Eshraghi et al. 2006 (their table 1). Trauma ranges from: no trauma (grade 0), elevation of the basilar membrane (grade 1), rupture of the basilar membrane (grade 2), dislocation of the electrode lead in the scala vestibuli (grade 3) to severe trauma with a total rupture the osseous spiral lamina or a tear in tissues of the stria vascularis/spiral ligament complex (grade 4).

Table 1.

Group	Macroscopic trauma	Fibrosis	Hair cell loss	SGC loss
	grade 0 to 4 *	% ST at B1		location B1
control	4 (# OSL B1)	1.4	B1-M1 IHC + OHC, M2 only OHC	+
control	4 (#OSL B1 + # BM M1)	5.0	B1-M1 IHC + OHC	+
control	4 (#OSL + BM elevation B1)	34.3	B1 only OHC	+
control	1	1.2	B1 IHC+OHC; B2-A3 OHC	-
control	0	44.1	NO	-
control	0	18.1	B1 only OHC	-
control	0	10.7	B1 only OHC	-
control	0	90.1	NO	-
dex	4 (#OSL + BM elevation B1)	17.5	B1 IHC+OHC; B2 partial OHC	+
dex	4 (#OSL + TM rupture)	0.4	B1 IHC and OHC	+
dex	0	0.1	NO	-
dex	1	6.4	NO	-
dex	1	3.9	B1 only OHC	-
dex	1	0.1	B1 only OHC	-

* grade 0= no macroscopic trauma; 1= elevation of BM; 2= rupture of BM; 3= dislocation of electrode in scala vestibuli; 4= fracture of OSL of modiolus, or tear in tissues of stria vascularis/spiral ligament complex
B1-A3 are different half-turn locations of the cochleal basal (B1, B2), middle (M1, M2) and apical (A1-A3)
#= fracture; OSL = osseous spiral lamina; BM= basilar membrane; TM = tectorial membrane
IHC= inner hair cell; OHC= outer hair cell

2.4.3. Fibrosis

The degree of fibrosis was examined in midmodiolar section images of the cochleas, in which all images contained the cochleostomy site. We quantified the degree of fibrosis as the percentage of fibrosis in the scala tympani in the most basal turn (B1). In most cases a fibrotic neosheath enveloped the electrode area. This electrode area was subtracted from the total scala tympani area.

2.5. Statistical analyses

Since the data were not normally distributed, the statistical analyses were performed by means of non-parametric tests, using SPSS for Windows (version 22.0.0.0). Group differences were calculated with the nonparametric Mann–Whitney test for independent samples. Linear regression analyses were performed to evaluate hearing recovery and the relation with histological findings.

3. Results

3.1. Hearing results

3.1.1. Click ABR threshold shifts and hearing recovery over time

Figure 3 shows click-evoked ABR thresholds for both treatment groups over time. Baseline thresholds were comparable for dexamethasone and control animals. Because the hearing results showed either recovery (>10dB) or no recovery in half of the animals in both experimental groups, we showed them separately. Largest threshold shifts occurred immediately after implantation (day 0). On average, combining both animals with recovery and no recovery in both treatment groups, there was a greater threshold shift directly after implantation in the dexamethasone-treated group (35 dB) than in the control group (25 dB) (Mann Whitney Test, postoperative thresholds, p= 0.02). One day after implantation thresholds of the two groups were comparable. Also, thresholds remained relatively stable after 1 week. In the case of recovery of hearing, this occurred in the first week. Thresholds in the animals with no recovery were about ~3540 dB nHL in both treatment groups after 4 weeks. In the animals which showed recovery, these were ~2025 dB nHL in the controls and ~10 dB nHL in the dexamethasone group (see Fig. 3). This suggests that this 'on-off' recovery effect is greater in the animals treated with dexamethasone. However, hearing recovery only showed a tendency to be larger in the dexamethasone group (Mann Whitney Tests, p= 0.08). Further, final hearing thresholds between the dexamethasone and the control animals were not different (Mann-Whitney Test, thresholds at day 28, p= 0.35).

3.1.2. Tone ABR and CAP threshold shifts

Figure 4 shows mean tone ABR thresholds before implantation, and both tone ABR and CAP thresholds 4 weeks after implantation. Tone ABR thresholds were similar for both groups before implantation (as observed with click ABRs). As expected considering the basal location of the electrode insertion, greatest threshold shifts after implantation occurred at the high-frequency region (8-16 kHz) in both groups. Both ABRs and CAPs show lower thresholds for the dexamethasone group (20-30 dB) at the high frequencies (8-16 kHz), but the differences were not significant (Mann-Whitney Test, ABR at 16 kHz, p=0.08; CAP at 8, 11, 16 kHz, p>0.2). Dexamethasone showed a significant protective effect (lower tone ABR thresholds)

on residual hearing at 1 kHz (Mann-Whitney Test, p=0.02) at 4 weeks after implantation, however, tone CAPs did not confirm this protective effect at 1 kHz.

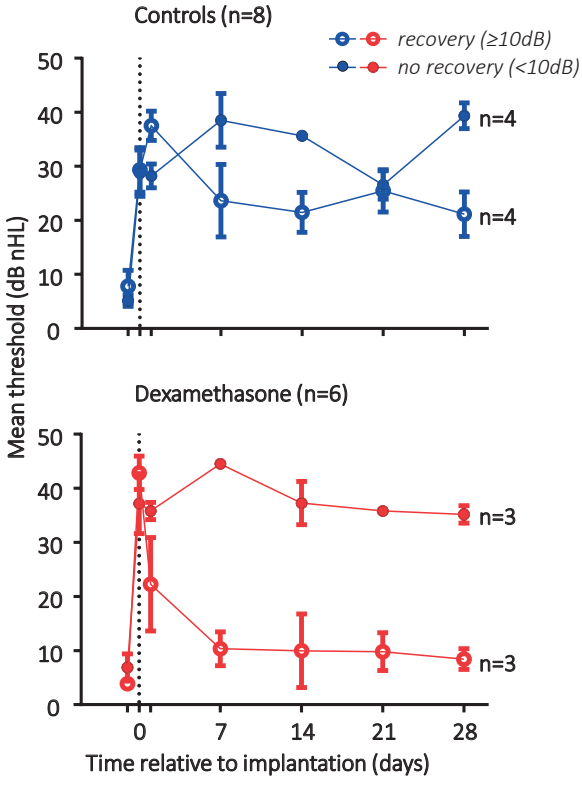

Figure 3. Mean click ABR thresholds in animals with recovery (open symbols) or no recovery (closed symbols) for both experimental groups (dexamethasone-treated; in red, controls; in blue). Error bars: SEM.

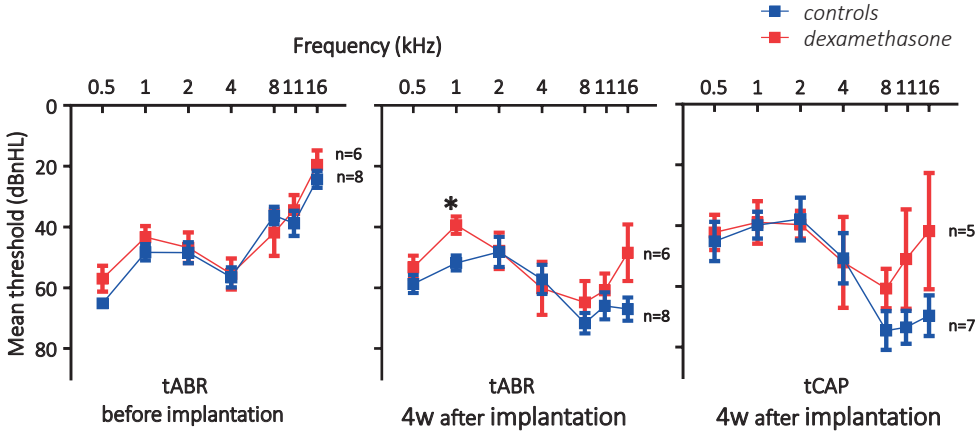

Figure 4. Mean tone-evoked ABR thresholds for dexamethasone-treated (in red) and control animals (in blue) before implantation and both tone evoked ABR and CAP thresholds 4 weeks after implantation. Error bars: SEM.

3.1.3. eABRs

Figure 5 shows mean thresholds (A), latencies (B) and amplitudes (C) of eABRs for dexamethasone-treated and control animals. There are no differences in thresholds and latencies between both groups over time. Amplitudes dropped in the first week after implantation and then increased up to 4 weeks after implantation. Amplitudes in the dexamethasone-treated animals seem larger compared to those in the control group from 2 to 4 weeks after implantation. At 2 weeks there is a tendency for this protective effect (MannWhitney Test, p=0.07), this was not significant at 3 and 4 weeks after deafening (MannWhitney Test, p>0.1).

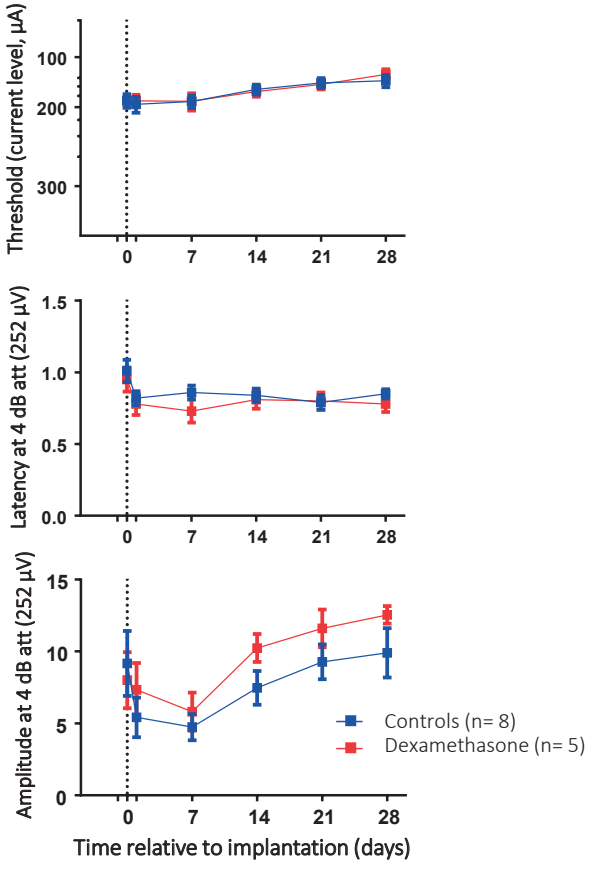

Figure 5. Mean eABR thresholds (A), latencies (B) and amplitudes (C) for dexamethasone (in red) and control (in blue) animals. The amplitude is based on the N_1-P_2 complex and the latency on the N_1 peak. The stimulus applied in the amplitude and latency data had a width of 20 µs and a current of 250 µA. In A, the vertical axis is scaled logarithmically. Error bars: SEM.

3.2 Histology

3.2.1. Degree of insertion trauma

Figure 6 shows representative examples of midmodiolar cochlear sections for both cases of mild and severe trauma caused by cochlear implantation. Figure 6A shows a case of mild trauma to the most basal region of the cochlea, with only a slight elevation of the basilar

membrane (grade 1 trauma). The majority of animals, a total of 9 (64%) showed no trauma or only this slight elevation of the basilar membrane. Figure 6B shows an example of severe trauma with total rupture of the osseous spiral lamina. The remaining animals (n=5; 34%) all had a ruptured osseous spiral lamina in the basal region of the cochlea (see also Table 1). Two out of 6 animals in the dexamethasone group and 3 out of 8 animals in the control group showed this severe trauma.

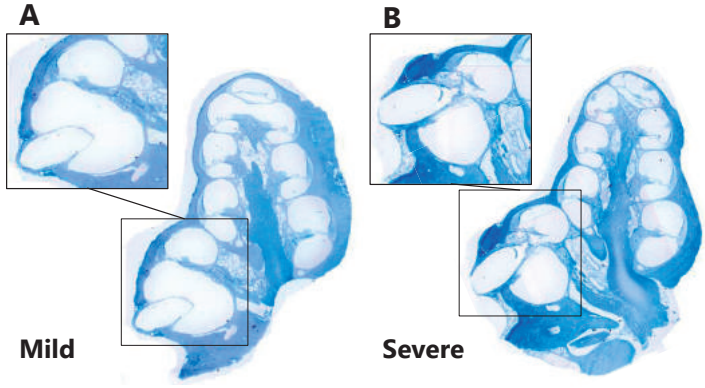

Figure 6. Midmodiolar sections of the cochlea for both representative cases of (A) mild and (B) severe structural insertion trauma, with the most basal turn magnified. Slight elevation of the basilar membrane at the basal turn in the case of mild trauma (A) and fracture of the osseous spiral lamina at the basal turn in the case of severe trauma (B). Both images show a fibrous neosheath surrounding the electrode area.

3.2.2. Fibrosis

The dexamethasone-treated animals showed less fibrosis, as quantified in the most basal region of the cochlea, than the control animals (Mann-Whitney Test, p=0.053, Fig. 7). Five out of eight animals in the control group versus only one out of six animals in the dexamethasone group showed fibrosis of >10% of the surface area of the scala tympani in the basal region of the cochlea (see Fig. 7).

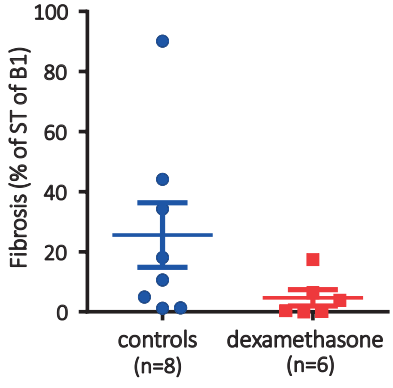

Figure 7. Degree of fibrosis as a percentage of the area of the scala tympani of the basal turn (B1) minus the electrode area itself for dexamethasone-treated (in red) and control animals (in blue). Error bars: SEM.

3.2.3. Cellular infiltration and/or infection

Dexamethasone did not cause additional damage and/or an infection in the cochlea. Analysis of midmodiolar sections of the cochlea (as shown in Fig. 2) showed some red and white blood cell infiltration in mainly the scala tympani in half of the animals in both treatment groups (data not shown). There were also no signs of infection during harvesting of the cochleas macroscopically in both dexamethasone or control animals.

3.2.4. Hair cell loss and auditory nerve degeneration

In animals with severe trauma to cochlear structures IHCs and OHCs were lost in the basal region of the cochlea, regardless of the treatment group (as described in Table 1). In most animals with mild trauma only OHC loss occurred in the basal region of the cochlea (see Table 1).

There were no differences in SGC loss between dexamethasone-treated and control animals (Mann-Whitney Test, p>0.2, see Fig. 8A). However, animals with severe cochlear damage showed significant SGC loss in the most basal region of the cochlea, corresponding to the hair cell loss described above (Mann-Whitney Test, p=0.001, see Fig. 8B).

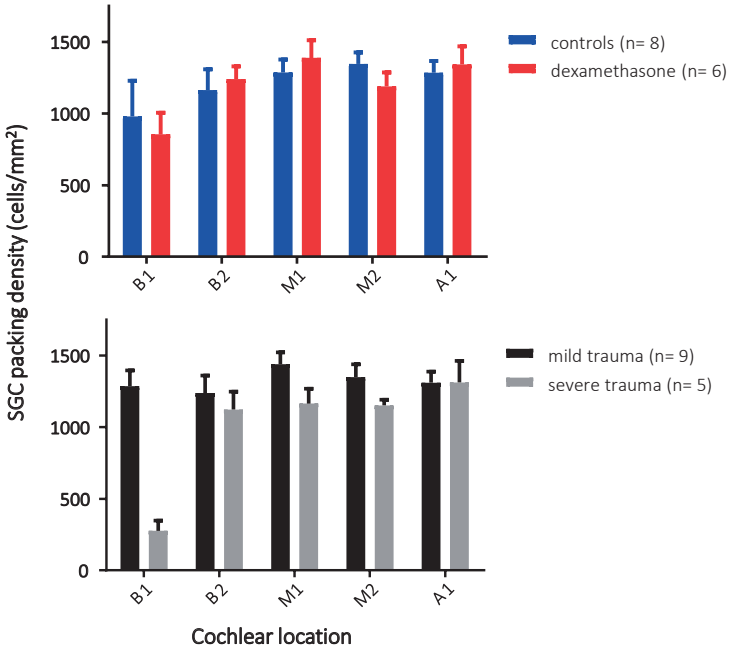

Figure 8. Mean SGC packing densities at cochlear locations B1 to A1 for both experimental groups (A; dexamethasone in red, controls in blue) and degree of insertion trauma (B, mild versus severe). Error bars: SEM.

3.3. Hearing recovery and the relation with histological findings

Figure 9 shows that the degree of functional insertion damage (i.e., the aABR threshold shift directly after implantation) correlates to the subsequent hearing recovery in the following 4 weeks (linear regression analysis, R^2=0.50; p=0.004).

Further, functional insertion damage was larger in the dexamethasone group and hearing recovery showed a tendency to be larger in the dexamethasone group (see also 3.1.1). Unexpectedly, functional insertion damage seemed to be negatively correlated to the degree of structural insertion trauma, since animals with severe trauma had lower threshold shifts, this was however not significant (closed symbols located in the left top quadrant, Mann Whitney Tests, p>0.1).

Finally, there was no correlation between the degree of fibrosis and the recovery of ABRs or CAPs (data not shown).

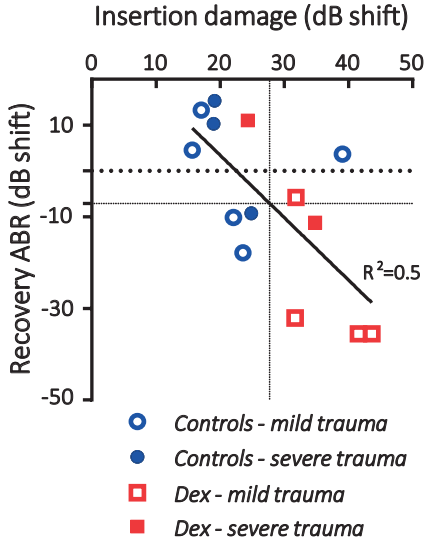

Figure 9. Recovery of click ABR thresholds for individual animals for each treatment group (dexamethasone-treated in red and controls in blue) and degree of trauma (mild trauma; open symbols, severe trauma; closed symbols).

Insertion damage: cABR threshold shift day 0 vs before implantation
Recovery: threshold shift day 28 vs 0

4. Discussion

Absolute threshold shifts after implantation were 35 and 25 dB for respectively dexamethasone-treated and control animals. Hearing recovery (>10 dB improvement of cABR thresholds) occurred in half of the animals in both groups, in the first week after implantation. Overall, dexamethasone showed no significant protective effect on hearing preservation (only a trend

after 4 weeks at 16 kHz). Further, we found a trend for less fibrosis in the dexamethasone treated animals. Finally, we found no significant correlation between the degree of structural insertion trauma to the organ of Corti and/or hearing recovery.

4.1 Residual hearing preservation

Stathopoulos et al. (2014) implanted a total of 36 normal-hearing guinea pigs with dexamethasone-eluting electrodes, coated with 74 to 100 µg of dexamethasone, with a follow-up of 3 months after implantation. They found no differences in hearing threshold shifts (aABRs at 2-32 kHz) between dexamethasone treated and control animals (initial hearing loss ~30-40 dB; recovery up to ~10-20 dB). They suggested the little degree of fibrosis (2-6 % in the basal turn) and the minimal structural damage caused by implantation in their animal model as a possible explanation. Accordingly, we described comparable aABR threshold shifts after implantation. However, both the degree of fibrosis and structural damage to the cochlea did not correlate to hearing thresholds in our study and therefore did not explain the lack of hearing protection of dexamethasone in our study.

Several other studies did find significant hearing preservation using dexamethasone-eluting implants. Astolfi et al. (2016) described CAP threshold shifts of ~10-15 dB directly after implantation for both dexamethasone-treated (86,7 µg) and control animals, with significant recovery of CAP thresholds (both click- and tone evoked thresholds) near to normal in the dexamethasone treated guinea pigs after 7 and 14 days (n=10 in both groups). Bas et al. (2016) described recovery of hearing close to contralateral normal ears for ABR and CAP measurements in normal-hearing guinea pigs implanted with both 1% or 10% (resp. 6 or 60 µg) dexamethasone-coated implants up to 3 months after implantation. Initial threshold shifts measured the first day after implantation were ~40-50 dB at 0.5, 1, 4 and 16 kHz for ABR and CAP recordings. Liu et al. (2015) reported greater ABR recovery in the 8 to 24 kHz region for dexamethasone-treated (20 µg) guinea pigs (n=18) compared to controls (n=17), from 4 weeks up to 6 months after implantation. Initial threshold shifts were ~40-50 dB at day 1, recovering to ~20-30 dB at 8 to 24 kHz for dexamethasone treated animals. Douchement et al. (2014) found preservation of residual hearing at 4 to 6 weeks after implantation for all tested frequencies (500 Hz-16 kHz), for both 1% and 10% (resp. 6 and 60 µg) in dexamethasone-treated gerbils. This effect was seen after 1 year only at 16 kHz.

We applied a dosage (60 µg) that has shown to be effective in three previously mentioned studies (they applied 6, 20, 60 and 86,7 µg). The protective effect of dexamethasone started within 4 weeks after implantation in 3 studies (Astolfi et al., 2016; Bast et al., 2016 and Douchement et al., 2014). However, Liu et al. (2015) reported significant recovery starting at 4 weeks after implantation. Pharmacokinetics studies on the eluting properties of the electrode leads showed greatest dexamethasone release in the first week after implantation (e.g. Liu et al., 2015; Astolfi et al., 2016). Since hearing thresholds in our study were stable after the first week, we do not expect them to improve further after 4 weeks. The above

mentioned studies were performed with larger number of animals. This might have increased the power of their analysis and may partially explain that we only found a trend of high frequency hearing protection.
In contrast, Wilk et al. (2016) found the opposite effect, describing greater hearing loss in dexamethasone-treated (60µ) guinea pigs 91 days after implantation, significant at 1 and 16 kHz. Absolute thresholds shifts at 3 months were 50-60 dB at the higher frequencies (>8 kHz) for dexamethasone-treated and 20-40 dB for control animals. Since hearing thresholds were already increased directly after surgery they hypothesized that the actual physical loading of the dexamethasone on the electrode may have been the reason behind this elevated hearing loss. However, the loading of the dexamethasone had no influence on the stiffness of the implant device in their study and therefore did not seem to explain the worse hearing thresholds in dexamethasone treated guinea pigs in their study. Accordingly, we found larger threshold shifts directly after surgery in dexamethasone-treated animals (see Figs. 3 and 9, greater threshold shifts at day 1 in dexamethasone-treated animals). Perhaps great release of dexamethasone during the first hours after implantation may cause changes in the cochlear homeostasis with as result worse hearing thresholds. In contrast to the results described by Wilk et al. (2016), due to greater subsequent recovery over time in most animals in the dexamethasone group, final thresholds were comparable to the control group after 4 weeks (see Figs. 3 and 9). The tendency of greater recovery in de dexamethasone group (see Figs. 3 and 9) could well be due to the actual treatment effect of dexamethasone in our study.
Finally, another possible explanation for the conflicting results on hearing preservation could be the influence of the degree of structural damage of the organ of Corti. The above mentioned studies did not report on the actual degree of structural damage in relation to the level of hearing preservation or treatment group. However, since in our experiment 3 out of 8 animals in the control group and 2 out of 6 animals in the dexamethasone group had severe damage to the cochlea, this does not seem to explain the greater threshold shifts directly after implantation in the dexamethasone-treated animals (see Table 1 and Fig. 3). Also, as mentioned above, the degree of structural insertion damage did not correlate to hearing recovery (Figure 9).

4.2 Fibrosis

Fibrous tissue growth after cochlear implantation is thought to be a factor that may negatively affect performance in cochlear implant recipients. In our study, dexamethasone seemed to reduce the degree of fibrosis in the most basal region of the cochlea compared with control animals, however non significant (p=0.053). This agrees with other studies, describing at least a trend for less fibrosis in dexamethasone-treated guinea pigs (Astolfi et al., 2016; Stathopoulos et al., 2014; Wilk et al., 2016).
Recent temporal bone studies on cochlear implant recipients showed that word recognition scores were not significantly correlated with fibrous tissue formation alone (Kamakura and

Nadol, 2016; Ishay et al., 2017). There was no correlation of fibrous tissue formation with intracochlear insertion trauma either (Kamakura and Nadol, 2016), which agrees with our current findings in guinea pigs. Kamakura and Nadol (2016) however did find a negative correlation between word recognition scores and new bone formation, mainly in the scala media and scala vestibuli. Also, new bone formation was positively correlated to the degree of damage of cochlear structures in their study. We did not find any new bone formation in our animals. This could be due to our relatively short follow-up period of 4 weeks. However, also in the animal studies with a follow-up up to 3 months new bone formation was not described (Stathopoulos et al., 2014; Wilk et al., 2016). So, the importance of fibrous tissue formation on hearing preservation outcome and cochlear implant performance is unclear.

In conclusion, the effect of intracochlear dexamethasone treatment using the cochlear implant device as a carrier is diverse in different studies regarding hearing results. We found no significant protective effect of intracochlear corticosteroid treatment on hearing preservation (only a trend after 4 weeks at 16 kHz). Further, we found a strong indication for less fibrosis in the most basal region of the cochlea in the dexamethasone treated animals ($p=0.053$). Hearing results did not correlate to the degree of structural damage in the cochlea. There was no greater risk of infection or worse hearing recovery in the guinea pigs with severe damage to cochlear structures. Therefore, based on our study we conclude that dexamethasone treatment was mildly effective at best. Importantly, dexamethasone seemed safe when administered directly into the cochlea.

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Chapter 5

A Guinea Pig Model of Selective Severe High-Frequency Hearing Loss

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Abstract

Hypothesis: Using an appropriate dose of an aminoglycoside antibiotic cotreated with a loop diuretic a guinea pig model of high-frequency loss can be obtained mimicking cochlear implant candidates with low-frequency residual hearing. We examined the stability of this model over time.

Background: A well-established method to create an animal model for profound deafness is cotreatment with an aminoglycoside antibiotic and a loop diuretic. Recent data indicated that reduction of the aminoglycoside dose might yield selective high-frequency hearing loss. Such a model is relevant for studies related to hybrid cochlear implant devices, for example, with respect to preservation of residual hearing.

Methods: Guinea pigs received an electrode for chronic recording of compound action potentials to tones to assess thresholds. They were treated with a coadministration of kanamycin (200 mg/kg) and furosemide (100 mg/kg), after which, the animals were sacrificed for histologic analysis at 2, 4, or 7 weeks.

Results: After 2 to 7 weeks threshold shifts were greater than 50 dB for 8 to 16 kHz in 15 of 17 animals, whereas threshold shifts at 2 kHz or lower were less than 50 dB in 13 animals. Major threshold shifts occurred the first 2 to 4 days; subsequently, some spontaneous recovery occurred and, after 2-3 weeks thresholds, remained stable. Inner hair cell loss still progressed between 2 and 4 weeks in the most basal cochlear region; thereafter, hair cell loss was stable.

Conclusion: An appropriate animal model for selective severe high-frequency hearing loss was obtained, which is stable at 4 weeks after ototoxic treatment.

1. Introduction

A cochlear implant (CI) is an effective auditory rehabilitation device in patients with severe hearing loss. The criteria for cochlear implantation continue to broaden: patients with a severe high-frequency loss and residual low-frequency hearing are considered candidates for implantation nowadays (Cohen, 2004). Often, these candidates are equipped with a hybrid device, providing electrical stimulation for the high-frequency region of the cochlea and acoustical stimulation for the low-frequency region (electroacoustic stimulation [EAS]). Animal studies in this research field, aimed at protection of the cochlea against electrode insertion trauma and the subsequent loss of residual hearing (e.g., Eshraghi et al., 2007; Choudhury et al., 2011), often use either normal- hearing or severely deafened animals. However, an animal model of selective severe high-frequency hearing loss would more accurately represent the situation in candidates for a hybrid device (Choudhury et al., 2011; Suberman et al., 2011).

Among various strategies used to obtain severe hearing loss, a combined administration of an aminoglycoside and a loop diuretic is often used (e.g., West et al., 1973; Brummett et al., 1975; Brummett et al., 1979; Xu et al., 1993; Nourski et al., 2004; Coco et al., 2007; Versnel et al., 2007; Stronks et al., 2011). The extent of the cochlear damage caused by this ototoxic treatment depends on dosing parameters. Dose-response curves of loop diuretics are steep, meaning that the cochlear condition goes from virtually unaffected to severely damaged by only doubling the dose (Brummett et al., 1975, 1979). In contrast, the dose-response curve of kanamycin is shallow, allowing to obtain small alterations of cochlear damage by dose changes (Brummett et al., 1979). Therefore, attempting to obtain selective high-frequency loss in guinea pigs, Stronks et al. (2011) lowered the kanamycin dose from 400 mg/kg, which is typically used to severely deafen guinea pigs, to 200 to 300 mg/kg. Indeed, this approach resulted in preservation of low-frequency hearing, whereas high-frequency hearing remained severely affected.

In the present study, we characterize this potential guinea pig model of selective severe high-frequency hearing loss by applying 200 mg/kg kanamycin and 100 mg/kg furosemide. Tone audiograms were assessed longitudinally, up to 7 weeks after the ototoxic treatment, with compound action potentials (CAPs) recorded from a chronically implanted round window electrode. Percentages of surviving hair cells were assessed at 2, 4, and 7 weeks after treatment. We were specifically interested in the stability of tone thresholds and hair cell losses over time.

2. Materials and Methods

2.1. Experimental Design

Albino female guinea pigs (strain: Dunkin Hartley; weighing 250-350 g) were obtained from Harlan Laboratories (Horst, The Netherlands) and housed in the Central Laboratory Animal Research Facility in Utrecht. Animals had free access to both food and water and were kept under standard laboratory conditions. All experimental procedures were approved by the University's Committee on Animal Research (DEC-UMC 2010.I.03.041).

We used 3 experimental groups and 1 control group. The experiments were performed in series of 3 to 6 animals, with a pseudorandom assignment of an animal to an experimental group. The 3 experimental groups were sacrificed for histologic analysis at different time points after ototoxic treatment: 2 (n = 5), 4 (n = 5), and 7 weeks (n = 7). The control group (n = 5) was sacrificed 12 weeks after a sham treatment. All 22 animals, which were included, had no signs of middle ear infection.

2.2. Surgery

For electrophysiologic recordings, all animals were implanted with a permanent round-window electrode in the right ear (Klis et al., 2002). In all 22 animals, electrophysiologic recordings were performed weekly after electrode placement, to measure baseline hearing thresholds and to assess stability of the measurements before ototoxic treatment. The animals were anesthetized with 40 mg/kg ketamine hydrochloride (Narketan, im) and 0.25 mg/kg dexmedetomidine hydrochloride (Dexdomitor, im). Postoperatively, the animals were injected with the nonototoxic antibiotic enrofloxacin (Baytril, 5 mg/kg, sc) and the nonsteroidal anti-inflammatory drug carprofen (Rimadyl, 5 mg/kg, sc).

At least 2 weeks after electrode implantation, allowing for recovery from surgical trauma, all animals in the experimental groups (weighing 450-650 g) were treated with kanamycin and furosemide to induce hearing loss. Kanamycin sulfate in isotonic saline (200 mg/kg) was administered subcutaneously; then, the right external jugular vein was exposed and cannulated, and 15 to 70 minutes after kanamycin injection, furosemide (100 mg/kg, Centrafarm) was slowly infused. The choice of kanamycin dose was based on previous results from our lab (Stronks et al., 2011), which showed that doses of 250 or 300 mg/kg resulted in large low-frequency hearing losses (i.e., 950 dB) in more animals than with 200 mg/kg. Control animals underwent a sham procedure, with isotonic saline replacing the ototoxic drugs. Electrophysiologic recordings were performed at the following times after ototoxic treatment: 1, 2, 4, and 7 days. Subsequently, recordings were continued once-weekly (up to 2, 4, 7, or 12 weeks), after which the animals were sacrificed for histology.

2.3. Electrophysiologic Measurements

Stimulus generation and data acquisition were controlled by custom-written software involving a personal computer and a Tucker-Davis Technology TDT3 system (modules RP2, PA5 (2x), and SA1). Acoustic stimuli were tone bursts consisting of trains of alternating polarity of 0.5, 1, 2, 4, 8, 11.3, and 16 kHz. Durations were 8 ms (1-16 kHz) or 12 ms (0.5 kHz) and rise/fall times were 1 ms (4-16 kHz), 1.5 ms (2 kHz), 2 ms (1 kHz), or 4 ms (0.5 kHz). They were presented in an open-field configuration with a Blaupunkt speaker (PCxb352; 4 Ohm; 30 W) positioned at 10 cm from the pinna. Using a pair of attenuators (TDT3 system; module PA5), we varied sound levels from about 100 dB SPL (frequency dependent) down to below threshold in 10-dB steps.

The CAP was obtained by adding the responses evoked by stimuli of opposite phase. The CAP amplitude was defined as the difference between the largest negative peak and the most pronounced subsequent positive peak. Figure 1 shows typical waveforms of the CAP at 1 and 8 kHz. At low frequencies, the neural response follows the phase of the tone, and due to the averaging procedure applied at the opposite phases to eliminate cochlear microphonics, the frequency of the multiple-peaked CAP waveform was twice that of the stimulus frequency. Thresholds at the higher frequencies (4-16 kHz) were defined as the stimulus level that evoked a 3 µV reproducible waveform (determined by interpolation). At the lower frequencies where CAPs were still clearly visible below 3 µV, we used lower criteria (0.5 and 1 kHz: 1.5 µV, 2 kHz: 2 µV).

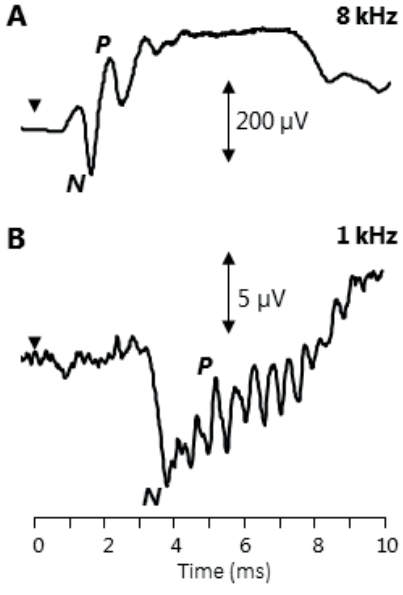

FIG. 1. Example CAPs evoked with 8 kHz at 80 dB SPL (A) and 1 kHz at 46 dB SPL (B) tone bursts. Triangles indicate tone onset. N indicates the largest negative peak, and P indicates the most pronounced subsequent positive peak.

2.4. Tissue Preparation

Immediately after the final recording, both left and right cochleas were fixed by intralabyrinthine perfusion with a fixative consisting of 3% glutaraldehyde, 2% formaldehyde, 1% acrolein, and 2.5% DMSO in 0.08 M sodium cacodylate buffer (pH 7.4), followed by immersion in the same fixative for 3 h at room temperature and subsequent histologic processing for light microscopy (de Groot et al., 1987). Semithin (1 Km) midmodiolar sections were examined with a Zeiss Axiophot light microscope, for spiral ganglion cell (SGC) analysis. The SGC loss was expressed as SGC packing density for each individual transection of the respective half-turn (b1, b2, m1, m2, and a1), as described by Van Ruijven et al. (van Ruijven et al., 2004). We plotted the SGC loss in the ototoxically treated groups relative to the mean packing densities in the control group.

Hair cell counts were obtained from block-surface preparations, after re-embedding of the cochlear halves in resin, by means of differential interference contrast (Nomarski) optics. The number of remaining inner hair cells (IHCs) and outer hair cells (OHCs) were counted as described by Van Ruijven et al. (2004). Hair cell loss was expressed as loss per 5% region on the basilar membrane and plotted into a cochleogram. To correlate the sound frequencies to a location on the basilar membrane, we used the following equation: distance (%) from base = 66.4- 38.2 * log [kHz] (Tsuji and Liberman, 1997).

2.5. Statistical Analysis

We used SPSS for Windows (version 15.0) for the statistical analyses. One-way ANOVAs (and also post-hoc Bonferroni analyses) were performed in comparing threshold shifts and hair cell loss over time. Linear regression analyses were used for assessing SGC loss over time and determining the predictive value of early threshold shifts on long-term hair cell survival.

3. Results

3.1. Stability of CAP Threshold Before Ototoxic Treatment

All 22 animals included in this study demonstrated stable thresholds before ototoxic or sham treatment as judged from 3 CAP recordings taken 1 week apart. Mean thresholds at the lower (0.5, 1, and 2 kHz) and higher (8, 11.3, and 16 kHz) frequencies were, respectively, 30 and 12 dB SPL (Fig. 4).

3.2. Variability in Threshold Shifts Between Animals

Individual tone audiograms showed a substantial amount of variability in threshold shifts among ototoxically treated animals, in particular for the low frequencies (Figs. 2 and 3). Fifteen of 17 animals showed threshold shifts greater than 50 dB for the highest frequencies (8, 11.3, and 16 kHz), and the 2 remaining animals showed such a threshold shift solely at 16

kHz (Fig. 2). Furthermore, in 13 of 17 animals, threshold shifts were less than 50 dB for the lowest frequencies (0.5, 1, and 2 kHz). In 15 of 17 animals, the audiograms showed threshold shift differences between high (8, 11.3, and 16 kHz) and low (0.5 and 1 kHz) frequencies greater than 20 dB.

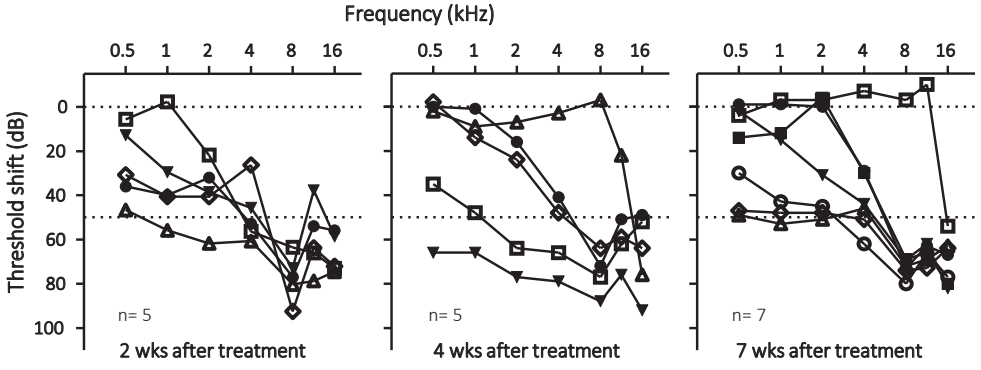

FIG. 2. Effects of ototoxic treatment after 2 (n = 5), 4 (n = 5), and 7 weeks (n = 7). Individual threshold shifts at 2, 4, or 7 weeks after ototoxic treatment, relative to the pretreatment thresholds for each individual animal. If thresholds were above the maximum sound level that could be applied, that level is used as threshold estimate. The larger threshold shifts at 8 kHz compared with 11 and 16 kHz were due to a higher maximal sound level, which we could stimulate with at 8 kHz.

3.3. Time Course of Major Threshold Shifts the First Days After Ototoxic Treatment

Figure 3 shows individual threshold shifts over 4 different time intervals in the first week after ototoxic treatment for 1 and 8 kHz. The largest threshold shift occurred on the second day after treatment. Shifts were considerably larger for 8 than for 1 kHz at the first 2 time intervals, and a slight recovery was observed at 1 kHz but not at 8 kHz in the third interval.

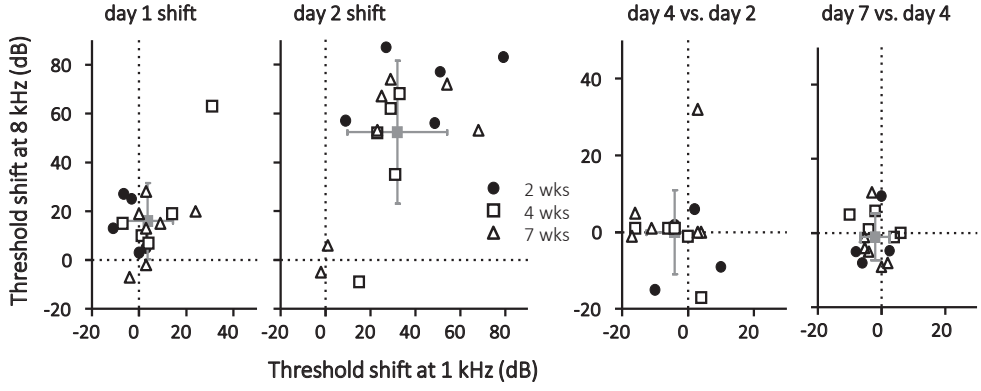

FIG. 3. Individual threshold shifts at 4 different time intervals in the first week after ototoxic treatment plotted horizontally for 1 kHz and vertically for 8 kHz. Means with standard deviations are shown in gray.

This accumulated to a difference in threshold shifts of approximately 35 dB (8 kHz: 65 versus 1 kHz: 30 dB).
The data shown for 1 and 8 kHz are representative for other low (0.5-4) and high frequencies (8Y-6 kHz), respectively. The lower frequencies showed a small insignificant threshold shift at day 1 (<10 dB; ANOVA, post hoc Dunnett, $p > 0.2$) and a large shift at day 2 (25-40 dB; ANOVA, post-hoc Dunnett, $p < 0.0001$). For the higher frequencies, threshold shifts were already substantial at Day 1 (15-25 dB; ANOVA, post hoc Dunnett, $p < 0.01$) and increased at Day 2 (35-50 dB; ANOVA, post hoc Dunnett, $p < 0.0001$). Hence, for the higher frequencies, thresholds increased mainly in the first 2 days and for the low frequencies mainly during the second day.

3.4. Stability of Thresholds Over Time

Above, we showed that major threshold shifts occur the first 2 days after ototoxic treatment. For the use of this animal model in future experiments an important question is whether thresholds are stable over time. Figure 4 shows mean absolute thresholds up to 7 weeks after ototoxic treatment, with 11 animals in the first 3 weeks (4- and 7-week groups) and 7 animals in Weeks 3 to 7 (7-week group). The panels on the left show that thresholds for 0.5, 1, and 2 kHz reached a maximum at Day 2 and showed a quick recovery of approximately 10 dB at Day 4, continuing up to 2 to 3 weeks after ototoxic treatment. For 11.3 and 16 kHz, highest thresholds occurred after 4 days, after which, a sloping recovery of approximately 10 dB occurred up to 3 weeks after ototoxic treatment. Four- and 8-kHz thresholds reached a maximum at Day 2 and, subsequently, were fairly stable over time. Paired comparisons of these maximum and minimum threshold levels for each frequency indicated that significant recovery occurred for 1, 2, 11.3, and 16 kHz, whereas recovery at 0.5, 4, and 8 kHz was not significant (paired t tests: 1, 2, and 11.3 kHz, $p < 0.05$; 16 kHz, $p = 0.07$; 0.5, 4, and8 kHz, $p > 0.1$). As shown in the panels on the right, for all frequencies, thresholds remained stable from 3 to 7 weeks after ototoxic treatment (paired t tests: 0.5-16 kHz, 3 versus 7 weeks, $p > 0.1$). Because the variability in thresholds over time for normal-hearing animals was about 7 dB, we consider a recovery of 10 dB relevant to take into account regarding the stability of this model over time.
In summary, major threshold shifts occurred early in the first week after ototoxic treatment. Subsequently, a mild spontaneous recovery occurred up to 3 weeks, after which, thresholds were stable over time.

3.5. Hair Cell Survival

Figure 5A illustrates the averaged hair cell counts obtained from block-surface preparations of the right cochleas, for all ototoxically treated animals. Data from the 2 animals with only a 16 kHz loss (Fig. 2) were excluded to form comparable groups for analyzing hair cell survival, that is, groups with matched mean audiograms at 2, 4, and 7 weeks after ototoxic treat-

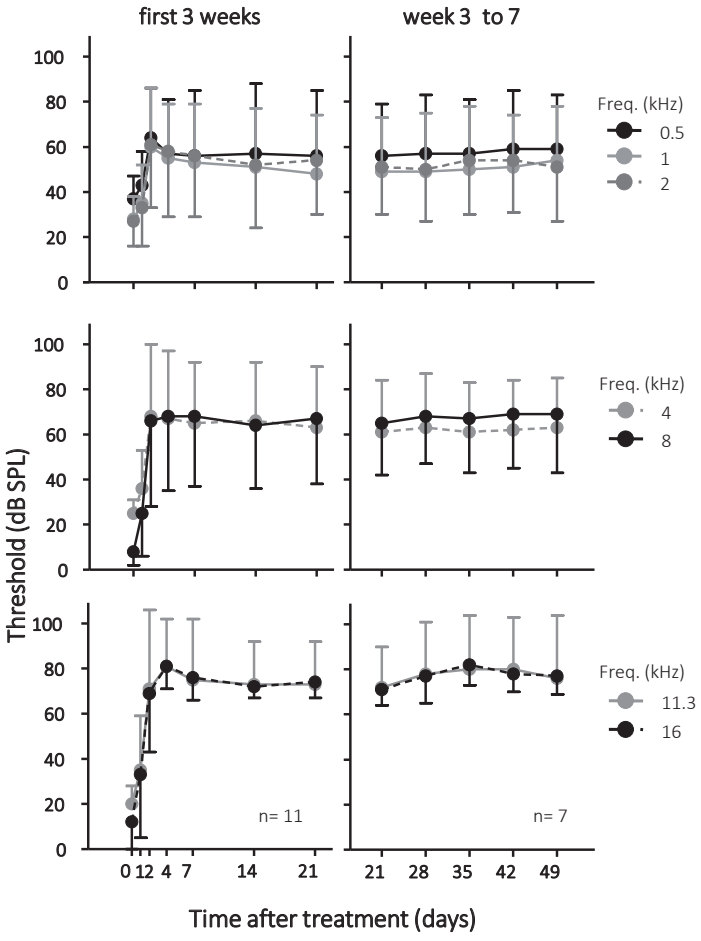

FIG. 4. Mean absolute thresholds over time; n = 11 animals in the first 3 weeks (left panel) and n = 7 animals in Weeks 3 to 7 (right panel), for each frequency (0.5-16 kHz). Means and SDs are shown.

ment. The shaded area indicates the region of the basilar membrane corresponding to the frequency band in which we performed CAP recordings (0.5-16 kHz).

OHC loss was pronounced in the middle and apical regions (50%-100%) and gradually turned into an almost complete loss in the basal region of the cochlea. The loss was most severe in the first row of OHCs (data not shown). In addition, OHC loss was comparable at 2, 4, and 7 weeks (ANOVA, for each 5% region on the basilar membrane, $p > 0.5$).

IHC loss ranged between 0% and 30% in the middle and apical region and gradually increased up to the basal region. Furthermore, IHC loss seemed to increase between 2 and 4 weeks in the most basal region of the cochlea. However, this was only significant at the 90% to 95% percent region (ANOVA, post hoc Bonferroni, $p < 0.05$, comparing IHC loss at 2 versus 4 and 7 weeks).

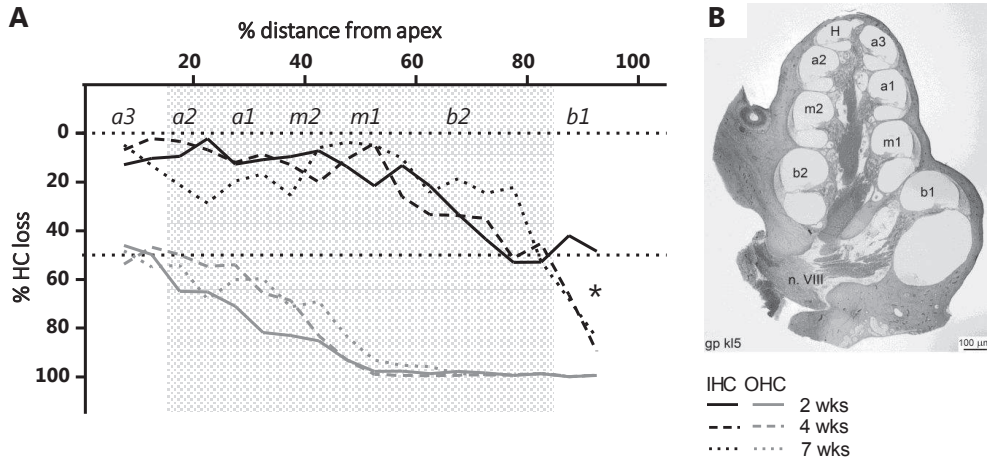

FIG. 5. A) Mean cochleograms at 2 (n = 5), 4 (n = 4), and 7 (n = 6) weeks after ototoxic treatment. Two animals with only a 16 kHz threshold shift were excluded to form comparable groups (matched audiograms at 2, 4, and 7 weeks after ototoxic treatment). The shaded area indicates the region of the basilar membrane corresponding to 0.5 to 16 kHz, which is the frequency band in which we performed CAP recordings. *p G 0.05. B) Midmodiolar section (1 µm) of the cochlea of a guinea pig. Transections of the cochlear half-turns are labeled b1 and b2 for the basal turns, m1 and m2 for the middle turns, and a1 to a3 for the apical turns. H: helicotrema; n. VIII: cochlear nerve.

To assess possible effects of electrode implantation on the round window, Figure 6 compares the mean IHC and OHC losses of left and right ears for 3 different regions on the basilar membrane (basal :60%-90%, middle: 30%-60%, apical: 10%-30%). Mean differences ranged from 0% to 5% and were larger and more variable in the ototoxically treated animals, as expected. Statistical analyses showed no significant differences in IHC or OHC loss comparing left and right ears (repeated measure ANOVA for both IHC and OHC loss, factor ear: $p > 0.2$, $F < 0.1$).

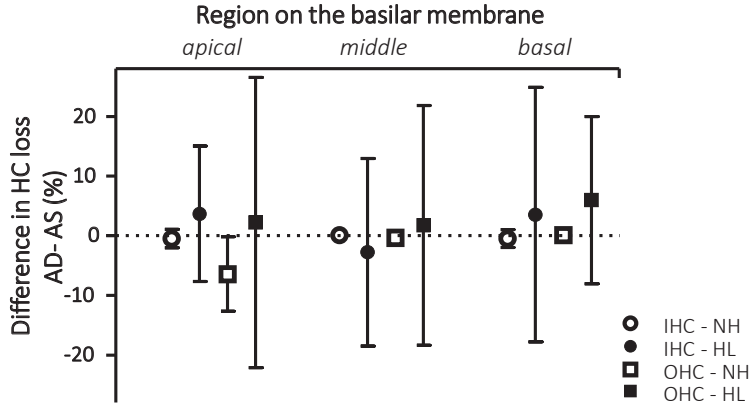

FIG. 6. Individual differences in IHC and OHC loss between right and left cochleas were averaged for normal hearing controls (normal hearing, NH; n = 5) and all ototoxically treated animals (hearing loss, HL; n = 17). Means and SDs are shown for 3 regions on the basilar membrane: basal (60%-90%), middle (30%-60%), and apical (10%-30%).

3.6. Spiral Ganglion Cell Survival

Spiral ganglion cell loss after ototoxic treatment was limited (~20%). This loss was largest in the most basal location (b1). Figure 7A shows the time course of the SGC packing density in location b1 for normal-hearing and ototoxically treated animals at 2, 4, and 7 weeks. It illustrates a minor decrease in SGC density with the period after ototoxic treatment, which we described by an exponential decay function with a time constant of 25 weeks (1/e = 37% SGC survival after 25 weeks, equivalent to 80% survival after ~5 wks).

Furthermore, we analyzed whether the SGC packing densities in location b1 depended on the survival of IHCs. The degree of IHC survival was calculated as the mean survival in the 10% region on the basilar membrane corresponding to location b1. First, we derived the position (distance in mm) for the cochlear turns along the basilar membrane (see ref. 17), and converted these distances in mm to percentage from the apex. Figure 7B shows the data for all groups at basal location b1, indicating a significant increase of SGC packing density with IHC survival (about 250 SGCs/mm2 from 0-100% IHC survival).

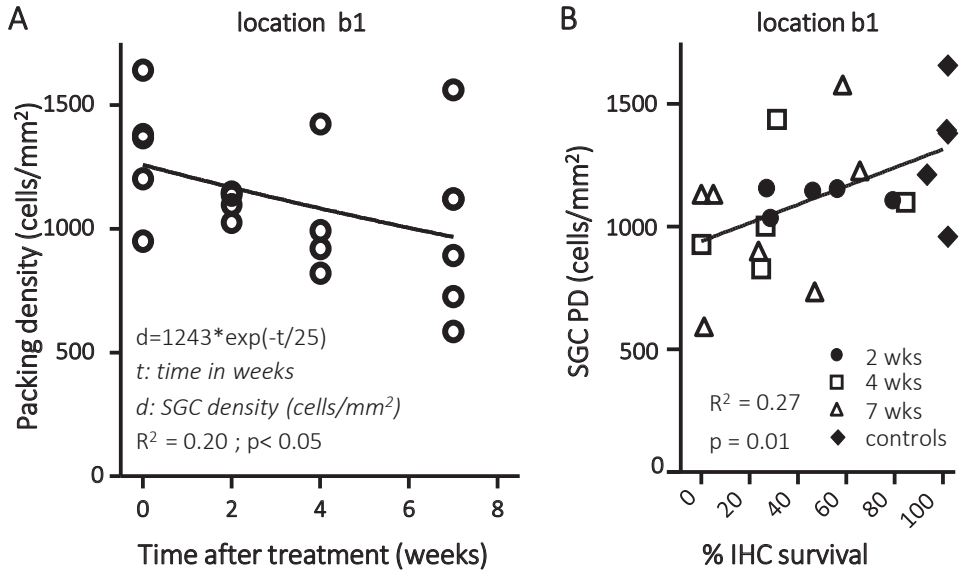

FIG. 7. A) SGC packing density at location b1 as a function of survival time after ototoxic treatment. Two animals with only a 16 kHz threshold shift were excluded. Densities of the right ears are shown (n = 20). An exponential function has been fitted to the data: the time constant is 25 weeks, indicating that after that period 37% (1/e) of the SGCs are still present. Linear regression of logarithm of SGC density versus survival time: $R^2 = 0.20$, $F_{1,18} = 4.6$, $p < 0.05$. B) Dependence of SGC packing density on IHC survival at location b1, for all animals (n = 22). $R^2 = 0.27$, $F_{1,20} = 7.0$, $p < 0.05$.

3.7. Correlation Electrophysiology and Histology

In almost all animals, threshold shifts at high frequencies were large (>60 dB), but threshold shifts at low frequencies were quite variable (Fig. 2), whereas limited shifts were intended. The largest threshold shifts took place the first 2 days after ototoxic treatment. We wondered whether early threshold shifts at low frequencies could be used to predict final thresholds and (apical) hair cell survival. Correlation analyses for the lower frequencies indicated that the early threshold shifts can indeed be used to predict final thresholds (linear regression analysis for 0.5, 1, and 2 kHz; $R^2 > 0.7$ and $p < 0.0001$; data not shown). For this reason, if animals with residual low- frequency hearing are desired, low-frequency threshold shifts should be low after 2 days. We then correlated final hair cell survival to low-frequency (0.5-2 kHz) CAP threshold shifts occurring the first 2 days after ototoxic treatment (Fig. 8). To determine hair cell survival, we first identified the best frequency location on the basis of the Tsuji and Liberman (1997) equation (see Materials and Methods). Next, we calculated the mean hair cell loss (OHCs and IHCs) of the corresponding frequency in the 5% region enveloping the best frequency location and the more basal 5% region. For each of the low frequencies, the final hair cell survival was highly correlated with the initial threshold shift ($R^2 > 0.5$; $p < 0.001$, see Fig. 8). The data indicate that for small threshold shifts hair cell survival was almost complete, whereas for early threshold, shifts greater than 40 dB hair cell survival was less than 60% (Fig. 8).

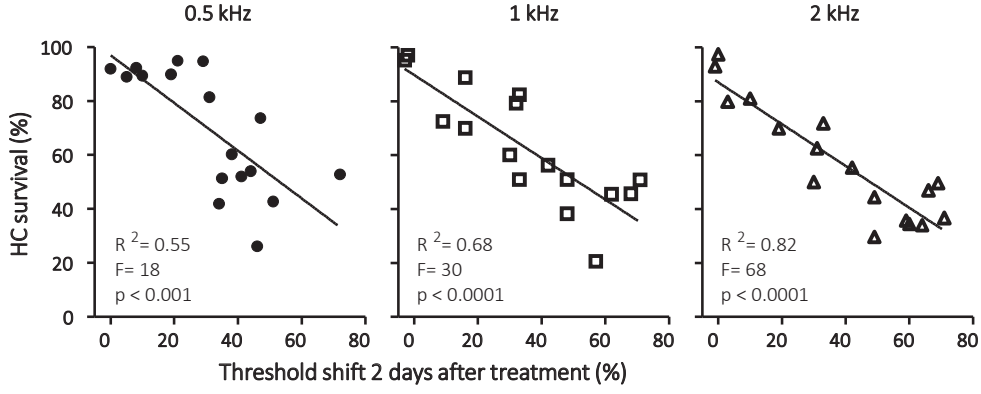

FIG. 8. The percentage of surviving hair cells related to the threshold shift after 2 days for 0.5, 1, and 2 kHz tones. OHC and IHC numbers of the corresponding frequency in the 5% region enveloping the best frequency location and the more basal 5% region (calculated with the Tsuji and Liberman (1997) equation) were averaged; subsequently, the obtained OHC and IHC percentages were averaged.

4. Discussion

We described a guinea pig model of selective severe high-frequency hearing loss, to be used in future animal experiments in the EAS research field. First, treating guinea pigs with the dose regimen of ototoxic drugs described previously resulted in severe high-frequency hearing losses in most animals. Second, this model was stable within 4 weeks after ototoxic treatment, in terms of both hearing threshold and hair cell survival. Third, early CAP recordings (after 2 days) predict final thresholds and hair cell survival, which is relevant information when using these animals in long-term and/or expensive experiments.

4.1. Variability

A considerable challenge regarding the reproducibility of an animal model of hearing loss is the great variability in the extent to which hair cells remain functional after ototoxic treatment among animals. Several studies described a significant variability in the response to treatment with ototoxic drugs varying from mild-to-severe hearing losses (cats: Xu et al., 1993; Coco et al., 2007; guinea pigs: Nourski et al., 2004; Versnel et al., 2007). We found a comparable variability (Figs. 2 and 3A), which can partially be explained by differences in the sensitivity to ototoxic drugs within individuals (Xu et al., 1993; see their discussion). Another possible factor could be age. A number of animal studies indicate a relationship between the susceptibility to drug ototoxicity and onset of auditory function, that is, the susceptibility is higher before than after onset of hearing.

Because the guinea pig is a precocial animal, in which the inner ear develops intra-uterinely, such susceptibility window for drug ototoxicity can be assumed not to exist postnatally (McDowell, 1982; Henley and Rybak, 1995). Evidence showing no difference in gentamicin ototoxicity comparing guinea pigs at 4 and 24 weeks of age supports this assumption (McDowell, 1982). Also, we did not find this correlation between the severity of hearing loss and body weight at the time of ototoxic treatment (data not shown).

Finally, aminoglycoside ototoxicity might depend on sex. However, reports on possible sex differences regarding aminoglycoside ototoxicity in animal research do not give a consistent view. Mills et al. (1999) showed that male rats are more susceptible to ototoxicity than female rats. Halsey et al. (2005) described the opposite in which female guinea pigs are more susceptible. Third, Tan et al. (2001) demonstrated no sex difference in the response to noise and/or amikacin treatment in guinea pigs. Because we only used female guinea pigs, this factor does not explain the variability.

In our view, the variability among animals is acceptable for future studies regarding severe high-frequency loss, in particular for longitudinal studies, which aim at changes in individual animals over time, for example studies of insertion trauma after cochlear implantation.

4.2. Stability Over Time

The largest threshold shifts after treatment with kanamycin and furosemide take place the first 2 days (Fig. 3), and these early recordings can be used to predict final thresholds and hair cell survival (Fig. 8). This is quite similar to findings in guinea pigs severely deafened after cotreatment of the same drugs but with a double dose of kanamycin (Versnel et al., 2007). The time courses of thresholds further

agree in that a small functional recovery occurs up to the third week, starting after the second day (200 mg/kg kanamycin, current study) or after the fourth day (400 mg/kg kanamycin, Versnel et al., 2007). The recovery is possibly due to clearance of kanamycin from the cochlea (Aran and Darrouzet, 1975).

OHC loss was complete in the basal region and gradually decreased toward apical regions (Fig. 5), which is typical for aminoglycoside treatments (Forge and Schacht, 2000), and this loss was stable within 2 weeks, which is consistent with the rapid loss generally found after cotreatment with loop diuretics (Russell et al., 1979; Xu et al., 1993; Versnel et al., 2007; Glueckert et al., 2008). In contrast, IHC loss still increased after 2 weeks in the basal turn (Fig. 5) as indicated by previous findings in guinea pigs with comparable kanamycin doses (200-300 mg/kg, Stronks et al., 2011). The IHC degeneration process is slower than with higher doses (400 mg/kg) where a rapid and substantial loss of inner hair cells is reached within a week (e.g., Versnel et al., 2007; Glueckert et al., 2008). We conclude that in our adjusted deafening procedure, in which we reduced the amount of kanamycin, the delayed and less severe IHC loss in the basal cochlear turn was due to lower concentrations of kanamycin in the IHCs.

4.3. Spiral Ganglion Cell Degeneration

The time course of SGC degeneration described in this study is much slower (time constants differed by a factor 3-4) than in animals which were treated with a double dose of kanamycin (400 mg/kg) in combination with furosemide (Versnel et al., 2007). This will be due to the higher number of IHCs surviving in the moderate version of ototoxic treatment (see previous subsection), which provide neurotrophic support to the spiral ganglion neurons to protect them from degeneration (Fritzsch et al., 2004). The correlation between SGC packing density and IHC survival (Fig. 7B) supports this notion. Whereas in this case of ototoxic insult, the SGC degeneration is probably caused by IHC loss; in other conditions, for instance aging, the SGC loss may well be independent of IHC loss (Jin et al., 2011).

4.4. Models of Selective Severe High-Frequency Hearing Loss

Recent articles described a gerbil model of selective high- frequency hearing loss (Choudhury et al., 2011; Suberman et al., 2011), using noise with certain high pass cutoff frequencies (ranging from 2 to 8 kHz). Suberman et al. (2011) reported that animals with mild-to- severe ABR threshold shifts have variable patterns of hair cell loss. This variability is consistent with various reports on noise-induced hearing loss (e.g., Cody and Robertson, 1983; Borg et al.,

1992). However, Choudhury et al. (2011) discussed that a slight increase in noise level yielded the intended results in 86% of the animals, with only 3 nonresponders out of 22 animals, indicating less variability.

Furthermore, as in our study, they described that the extent of cochlear damage was comparable for both ears. This is an important finding because the contralateral ear often serves as a control ear, for example, when assessing additional damage caused by cochlear implantation. Altogether, the gerbil noise model and the guinea pig pharmacologic model described in this article seem to yield comparable results. We conclude that application of a moderate dose of kanamycin in combination with furosemide yields an appropriate guinea pig model for selective severe high- frequency hearing loss, which is stable after 4 weeks, and can be used in studies regarding EAS research.

Acknowledgements

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Chapter 6

Hearing Preservation Surgery: Cochleostomy or Round Window Approach? A Systematic Review

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Abstract

Objectives/Hypothesis: An increasing number of patients with low-frequency residual hearing are fitted with a cochlear implant. The challenge is to optimize cochlear implant device properties and develop atraumatic surgical techniques to preserve residual hearing. In view of the ongoing debate about the optimal procedure for opening the cochlea during cochlear implantation, we reviewed the evidence on the round window and the cochleostomy insertion techniques and compared their effects on postoperative residual hearing.

Design: Systematic review.

Methods: Electronic databases were systematically searched for relevant studies published up to January 2012. All studies reporting on residual hearing and hearing preservation surgery were included.

Results: Sixteen studies, with a total of 170 patients, were included. There were no studies directly comparing both surgical insertion techniques. The methodologic quality of the studies was poor and might be subjected to a high risk of bias. Because there were no studies directly comparing the 2 techniques and controlling for possible influencing factors, differences between studies might also be influenced by intersurgeon variance in many facets regarding cochlear implantation surgery. The available data show a postoperative low-frequency hearing loss ranging from 10 to 30 dB at 125, 250, and 500 Hz, regardless of surgical technique. The number of patients with a postoperative complete hearing preservation ranged from 0% to 40% for the cochleostomy group and from 13% to 59% in the round window group.

Conclusion: The available data do not show that there is a benefit of one surgical approach over the other regarding the preservation of residual hearing. To provide solid evidence, a double-blind randomized trial is needed, which compares the clinical outcomes, notably the degree of hearing preservation, of both surgical approaches.

1. Introduction

The expanding indication criteria for cochlear implantation because of the improved postoperative hearing results lead to a worldwide increase in patients with residual hearing who are fitted with a cochlear implant. In the last decade, several research groups have explored various methods of implanting different electrode arrays in the cochlea to preserve the residual hearing and combine acoustic and electric speech processing (Gstoettner et al., 2004, 2006; Gantz et al., 2009; Lenarz et al., 2009; Skarzynski et al., 2007, 2009;). The different electrode arrays (hybrid), specifically developed for this purpose, vary in several aspects, shorter length, more flexible, and thinner.

Although much attention has been given to minimize trauma by optimizing the electrode design, a minimal traumatic opening of the cochlea and insertion of the electrode is essential for hearing preservation. Over the last years, 2 major atraumatic surgical techniques have been promoted, the round window approach and the ''soft surgery'' cochleostomy technique. The latter has been proposed by Lenhardt in 1993, who performed a minimal cochleostomy anterior and inferior to the round window and used hyaluronic acid (Healon) to lubricate the electrode and seal the cochleostomy during insertion (Lehnhardt, 1993). Nowadays, this technique has been slightly modified by different surgeons, but the general ''soft surgery'' principles of drilling a minimal cochleostomy, avoiding suction of the perilymph, and sealing the cochleostomy hole remained identical. Although the round window insertion was the initial technique of cochlear implant electrode placement, the technique became less used because of concerns that the insertion angle in conjunction with the former rigid, straight electrodes, might lead to trauma of the osseous spiral lamina. With the development of more flexible and perimodiolar electrodes new interest for this technique has emerged. Because the round window technique involves only a minimal incision of the round window membrane, advocates of this technique have pointed out the shortcomings that are associated with drilling a cochleostomy, such as acoustic trauma, the presence of bone dust and the higher chance of electrode insertion into the scala vestibuli.

A temporal bone study comparing both insertion techniques demonstrated that the insertion angles between the two techniques are comparable. With both techniques no signs of histologic damage to the modiolus, osseous spiral lamina or basilar membrane were seen (Briggs et al., 2006). The influence of the technique on postoperative residual hearing is being argued.

In view of this ongoing debate about the optimal procedure for opening the cochlea during cochlear implantation to preserve residual hearing our main research question was: Is there a difference in postoperative residual hearing comparing round window and cochleostomy insertion techniques in patients with low-frequency residual hearing fitted with a cochlear implant?

2. Methods

2.1. Search Strategy and Study Selection

We conducted a systematic search in the PubMed, Embase, and Web of Science databases from inception up to January 9, 2012, by combining the search terms ''residual hearing'' and ''hearing preservation surgery,'' and their synonyms in title and abstract fields (Appendix 1). We checked the bibliography of all relevant articles and reviews to identify supplemental studies. Two authors independently screened all titles and abstracts of the retrieved publications, and subsequently, they screened the full texts of eligible studies for selection (Fig. 1). Studies were included if individual or group average preoperative and postoperative hearing thresholds and/or the number of subjects with complete or partial hearing loss were reported. If for specific electrode arrays, only 1 electrode insertion technique (either round window or cochleostomy) has been reported in the literature, the studies reporting these electrode arrays were excluded because no comparisons between the 2 insertion techniques could be made for that specific electrode. Disagreement between the authors during selection was resolved by discussion. We used no language restrictions during our search and selection.

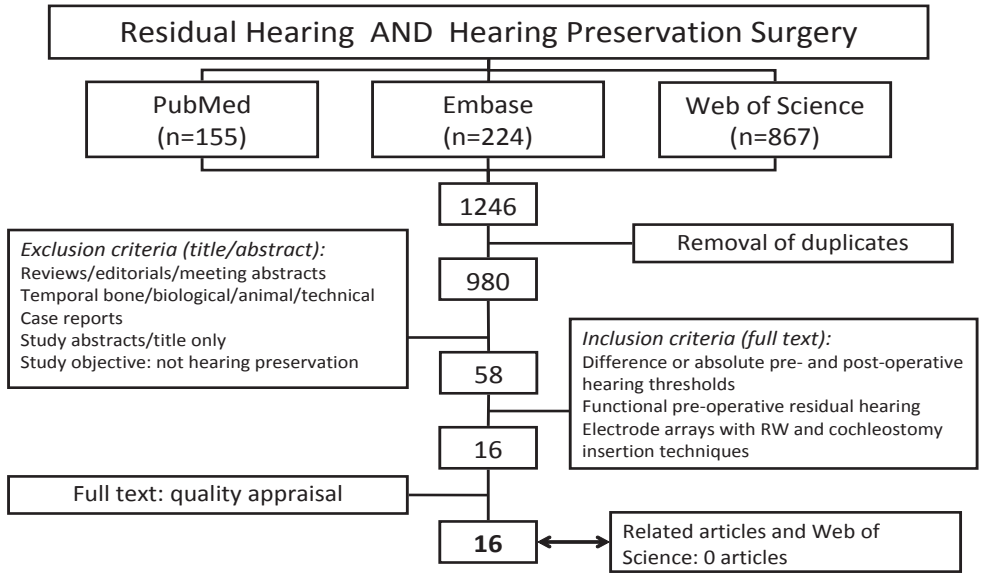

FIG. 1. Flow-diagram of search strategy. Search date: January 9, 2012. RW: round window.

2.2. Quality Assessment

Two authors independently assessed the risk of bias of all included studies with the following criteria: 1) Was patient allocation to a treatment or a control group randomized and concealed?

2) Were patients, caregivers, and outcome assessors blinded? 3) Are, apart from differences in surgical technique (round window or cochleostomy) and different electrode arrays, all participants treated according to the same, clearly defined, surgery protocol? 4) Equal evaluation of outcomes: were preoperative and postoperative audiograms performed in all participants according to the same, clearly defined, protocol? 5) Was the number of missing data and explanation given?

2.3. Data Extraction

Information was gathered for each included study on design, study population, and its preoperative hearing thresholds, number of included patients, insertion techniques, cochlear implant electrode arrays, and individual or group average preoperative and postoperative hearing threshold or threshold shift. If individual preoperative and postoperative audiograms were presented, we extracted the individual data from these audiograms. The authors from articles only presenting group average preoperative and postoperative hearing thresholds or group average threshold shifts were contacted and were asked to supply additional data. If the individual data remained unavailable, the group averages were used.

3. Results

3.1. Search Results and Quality Assessment

Our search identified 980 unique titles, of which, 922 articles were excluded after screening titles and abstracts (Fig. 1). The 58 remaining articles were retrieved in full text for formal review. After independent review, no reports, which compared the round window insertion with the soft surgery cochleostomy technique, were identified. Sixteen articles met the inclusion criteria and were eligible for further analysis (Table 1). These studies originated from 9 centers, with 8 studies from 2 centers.

Table 1 shows the study characteristics and also provides details on the used electrode array and insertion technique in each study. They together reported the individual data for 170 patients (range per study, 4 to18). In 6 reports, both insertion techniques were described (Table 1). In most of these studies, the preferred insertion technique was the round window technique. If a good visualization of the round window was impossible, a cochleostomy was performed in these patients. Seven studies reported on the hearing preservation after partial insertion of the Med-El Standard (31.5 mm) electrode array or full insertion of the Med-El Medium (24 mm) electrode array (Table 1). Three of these studies presented individual data from one center using the cochleostomy insertion technique (Gstoettner et al., 2004, 2005, 2006), the other 4 studies reported data from 1 center using the round window insertion technique (Skarzynski et al., 2004, 2007a,b, 2010). Because the properties are comparable for both electrodes, we considered partial insertion of the standard electrode comparable to

full insertion of the medium electrode and analyzed them as 1 group. Six articles evaluated hearing preservation after full insertion of the Med-El FlexEAS (24 mm) electrode array. In 2 of these studies, some patients with a fully inserted Med-El FlexSoft (31.5 mm) electrode were included. This 31.5-mm-long electrode was evaluated in 4 studies. The reported follow-up periods varied widely across the studies, between 1 and 70 months (Fig. 2).
Table 2 shows the results of the risk of bias assessment. The overall methodologic quality of the 16 articles was poor. Only prospective or retrospective case series, evaluating the postoperative residual hearing preservation after round window insertion or ''soft surgery'' cochleostomy were identified.

Table 1. Study characteristics

Study	Round window (n)	Cochleostomy (n)	Electrode	Insertion depth (mm)	Intraoperative steroids	Hyaluronic acid	Fascia	Mean follow up months (range)	Comments
Arnoldner 2010	8	2	EAS	18-22	●	●	●	8 (1-16)	Cochleostomy if RW was not possible
Arnoldner 2011	1	1	EAS/Soft	23-31	●	●	●	30 (24-36)	
Bruce 2011	-	12	Soft	Full	●	●	?	11.6 (0.25-23)	
Gifford 2008	6	0	EAS/ Hybrid	?	?	?	●	8 (2-13)	Only results for EAS patients were used
Gstoettner 2004	-	16	S/M	16-24	●	●	●	26.6 (4.3-55.9)	
Gstoettner 2005	-	1	S/M	18-22	●	●	●	28.7 (1.7-58)	
Gstoettner 2006	-	15	S/M	18-24	●	●	●	27.2 (6-70)	
Gstoettner 2009	7	1	EAS	18-21	●	●	●	9.73 (6-16)	Cochleostomy if RW was not possible
Helbig 2011a	5	13	EAS	?	?	?	?	12	
Helbig 2011b	12	4	Soft	26-31	●	●	●	15 (4-33)	Cochleostomy if RW was not possible
Lee 2010	6	2	M/EAS	180°-390°	●	●	●	22 (2-66)	
Podskarbi-Fayette 2010	18	-	S/M/Soft	18-22	Post	?	?	12	
Skarzynski 2004	6	-	S	18-22	?	?	?	1	
Skarzynski 2007a	1	-	S	6-8 el	Pos	?	?	12	
Skarzynski 2007b	9	-	S/M	± 20	?	?	?	11.2 (3-12)	
Usami 2011	4	-	EAS/Soft	full	●	?	?	9.4 (7-16)	

● = yes, - = no, ? = unknown. EAS: Med-El FlexEAS array, el: electrodes, Soft: Med-El FlexSoft array, M: Med-El Medium array, S: Med-El Standard array

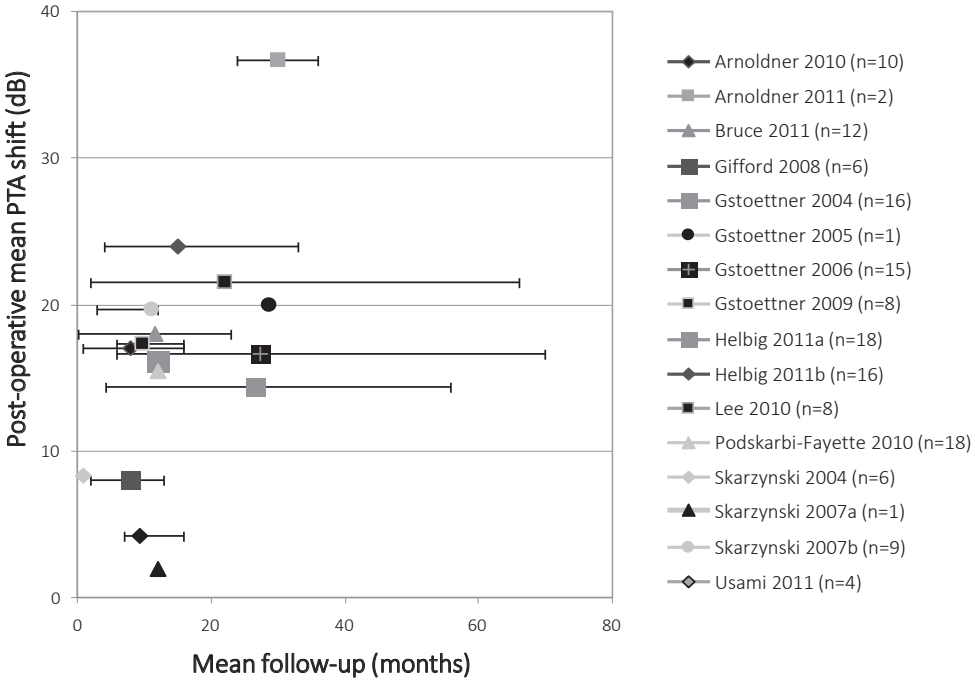

FIG. 2. Mean PTA threshold shifts and follow-up range of the 16 included studies. PTA: pure tone average (125, 250, and 500 Hz). Numbers of patients included in this figure are noted in the legend for each study individually (n).

3.2. Data Analysis

To compare the 2 insertion techniques based on the results presented in the various studies, it was necessary to make a few assumptions. First, most studies presented the number of patients with a low-frequency threshold shift of up to 10 dB or above. Therefore, we have used the cutoff value of 10 dB for our definition of complete (≤10 dB) or partial (>10 dB) low-frequency hearing preservation. Second, average low-frequency hearing loss was defined as the hearing loss over the frequencies 125, 250, and 500 Hz because data from these frequencies were reported most frequently in the various articles. Third, the absolute low-frequency hearing threshold shift could only be determined for the frequencies 125, 250, and 500 Hz because hearing thresholds for these frequencies were reported for most patients. Furthermore, in our analyses, we only included subjects for whom individual and/or unique data were reported. Therefore, in some studies, the number of included subjects in our analyses (Table 1) is smaller than the number of subjects presented in the original articles. In the study by Helbig et al. (2011a), only pooled data were reported. For our evaluation of the threshold shift of the FlexEAS array, we therefore had to rely on the pooled patient data presented by Helbig et al. (2011a) and individual data presented in the other articles using the FlexEAS array. To combine the individual absolute low-frequency hearing threshold

Table 2. Risk of bias assessment

Study	Comparison RW vs. C	Randomization	Blinding	Equal treatment	Equal evaluation of effect (test)	Missing data	Comments
Arnoldner 2010	○	○	○	●	●	N/A	
Arnoldner 2011	○	○	○	●	●	N/A	
Bruce 2011	○	○	○	●	●	N/A	
Gifford 2008	○	○	○	●	●	N/A	
Gstoettner 2004	○	○	○	●	●	N/A	
Gstoettner 2005	○	○	○	●	●	N/A	Data partially also presented in Gstoettner 2004
Gstoettner 2006	○	○	○	●	●	N/A	
Gstoettner 2009	○	○	○	●	●	●	
Helbig 2011a	○	○	○	●	●	●	
Helbig 2011b	○	○	○	●	●	N/A	
Lee 2010	○	○	○	●	●	N/A	
Podskarbi-Fayette 2010	○	○	○	●	●	●	
Skarzynski 2004	○	○	○	●	●	N/A	
Skarzynski 2007a	○	○	○	●	●	●	
Skarzynski 2007b	○	○	○	●	●	N/A	
Usami 2011	○	○	○	●	●	N/A	

● = good (low risk of bias), □ = moderate (moderate risk of bias), ○ = poor/unknown (high risk of bias). C: cochleostomy, RW: Round Window. Missing data: ●: <10%, □: 10-20%, ○: >20%. N/A: not applicable for retrospective studies

shifts in these studies with the pooled group average threshold shifts reported by Helbig et al. (2011a), we calculated weighted means and standard deviations for the 3 frequencies, using the equations presented in Figure 3. Fourth, in the literature, a wide variation in follow-up is reported. Because of this wide range, we were not able to analyze the results for both short and long follow-up terms separately but had to combine the postoperative results with the varying follow-up. If studies reported hearing thresholds at various follow-up moments, we only included the latest evaluation in our analyses. Finally, as already stated in the introduction, several modifications of the 2 techniques have been made by the different surgeons. This has resulted in numerous variables, such as the use of hyaluronic acid (Healon), fascia, or intracochlear and systematic corticosteroids, for which in this review cannot be controlled. These factors are, however, presented in the study characteristics table (Table 1).

A

$$\overline{X}_W = \frac{\sum_{i=1}^{n} w_i X_i}{\sum_{i=1}^{n} w_i}$$

B

$$sd_w = \sqrt{\frac{\sum_{i=1}^{N} w_i (x_i - \bar{x}_w)^2}{\frac{(N' - 1)\sum_{i=1}^{N} w_i}{N'}}}$$

FIG. 3. Equations used to calculate the weighted mean (A) and weighted standard deviation (B).

3.3. Evaluation of Low-Frequency Hearing Preservation

Figure 4A shows mean hearing losses for the low frequencies (125, 250, and 500 Hz) after surgery for subjects implanted with the Med-El Standard/Medium electrode array (left panel), Med-EL FlexEAS (middle panel), and the Med-El FlexSoft electrode array (right panel). Overall, mean hearing losses of subjects implanted through the round window or cochleostomy approach were comparable, ranging from about 10 to 30 dB at 125, 250, and 500 Hz. The upper panel of Figure 4B shows the mean preoperative pure-tone average (PTA) hearing thresholds for both cochleostomy and round window implantees for all 3 electrodes. The results in the lower panel indicate that there are no differences in mean postoperative PTA threshold shifts comparing both the insertion technique and the type of electrode; mean PTA shifts ranged between 15 and 23 dB for all subgroups (Table 3).

Table 3. Mean threshold shifts (125, 250, 500 Hz and PTA) for both cochleostomy and round window insertion methods

Freq		Cochleostomy			Round window		
(Hz)		Standard/M	FlexEAS*	FlexSoft	Standard/M	FlexEAS*	FlexSoft
125	mean	14 (9-19)	17 (11-23)	14 (2-26)	8 (3-13)	9 (3-15)	10
	n	*34*	*19*	*9*	*35*	*35*	*1*
250	mean	15 (11-19)	24 (14-34)	18 (9-28)	15 (9-20)	15 (9-22)	10
	n	*37*	*19*	*13*	*35*	*35*	*1*
500	mean	16 (11-21)	28 (20-37)	16 (9-23)	22 (16-28)	21 (14-29)	15
	n	*37*	*17*	*13*	*35*	*35*	*1*
PTA	mean	15 (11-20)	20 (15-24)	19 (10-29)	15 (10-19)	15 (9-22)	23 (12-35)
	n	*34*	*17*	*14*	*35*	*35*	*12*

Mean threshold shifts and accompanying 95% confidence intervals (125, 250, 500 Hz and PTA) in dB for both cochleostomy and round window insertion methods. 95% confidence intervals are shown between parenthesis. M: Med-El Medium array, n: number of subjects, PTA: pure-tone average (125, 250 and 500Hz), *: Weighted mean

3.4. Degree of Low-Frequency Hearing Preservation

Besides absolute low-frequency threshold shifts after surgery, another frequently described outcome parameter regarding the degree of hearing preservation is the percentage of subjects reaching a complete or partial hearing preservation versus a complete loss of residual

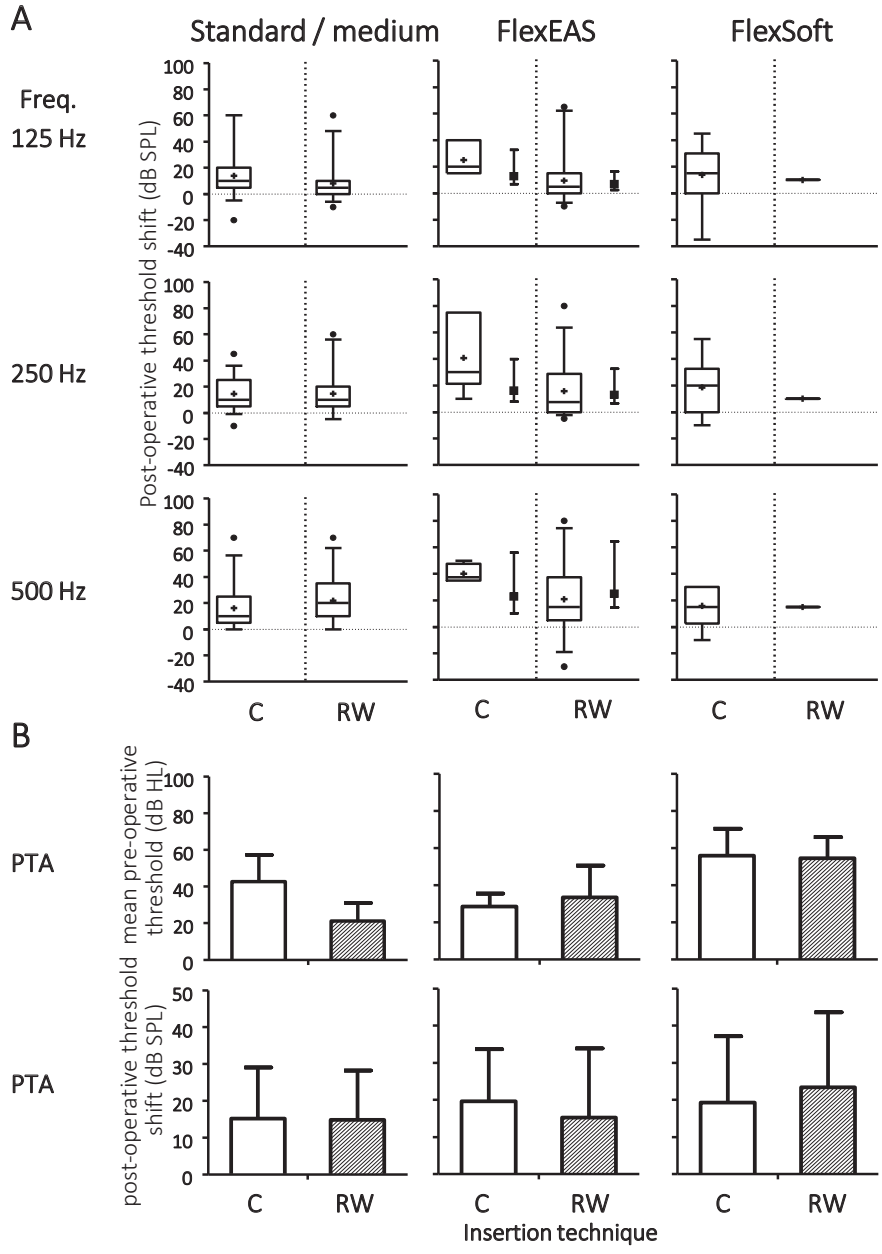

FIG. 4. A) Postoperative threshold shifts for 125, 250, and 500 Hz, after partial insertion of the Med-El Standard or full insertion of the Med- El Medium, FlexEAS and FlexSoft electrode arrays. Lines within the boxes indicate the median and the positive sign indicates the mean postoperative threshold shift. Whiskers denote the 95% confidence intervals. B) Mean preoperative PTA (125, 250, and 500 Hz) threshold and postoperative PTA threshold shift for the 2 insertion techniques presented for the different electrode arrays. Upper panel: mean preoperative PTA threshold. Lower panel: mean postoperative PTA threshold shifts. Error bars indicate the standard deviation. C: cochleostomy, RW: round window.

hearing. Complete hearing preservation is often reported as the mean PTA shift at the lower frequencies (125, 250, 500, and sometimes also 750 Hz) of 10 dB or lesser (Gstoettner et al., 2004, 2005, 2006, 2009; Kiefer et al., 2005; Baumgartner et al., 2007; Skarzynski et al., 2007; Arnoldner et al., 2010, 2011). Table 4 describes the number of subjects with a complete or partial hearing preservation or a complete loss of residual hearing, for each electrode separately. Complete hearing preservation ranged from 0% to 40% in patients implanted by means of the cochleostomy approach and from 13% to 59% in patients implanted through the round window. A complete loss of residual hearing occurred in 0% to 26% in the cochleostomy group and in 3% to 20% in the round window groups.

Table 4. Number of subjects with a PTA hearing loss ≤ 10 dB, > 10 dB or a complete loss of residual hearing

	Mean PTA shift (dB)*	Patient number	Hearing preservation		
			Complete	Partial	Complete loss
			≤ 10 dB	> 10 dB	not measurable
Med-El Standard/Medium					
C	15	43	17 (40%)	15 (35%)	11 (26%)
RW	15	34	15 (44%)	18 (53%)	1 (3%)
Med-El FlexEAS					
C	27	4	0 (0%)	4 (100%)	0 (0%)
RW	15	32	19 (59%)	11 (34%)	2 (6%)
Med-El FlexSoft					
C	19	19	4 (21%)	10 (53%)	5 (26%)
RW	24	15	2 (13%)	10 (67%)	3 (20%)

Number of subjects with a complete (≤ 10 dB) or partial (> 10 dB) loss over the frequencies 125, 250 and 500 Hz (PTA) indicated for three electrode arrays. C: cochleostomy, RW: round window, PTA: pure tone average (125, 250 and 500 Hz),*: Patients with complete loss were excluded.

4. Discussion

The results of this review show that there seems to be no clear benefit of a certain surgical approach regarding cochlear implantation, that is, the round window versus cochleostomy approach. To our knowledge, this is the first systematic review comparing both surgical approaches. However, some potential limitations should also be addressed. First, only (prospective) cohort studies and case series provided information on our research question. There were no studies directly comparing both surgical approaches. A direct comparison between the 2 approaches could therefore not be made, and for our analyses, we had to rely on indirect information provided by studies with different research questions. Because of the absence of randomized study designs, concealed treatment allocation, and unclear patient selection procedures, the included studies might be subject to a high risk of bias. As there are

no direct comparisons between both approaches reported in the literature and the quality of methods of the indirect evidence is either poor or has been reported poorly, there are as yet no valid data to support any statement on the superiority of either approach. Although the results of this review might suggest an advantage regarding less patients with complete hearing loss after a round window insertion (Table 4), this difference might be influenced by the high risk of reporting and selection bias. Second, to allow for comparisons of various studies, all calculations were based on the reported information and certain assumptions. To make the various comparisons between the 2 techniques, the differences in follow-up between the included patients had to be disregarded. Because several studies reported variable follow-up durations, ranging from weeks to months, this could have influenced the results reported in the individual studies and in our analyses (Fig. 3). Third, many factors possibly influencing the results on residual hearing were variable. For example, different definitions of residual hearing were used. Also, various cochlear implant devices with various implantation depths were described. To address these issues, we only included patients with preoperative audiograms fitting the audiometric inclusion criteria for the EAS and EAS extended groups (Lenarz et al., 2009) and showed all data for several electrodes separately. In this report, only studies reporting on the Med-El Medium, EAS, Flex, and partially inserted Med-El Standard electrodes fulfilled these inclusion criteria. This comparison between the 2 insertion techniques could therefore not be made for other electrode arrays. Finally, because of the limited number of centers reporting data on this matter, especially for our comparison between the 2 insertion techniques with the standard/medium electrode array, a predominant data-inflow from one center might influence the results. With the relative low number of different centers, included in the various analyses in our study, and the differences between studies, it was not possible to control for differences between centers regarding for example candidacy, follow-up and postoperative results reporting or other important factors possibly influencing hearing preservation, such as intersurgeon or variability in soft surgery protocols used.

5. Conclusion

The results of this review demonstrate that the current available literature regarding the influence of different insertion methods on the degree of hearing preservation or loss is limited. The results confirm that randomized clinical trials, in which possible confounding factors, such as electrode array and the use of corticosteroids, are properly controlled for are required to determine the actual difference in clinical outcome between both surgical approaches. As yet, there are no valid data to support any statement on the clinical superiority of either approach.

Appendix

(Residual hearing OR low frequency hearing OR Residual acoustic hearing OR Partial deaf* OR High frequency deaf* OR High frequency hearing OR sloping hearing OR sloping high frequency OR partial hearing) AND (Round window OR cochleostomy OR cochleotomies OR cochleotomized OR Soft surgery OR Atraumatic OR Less traumatic OR Hearing preservation surgery OR hearing preservation surgical OR hearing preservation technique OR hearing preserving OR electroacoustic OR Electric acoustic stimulation OR EAS OR hybrid s OR hybrid l OR hybrid implant OR hybrid cochlear implant OR hybrid cochlear implantation OR Hybrid CI OR Nucleus Hybrid OR short electrode OR medium electrode OR flex electrode OR Iowa/ Nucleus OR combined electric acoustic OR combined electro acoustic stimulation OR DUET OR Cochleostomies OR Cochleostomized OR flexEAS OR Hybrid-L24 OR [soft AND surg*])

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Chapter 7

Retrospective study on residual hearing and speech perception in patients with low-frequency residual hearing after cochlear implantation

Sarah Havenith, Gijsbert A. van Zanten, Vedat Topsakal

Abstract

Objectives/Hypothesis: There still is much debate about the preservation of residual hearing in patients with low-frequency residual hearing fitted with a cochlear implant. In view of the ongoing debate on the optimal procedure to preserve residual hearing we present a descriptive overview of our postoperative results in implantees with residual hearing. Also we investigated possible predictors for outcomes on residual hearing and implantees speech perception performance.

Patients: Thirteen adult subjects with residual hearing were included. Patients underwent cochlear implantation surgery with an attempt to preserve residual low-frequency hearing.

Main outcome measures. Postoperative hearing preservation and speech perception. Preoperative residual hearing thresholds were analyzed as a possible predictor for postoperative hearing preservation. Also known predictors for speech perception performance (duration of deafness and preoperative speech perception scores) were assessed. Finally correlations between hearing preservation and speech perception scores were calculated.

Results: The mean low-frequency pure tone average (PTA) shift (at 125, 250 and 500 Hz) was 20dB at 2 months and 27dB at the last follow-up moment (2m-5y) after implantation. Further, 54% (7/13) of the patients in our study showed only minimal hearing preservation or complete loss of residual hearing at the last follow-up moment (2m-5y). Better preoperative residual hearing predicted better postoperative low-frequency hearing thresholds in our patient cohort at 2 months after surgery. This effect seems to persist at longer term. There was one poor performer with speech perception scores of ~15% up to three years after implantation. In the other patients the mean speech perception score was 79%. The degree of hearing preservation did not correlate with postoperative speech perception scores.

Conclusion: Hearing preservation rates were comparable with current literature. We identified the degree of preoperative residual hearing as a predictor for postoperative low-frequency residual hearing, which could be helpful in the preoperative counselling of EAS candidates. Also, the degree of hearing preservation did not correlate to postoperative speech perception scores.

1. Introduction

Since the start of cochlear implantation surgeons always paid attention not to unnecessarily harm anatomical structures. This was published by Lehnhardt (1993) as a set of rules for soft surgery. It made sense to protect every structure related or unrelated to the surgery goals because surgeons were used to this general rule of medicine. With the idea of simultaneous electric and acoustic stimulation in the same ear yet another reason was born to preserve as much structures as possible. This type of surgery aimed for combined electric and acoustic speech processing (EAS) in patients with low-frequency residual hearing.

In the recent past numerous research groups have explored various factors that may influence the degree of residual hearing preservation during cochlear implant (CI) surgery. Many factors are related to the surgical techniques aimed at preventing trauma to the inner ear. Much attention has been given to optimizing the electrode design (so called hybrid devices, less traumatic arrays, variable length, flexibility and thickness). Also a minimal traumatic opening of the cochlea is discussed (cochleostomy technique versus round window insertion) (e.g. Gstoettner et al. 2006; Gantz et al. 2009; Skarzynski et al. 2007, 2009; Lenarz et al. 2009). There is however no clear consensus in recent literature on this dilemma (e.g. Havenith et al. 2013). Another possible protective factor is the use of dexamethasone per(i)-operatively. The use of dexamethasone with the aim to preserve postoperative residual hearing is common clinical practice. However, no consensus is available on dosage, timing and routes of administration (locally in the middle ear and/or systemic delivery) of this drug (Rajan et al. 2012; Santa Maria et al. 2014; Causon et al. 2015). Several animal studies on this topic suggest a protective effect of dexamethasone applying it locally on the round window membrane, directly into the cochlea (mini-osmotic pump or coated implant devices) and/or systemically (i.e. Eshraghi et al. 2007; Ye et al. 2007; James et al. 2008; Vivero et al. 2008; Chang et al. 2009; Maini et al. 2009; Bas et al. 2016).

In cochlear implantation for the pre- or postlingually profoundly deaf patient well-known predictors for better speech perception performance are shorter duration of deafness and better preoperative speech perception scores (i.e. Lazard et al. 2012; Blamey et al. 2013; Kraaijenga et al. 2016). For several other predictors results vary. In postlingually deafened cochlear implant recipients preoperative residual hearing of the implanted ear might be a predictor for speech perception performance (van Dijk et al. 1999; Roditi et al. 2009), however Green et al. (2007) and Lazard et al. (2012) did not find this variable to be an independent predictor. We studied the degree of postoperative hearing preservation and speech perception scores of our own series with one specific implant type and one surgeon. Further we analyzed whether known variables predicted postoperative speech perception performance in this cohort of EAS candidates. Also, we were interested if preoperative residual hearing predicted the level of postoperative residual hearing with improving patients counselling as a goal.

2. Methods

2.1. Study design

Retrospective cohort study at a tertiary academic referral center.

2.2. Patients

We included all patients with low-frequency residual hearing fitted with a Med-El hybrid cochlear implant device (Flex^{EAS} or Flex^{24} electrode) implanted in our center in the period 2009 up till 2014 . All patients were counseled for hearing preservation goals and were informed about the possible loss of this residual hearing postoperatively. Exclusion criteria were age at implantation <18 years and deafness caused by meningitis.

2.3. Hearing assessments

Patients with a preoperative pure tone average (PTA; 125, 250 and 500 Hz) <80 dB HL were considered candidates for electroacoustic stimulation. The preoperative pure tone thresholds of individual patients included in this study are shown in Figure 1. Pure tone thresholds were available from 15 years before implantation up to 5 years after implantation, varying among patients. Low-frequency residual hearing was defined as the PTA at 125, 250 and 500 Hz (PTAlow). Most studies on residual hearing reported on these frequencies, which makes our results comparable to literature. However, for the analysis of the natural course of the low-frequency hearing in our patients we used mean thresholds at 250, 500 and 1000 Hz, since thresholds at 125 Hz were missing at several time points in most patients.

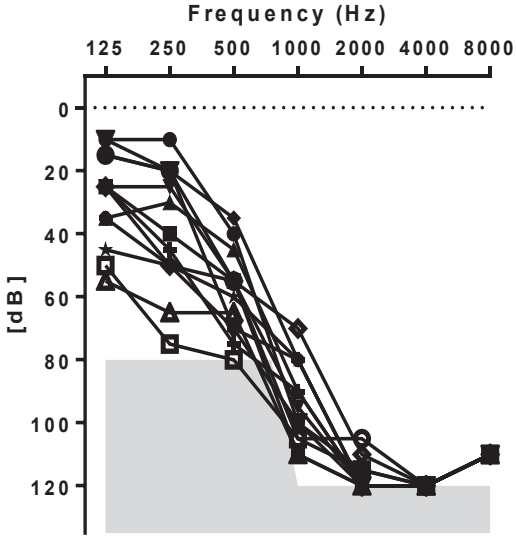


Fig. 1. Individual pure tone thresholds (n=13). Grey area indicates cut-off threshold at each frequency used as selection criteria for electroacoustic stimulation (EAS). Patients with a low-frequency PTA (125, 250 and 500 Hz) <80dB were EAS candidates.

Further, speech perception scores were obtained using the Dutch Society of Audiology standard consonant-vowel-consonant (CVC) word list at 65 dB SPL (Versfeld et al., 2000). In this auditory-only presented open-set test, speech perception was scored as the percentage of phonemes and words correct. In this study we reported on the percentage of words correct. We reported on the actual-user-condition in each patient at each time point, in order to reflect the performance status of the patient most accurately. In most patients this was the CI-only condition in the operated ear with the use of a contralateral hearing aid (bimodal condition, see table 1). Four out of thirteen patients were EAS users, in which we reported on the best-aided condition (cochlear implant and hearing aids in both ears).

2.4. Surgery

All implantations were performed by the same surgeon (VT). The retro-auricular incision with a cortical mastoïdectomy and posterior tympanotomy was performed in all patients. Electrodes were inserted via the round window. In one case it seemed impossible to get access through the round window and insertion was converted to the cochleostomy approach (antero-inferiorly). When given, intratympanic dexamethasone (4mg/ml, 1ml solution) was administrated after drilling the posterior tympanotomy and uncovering the round window membrane by removing its bony overhang. Subsequently the implant bed was prepared and the estimated time for intratympanic dexamethasone exposure was approximately 15 minutes. Then the corticoid solution was sucked away and the round window membrane was opened with a linear slit. A lubricant (hyaluronic acid) was always used during slow insertion. Further, in a subgroup of patients, 60 mg of prednisone was given daily during the first week after implantation. The per(i)operative treatment with corticosteroids differed between patients, this is outlined in Table 1.

2.5. Data processing

2.5.1. Hearing preservation calculation

We calculated the degree of hearing preservation in individual patients. For calculating the percentage of hearing preservation (HP) we used an equivalent of the equation described by Skarzynski et al. (2013):

HP = (PTAmdh- PTApost) / (PTAmdh- PTApre) * 100

The PTA refers to pure tone average; we calculated pre- and postoperative pure tone thresholds at 250, 500 and 1000 Hz, since thresholds at 125 Hz were missing at several time points in most patients. The percentage of hearing preservation is related to the maximal detectable hearing levels (PTAmdh). We applied the maximal detectable hearing levels as measured in our clinic, which differ slightly from those described by Skarzynski et al. (2013). Hearing preservation rates are reported on relative to the time of implantation, with the preoperative PTA at time point 0 set at 100% in each patient.

Table 1.

	Patient characteristics						Operation technique		CI characteristics		
	Age CI	Cause of deafness	Age onset of deafness	Duration of deafness	Gender	Ear	Insertion technique	Corticosteroïd treatment*	Electrode	EAS user	HA contralateral
	(y)		(y)	(y)	M/F	AD/AS	RW / C	Y/N		Y/N	Y/N
1	77	progressive fam	58	19	F	AS	RW	N	Flex ^{EAS}	Y	Y
2	45	progressive fam	4	41	F	AD	RW	N	Flex ^{EAS}	Y	Y
3	63	progressive fam	33	30	F	AS	RW	N	Flex ^{EAS}	N	Y
4	61	genetic	22	39	F	AS	RW	N	Flex ^{EAS}	N	Y
5	64	progressive fam	50	14	F	AS	RW	Y^1	Flex ^{EAS}	Y	Y
6	53	progressive fam	22	31	F	AD	RW	Y^1	$Flex^{24}$	N	Y
7	49	progressive fam	28	21	M	AS	RW	Y^1	$Flex^{24}$	N	Y
8	58	progressive fam	37	21	F	AD	RW	Y^1	$Flex^{24}$	N	Y
9	50	progressive fam	43	7	M	AS	RW	Y^2	$Flex^{24}$	N	N
10	73	progressive fam	60	13	M	AS	RW	Y^2	$Flex^{24}$	N	Y
11	59	progressive fam	30	29	F	AS	RW	Y^2	$Flex^{24}$	N	Y
12	82	progressive fam	60	22	M	AD	RW	Y^3	$Flex^{24}$	Y	Y
13	53	progressive fam	25	28	M	AD	C	Y^3	$Flex^{24}$	N	Y

Overview of patients characteristics, operation technique and cochlear implant characteristics for individual patients. * Corticosteroid treatment protocols: Y^1; 4 mg of dexamethasone intratympanically, Y^2; 4 mg of dexamethasone intratympanically and prednisone 60mg/day during 7 days systemically, Y^3; prednisone 60/day during 7 days systemically. CI= cochlear implant; AS=left ear; AD=right ear; RW=round window; C=cochleostomy; F=female; M=male. 2.5.2. Predictive value of preoperative data

2.5.2. Predictive value of preoperative data

We investigated whether there was a correlation between the degree of loss of residual pure tone hearing and speech perception performance. We reported on this possible correlation both at short-term (reporting on both residual hearing and word scores at 2 months after implantation) and long-term (reporting on residual hearing and word scores individually matched at 1 to 5 years after implantation). In the long-term analyses, for the four patients with mean postoperative low-frequency PTAs reaching the maximum detectable hearing levels (described above) we showed residual pure tone hearing at 2 months after implantation and speech perception word scores at their last FU moment.

Finally, several possible predictors on postoperative speech perception performance were assessed. For these analyses, we reported on speech perception word scores measured at the last follow up moment in each individual patient, ranging from 2 to 6 years after implantation.

We tested both known predictors; preoperative speech perception scores and duration of deafness. We also assessed whether preoperative residual hearing loss predicted postoperative residual hearing loss and/or speech perception performance.

2.6. Data Analyses

Statistical analyses were completed using SPSS version 22.0 software. Group differences were calculated with the nonparametric Mann–Whitney or Kruskal-Wallis tests for independent samples, and with the Wilcoxon-signed-ranks test for related samples. Linear regression analyses were performed to evaluate the predictive value of preoperative data.

3. Results

3.1. Patient data

A total of 13 patients were included in this study. The electronic patient file was consulted for each case (e.g. cause and age of deafness, gender, see table 1). Also, several characteristics regarding the operation technique and the cochlear implant device are outlined.

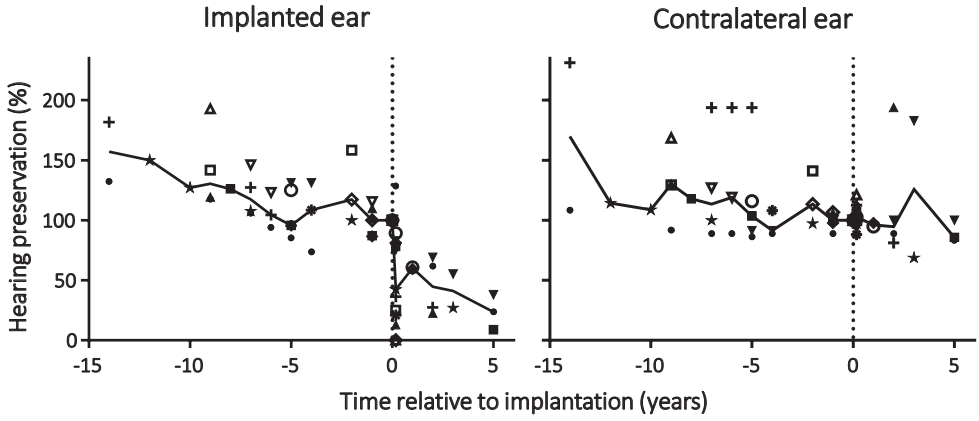
Pure tone thresholds were available up to 5 years after implantation. At longer term after implantation (ranging from 1 to 5 years) we had data from eight patients available. In 5 patients postoperative hearing thresholds were measured 2 months after implantation only. Four of these patients had mean low-frequency PTAs reaching the maximum detectable hearing levels in this frequency range. Since low-frequency hearing loss was already substantial in these patients (reaching the maximal detectable hearing level) and they did not use the acoustic part of the EAS device, this probably is the reason that residual hearing was not followed up over time. Because we did not expect these thresholds to improve over time, these patients were included in the long-term residual hearing analyses.

Speech perception scores were reported from the first fitting at ~1-3 months up to a final follow-up period of 2 to 6 years after implantation. There was one outlier with a poor speech perception performance. We excluded this patient in the evaluation of the predictive value of preoperative data.

3.2. Residual low-frequency hearing

Hearing thresholds were measured preoperatively and postoperatively at 2 months for each patient, up to a period 1 to 5 years for 8 out of 13 patients, see Figure 3. The mean low-frequency PTA shift (at 125, 250 and 500 Hz) was 20 dB at 2 months and 27 dB at the last follow-up moment (2m-5y) (data not shown).

Hearing preservation (HP) rates were calculated using an equivalent of the equation described by Skarzynski et al. (2013), as mentioned above. They were categorized as complete HP (>75 %); partial HP (25-75 %); minimal HP (1-25 %); and complete loss of hearing (0 %),



	Short term (2 m)	Last FU **(2-60 m)**
Complete HP	6 (46%)	1 (7%)
Partial HP	2 (15%)	5 (39%)
Minimal HP	3 (24%)	5 (39%)
Loss of hearing	2 (15%)	2 (15%)

Fig. 2. Hearing preservation as % of preoperative thresholds at 250, 500 and 1000 Hz at different time points relative to pre-implantation thresholds at time 0 (n=13). Results for both the implanted (A) and contralateral (B) ears are shown. The dashed line indicates the time of implantation (at time point 0). Hearing preservation rates were calculated using an equivalent of the equation described by Skarzynski et al. (2013); HP %=(PTAmdh-PTApost) / (PTAmdh-PTApre)* 100. The connected line shows the median at each time point. PTAmdh indicate maximal detection levels. Hearing preservation (HP) was categorized as complete HP (>75%); partial HP (25-75%); minimal HP (1-25%); and complete loss of hearing (0%).

see Figure 3. The degree of hearing preservation at 2 months ranged from a total loss of hearing in 2 patients (15 %) to complete HP in 6 patients (46 %). In eight patients audiological follow-up was performed at 1 to 5 years after implantation. In seven of these patients the residual hearing declined over time and a small improvement of hearing thresholds was only seen in one patient at 2 years after implantation.

Also, figure 2 illustrates the natural course of the mean low-frequency hearing in both the implanted and the contralateral ears. Hearing thresholds decline in the period before implantation. This decline was worse in the implanted ears compared to the contralateral ears, respectively 20 dB and 10 dB, calculated individually between the initial follow-up moment relative to the moment of implantation (Wilcoxon Signed Ranks Test: p=0.01, data not shown). Further, figure 2A shows a median drop in hearing preservation rates shortly after implantation, after which hearing preservation rates continue to decline (measured up to 5 years after implantation). In the contralateral ears hearing preservation rates show a slow continuous decline (Fig. 2B).

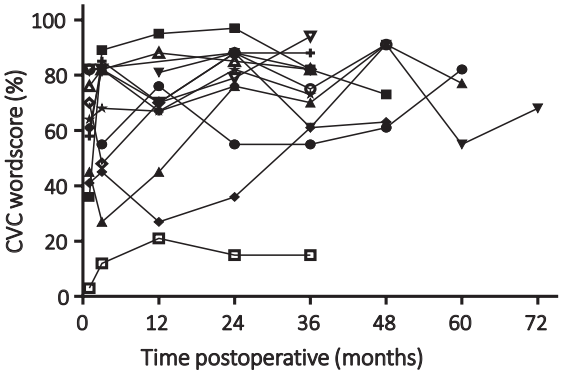


Fig. 3. Individual speech perception word-scores (%) after implantation (n=13). CVC= consonant-vowel-consonant. Actual condition used by the patient is reported.

3.3. Postoperative speech perception

Individual speech perception word scores over time are shown in Figure 3. There is one poor performer with speech perception word scores of ~15 % up to three years after implantation. In the other twelve patients the mean speech perception word score at the individual last follow-up moment was 79 %.

3.4. Predicting postoperative low-frequency residual hearing

Figure 4A shows a strong positive correlation between the postoperative and the preoperative PTAlow at 2 months after implantation (linear regression; p=0.001). Patients with better preoperative residual hearing also have a better postoperative PTAlow. At later time points

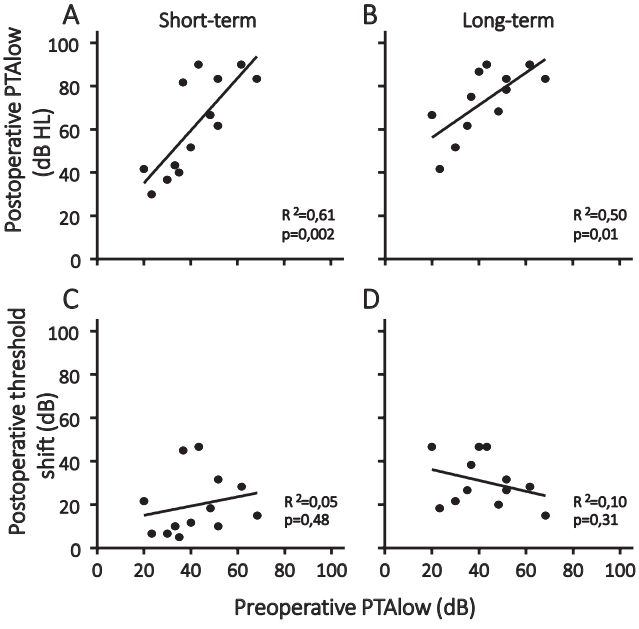


Fig. 4. A, B: Preoperative PTAlow at 125, 250 and 500 Hz, as a predictor for the postoperative PTAlow at 2 months (n=13) or 1-5 years (n=12) after implantation. In the latter group 2 months data from 4 patients were included, with postoperative PTAlow levels reaching the maximum hearing level. C,D: preoperative PTA low versus absolute postoperative threshold shifts. Results of linear regression analyses are shown.

after implantation (ranging from 1 to 5 years and including the 4 patients with little to no residual hearing at 2 months), this correlation is still present (Figure 4B, linear regression; p= 0.01).

However, absolute threshold shifts were not related to the preoperative PTAlow (Figure 4C,D, linear regression; ns). At 2 months after surgery median PTAlow thresholds for the patients with a preoperative PTAlow between 20-40 dB was 10 dB versus 23 dB in patients with a preoperative PTAlow between 40-70 dB, this difference was however non-significant (Mann-Whitney test, p= 0.10). At the last follow-up moment absolute threshold shifts were comparable for these groups, respectively 27 and 28 dB (Mann-Whitney test, p= 0.94). To conclude, absolute threshold shifts were not smaller in patients with better preoperative residual hearing.

3.5. Predicting postoperative speech perception scores

We found no correlation between the postoperative words scores and postoperative low-frequency hearing thresholds at both short- (2 months) or long-term (1 to 5 years) after implantation (Figure 5A, B).

Further, the postoperative word scores did not correlate to the preoperative low-frequency residual hearing (Figure 6A). Other known predictors for cochlear implant performance, i.e. preoperative speech perception scores and duration of deafness, also did not correlate either with postoperative word scores in our patient cohort (see Figure 6B, C).

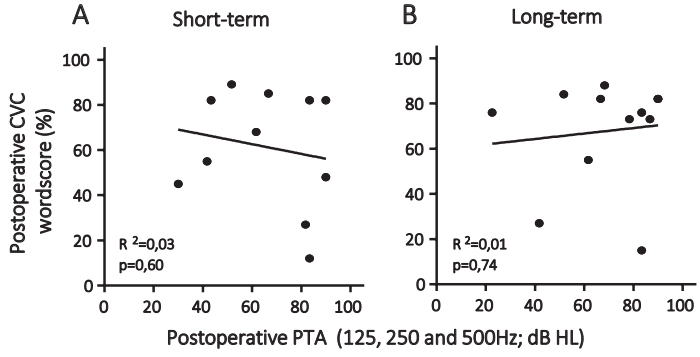


Fig. 5. Correlation postoperative PTAlow. A: at short-term (2 months after implantation, (n=11). B: at long-term (matched at 1 to 5 years after implantation, (n=12). For the long-term analysis patients with almost complete low-frequency hearing loss at 2 months after

3.6. Postoperative residual hearing loss comparing different dexamethasone treatments

Figure 7 shows the individual degrees of hearing preservation related to the dexamethasone treatment (peri-)operatively. We found no protective effect of dexamethasone on the postoperative residual hearing comparing these groups (Kruskal-Wallis test, p= 0.67), nor comparing any dexamethasone treatment versus no dexamethasone treatment (Mann-Whitney test, p= 0.39).

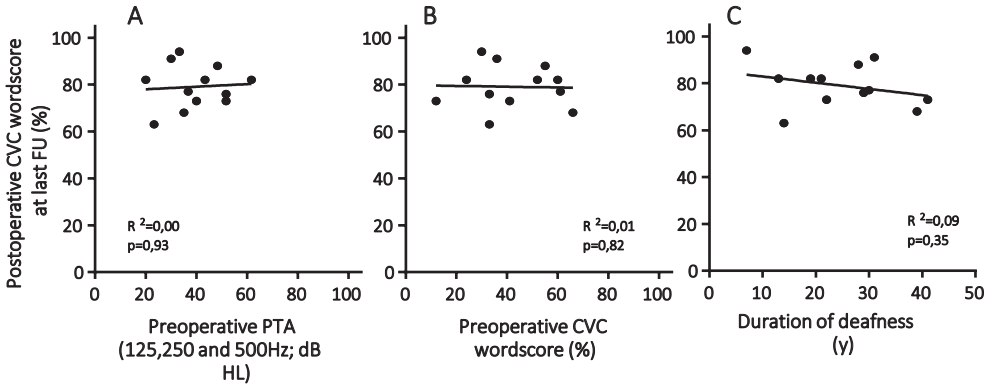


Fig. 6. A, B and C: Assessment of three possible predictors for cochlear implant performance (n=12). One patient with poor performance was excluded. Postoperative CVC wordscore at last follow-up range from 2 to 6 years. Dots show individual data points. Results of linear regression analyses are shown.

3.7. Hearing preservation and EAS users

Table 1 shows that in our cohort about one third of all patients (4 out of 13) use the acoustic stimulation in the implanted ear. All of these patients had mean low-frequency thresholds better than 80 dB at the last follow-up moment (2m-5y). However, in the group non-EAS users also 5 out of 9 patients had postoperative low-frequency residual hearing better than 80dB, and thus fitted the criteria for EAS. After a trial, these patients did not opt for acoustic stimulation in the implanted ear, but used the bimodal condition with a hearing aid in the contralateral ear. The mean degree of hearing preservation at the last follow-up moment in the EAS users was 41 % versus 30 % in the non-users group (Mann-Whitney Test, p= 0.27). Word recognition scores at individual last follow-up moments were comparable in both groups (75 % in the EAS-users versus 72 % in the non-users).

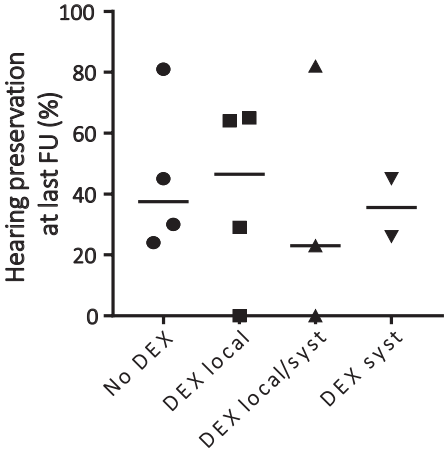


Fig. 7. Individual hearing preservation rates at the last follow-up moment ranging from 2 months to 5 years after implantation for each animal grouped for type of dexamethasone treatment. No DEX: not given, DEX local: 4 mg intratympanically (IT), DEX local/syst: 4 mg IT and 60mg/dy during 1 wk postoperatively (PO), DEX syst: 60mg/dy during 1 wk PO. Kruskal-Wallis test, p=0,67, no differences between the groups.

4. Discussion

This retrospective study provides a descriptive overview of the hearing preservation results in thirteen cochlear implant recipients with low-frequency residual hearing. The mean low-frequency PTA shift (at 125, 250 and 500 Hz) was 20 dB at 2 months and 27 dB at the last follow-up moment (2m-5y) in this patient cohort. At the last follow-up moment, ranging from 2 months to 5 years, 46 % of the patients had complete or partial hearing preservation rates. Preoperative residual hearing was identified as a predictor for residual hearing outcome, not for postoperative speech perception scores. Better preoperative residual hearing predicted better postoperative low-frequency hearing at 2 months after surgery. This effect seems to persist at longer term. Finally, speech perception scores did not correlate with postoperative residual hearing .

4.1. Degree of hearing preservation

The mean PTAlow shifts of 20 dB at 2 months and 27 dB at the last follow-up moment (2m-5y) in our patient cohort are comparable to other studies (Havenith et al. 2013; mean PTAlow shifts of 15-27 dB, their figure 4). Further, 54 % (7/13) of the patients in our study showed (virtually) no hearing preservation at the last follow-up moment ranging from 2 months to 5 years. Long-term results in literature are 57 % (Helbig et al. 2016), 50 % (Santa Maria et al. 2014) and 28 % (Mertens et al. 2014). Helbig et al. (2016) investigated the long-term effect on hearing preservation and reported that mean postoperative low-frequency threshold shifts did not significantly change over time, measured beyond 24 months after surgery. They discuss that in cases with a total hearing loss this loss may occur more or less 'sudden'. Mertens et al. (2014) however describe a continuous decline of residual hearing measured from the first fitting up to 6 years postoperative. Both cases of total hearing loss in our group of patients were already measured at the first follow-up moment, at 2 months after implantation, after which they were not tested again. Figure 2 shows changes in individual hearing preservation rates over time, with further decline in 7 patients and show a minor improvement in only 1 patient, suggesting an overall decline of the hearing preservation rates at the long-term as described by Mertens et al. (2014). In figure 2, the natural course of the low-frequency hearing shows a decline over years, measured up to implantation. Therefore, the natural course of gradual decline in low-frequency hearing is likely to contribute to the residual hearing loss occurring during the years after surgery. In contrast, the sudden decline of residual hearing shortly after surgery is probably more likely related to insertion damage caused during and/or after implantation.

4.2. Predicting hearing preservation outcome

We found no differences in the groups regarding per(i)-operative treatment with or without dexamethasone (see Figure 7). Since the different treatment regimens of dexamethasone

and/or prednisone were not based on clear preoperative criteria, we cannot conclude on its effectivity based on these results. Also, because of the small cohort of patients and small diversity regarding the surgical techniques used we did not include all possible predictive factors regarding residual hearing preservation as discussed in literature (i.e. insertion technique, cause of deafness, surgical techniques used). The reports in literature on possible predictors are not consistent, for example, regarding cochleostomy or round window insertion (Havenith et al. 2013; Andunka et al. 2014; Santa Maria et al. 2014; Causon et al. 2015). Besides the above mentioned per(i)-operative related variables we assessed whether preoperative residual hearing was a predictor for postoperative residual hearing (see Figure 4). This is, to our knowledge, not reported on in other studies. We found a positive correlation between the preoperative low-frequency hearing and postoperative low-frequency hearing; better postoperative thresholds in patients with better residual hearing before surgery. It could be that better preoperative hearing indicates more cochlear reserve or capability to resist to insertion trauma. However, the latter seems not the case since the preoperative PTAlow did not correlate with absolute threshold shifts after implantation (Figures 4C, D).

4.3. Correlation speech perception scores and postoperative residual hearing

Besides our aim at finding possible predictors for postoperative residual hearing, which can be used in the preoperative counselling of patients, we were also interested in the performance status of the patients. There was only one poor performer with speech perception word scores of ~15 % up to three years after implantation. This patient had the lowest preoperative speech perception score of 3 %, limited low-frequency residual hearing (PTAlow of 68 dB) and depended on lipreading. Imaging showed signs for fenestral otosclerosis, however without cochlear involvement. Likely, this poor performance may be caused by other pathology than hair cell loss. In the other patients the mean speech perception score at the individual last follow-up moment was 79 %.

The 2 patients which lost their residual hearing had excellent speech perception scores of both 82 % measured up to 3 years after implantation. Hence, we found no correlation between the postoperative low-frequency residual hearing and speech perception scores at short or long-term after implantation (see Figure 6). This raises the question of the actual importance of preserving residual hearing on cochlear implant performance.

Helbig et al. (2016) studied long-term hearing preservation outcomes up to 11 years in 96 patients, 103 ears, with partial deafness. They also found that long-term monosyllable recognition scores were successful regardless of the loss of residual hearing (complete HP 70 %; partial HP 75 %; minimal HP 75 % and total loss of hearing 65 %). However, Gifford et al. (2013) presented the results of 39 patients with preserved low-frequency hearing implanted with electrodes with insertion depths varying from 10 to 31 mm. They did find that preserved low-frequency hearing improves speech understanding. Further, O'Connell et al. (2017) found a positive correlation between greater angular insertion depths and better

word recognition scores. Deeper insertions were, however, associated with worse short-term hearing preservation. They discuss the clinical dilemma of whether to implant a shorter electrode and preserve more residual hearing or to implant a longer device with may have the advantages for better hearing performance. However, other studies have shown that implanting a longer electrode (with the advantage on hearing outcome) did not necessarily cause worse hearing preservation (Punte et al. 2010; Havenith et al. 2013, see their table 3). Regarding the depth of implantation and choice of electrode type, Landsberger et al. (2016) suggested that temporal coding with a cochlear implant is optimally provided by electrodes placed deeper into the cochlea, e.g. well into the second cochlear turn. Since cochlear dimensions differ between patients (Escudé et al. 2006; Erixon et al. 2009), Tamir et al. (2012) discussed that the actual electrode lengths divided as short or long should be nuanced and related to the actual cochlear size in each individual patient. Hence, the individual choice of the most optimal electrode can be assessed preoperatively by means of imaging, which is already common practice in some cochlear implant centers. Therefore, deep insertion is not a threat to hearing preservation, indeed it is thought to improve speech recognition scores (i.e. Landsberger et al. 2016; O'Connell et al. 2017).

In our cohort of EAS candidates, 69 % fitted the criteria for EAS (9/13), of which half of them were actual EAS users (4/13). Since this is only a small cohort with little variance in patient characteristics (see Table 1) we cannot account for any variables which may predict which patient fitting the criteria for EAS will actually benefit from it. A possible explanation for little to no benefit of combined electric-acoustic stimulation in half of our patients might be suboptimal programming and fitting of the EAS device. Finally, perhaps not the postoperative residual hearing but other factors as the etiology of the hearing loss are of greater influence in predicting the benefit of EAS.

5. Conclusion

We presented the data of 13 patients fitted with a MED-EL-Flex Hybrid cochlear implant device in our center. Results showed a comparable degree of low-frequency hearing preservation with current literature, with complete or partial hearing preservation in 46% of the patients at the last follow-up moment (ranging from 2 months to 5 years after implantation). Further, we identified the degree of preoperative residual hearing as a predictor for residual hearing preservation; better preoperative residual hearing predicted better postoperative residual hearing. This can be helpful in the preoperative counselling. Finally, speech perception scores did not correlate with the degree of hearing preservation, which raises the question of the actual importance of hearing preservation for postoperative speech perception.

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Chapter 8

General discussion

1. Introduction

Disabling hearing loss is an important health issue worldwide. Exact numbers of hearing disability are not available for the Netherlands. In 2011 about 800.000 people visited the general practice because of hearing loss, which is thought to be an underestimation (Gommer et al., 2013). For specific subpopulations, i.e. adolescents and elderly, this number is estimated to grow. There are indications that the prevalence of noise induced hearing loss increases in adolescents due to the increased exposure to potentially damaging sound levels (Shargorodsky et al., 2010). Also, the prevalence of age related SNHL is expected to increase due to aging (due to both an increase of the percentage of elderly in the population and the increase of life expectancy).

Annually, ~450 patients with severe to profound SNHL receive a cochlear implant in the Netherlands. This number is expected to grow the following years. Due to a generally improving hearing performance of CI users, the criteria for cochlear implantation are expanded. Patients with a substantial degree of residual hearing are implanted nowadays. Protection of cochlear structures is regarded of general importance, for both CI recipients with or without residual hearing, with as goal to improve cochlear implant hearing performance. Therefore, much attention is given to optimize surgical strategies. Some of these strategies are common clinical practice and are assumed to improve the outcome in cochlear implant patients. In this thesis we investigated the effect of some of these strategies on a biological level (protection of the auditory nerve in guinea pigs) and a clinical level (residual hearing outcome and hearing performance in cochlear implant patients). On the one hand, this thesis looks back and evaluates outcome variables in patients, thereby contributing to a scientific basis of the best clinical practice. On the other hand, animal research providing more insight on the actual effect of treatments on the auditory nerve contributes to improvement of current strategies used in patients and the development of clinical applications in the future.

2. Otoprotective drugs: from animal research to clinical applicability

2.1. Neurotrophic factors

In **chapters 2 and 3** we have examined if round window membrane application of absorbable gelatin sponge (gelfoam) infiltrated with a neurotrophin resulted in SGC survival in deafened guinea pigs. Two weeks after deafening, gelfoam cubes infiltrated with 6 µg of BDNF were deposited onto the round window membrane. We kept the round window membrane intact in the first experiment (**chapter 2**) and perforated it in the second experiment (**chapter 3**). The functionality of the auditory nerve was measured with electrically evoked auditory brainstem responses (eABRs). We found that local BDNF treatment enhanced the survival of SGCs in the most basal region of the cochlea up till four weeks in both experiments. In the

animals treated with BDNF on the perforated round window, eABR amplitudes were similar to normal-hearing control animals. However, local application on the intact round window did not show this effect of improved functionality of the auditory nerve. This difference seemed not be caused by the number of SGCs or round window stimulation properties of the electrode. It can be explained by a greater responsiveness of the SGCs.

When considering clinical application of BDNF, our local delivery method was safe and effective. In cochlear implant surgery the round window membrane is already opened and the BDNF-containing gelfoam can easily be placed before and/or after inserting the electrode array. Further, to possible enhance its effects and/or apply it for other indications the BDNF-containing gelfoam can be placed onto the round window through a tympanostomy opening in the outpatient clinic under local anesthesia. Comparable intratympanic drug delivery techniques are already performed routinely, for example in Ménière patients treated with glucocorticoids or gentamicin (e.g. Patel et al., 2016). Perforating the round window membrane before placement of the gelfoam is a greater challenge, since there is a risk of perilymph leakage (so called 'gusher') after perforating the round window membrane (observed during cochlear implantation; e.g. Kamogashira et al., 2017). It is however possible to perform it, in a specific subpopulation, under local anesthesia. Repeated applications seem not be necessary, because sustained preservation of SGCs was observed up till 8 weeks after cessation of the treatment (e.g. Ramekers et al., 2015).

Currently, animal research is performed on smaller neurotrophic factor molecules (small-molecule TrkB receptor agonists), which could possibly penetrate the round window membrane in a greater extent and therefor enhance their effectivity (Yu et al., 2013). Increasing the permeability may avoid the need to perforate the round window membrane. Another less invasive option is to infuse a neurotrophin solution into the middle ear space by means of an injection through the tympanic membrane preoperatively. However, intratympanic solutions are easily washed out of the middle ear through the Eustachian tube. Further, a limiting factor could be that the round window is covered due to a partial or complete pseudomembranous or bony obstruction that is seen in 20%-25% of the patients (Silverstein et al, 1997; Alzamil et al., 2000). However, then a part of the solution infused intratympanically may still enter the inner ear due to penetration through the oval window via the annular ligament of the stapes footplate (described by King et al., 2013; using gentamicin), or the gelfoam can be placed on the (perforated) stapes footplate. Substances placed on the oval window will enter the scala vestibuli instead of the scala tympani (as in round window application). The scala vestibuli is anatomically further away from the SGCs, the target cells for neurotrophin treatment. Because it is observed in preclinical research that substances cross readily between the scala vestibuli and scala tympani by passing through the spiral ligament, drugs may reach the SGCs by this route. However, since the surface area of diffusion via the oval window is smaller compared to the round window membrane the amount of drug entering by this route is thought to be smaller (Salt and Plontke, 2009). Therefore, the possible otoprotective

effect of neurotrophins by means of placement on the oval window is expected to be smaller compared to round window application.
Another strategy in delivering neurotrophic factors to the inner ear is to coat the electrode array with neurotrophic factor, providing a slow continuous release starting directly after implantation (Richardson et al., 2009). Combining both local intratympanic and intracochlear delivery methods may enhance its effects of protecting the auditory nerve, improving the number and integrity of SGCs, and therefore possibly improve cochlear implant hearing performance.
Nakagawa et al. (2014) placed a growth factor (IGF-1) containing hydrogel solution onto the round window niche in patients with sudden sensorineural deafness refractory to systemic steroids and found this treatment to be comparable to intratympanic corticosteroid therapy. Only self-limiting minor adverse effects occurred (e.g. otitis media), considering it to be safe therapy. Further, Bezdjian et al. (2016) reviewed the clinical use of neurotrophic factors. They are mainly used in neurodegenerative disorders (e.g., amyotrophic lateral sclerosis and Parkinson's disease) and in diabetic neuropathy. They were considered safe in 90% of the studies. This makes the translation from animal studies to clinical application promising for the near future.

2.2. Glucocorticoids

Treatment with glucocorticoids, e.g. dexamethasone, per(i)operatively is common practice in cochlear implant surgery, especially in patients with low-frequency residual hearing. It is an accepted treatment, among several others, to optimize hearing preservation in cochlear implant surgery. However, reports on its actual effectivity are contradictive and treatment protocols differ (in type of delivery, dosages and timing).
Concerning the type of delivery there is much clinical interest for dexamethasone-coated implant devices. Direct intracochlear delivery has thought to enhance its effectivity. A possible adverse effect of this delivery could be an increased risk of infection due to its immunosuppressive effects, which especially may be of importance in the case of severe damage to cochlear structures. The effect of intracochlear dexamethasone treatment using the cochlear implant device as a carrier is diverse in different animal studies regarding hearing results and fibrous tissue formation (Douchement et al., 2014; Stathophoulos et al., 2014; Liu et al., 2015; Astolfi et al., 2016; Bas et al., 2016; Wilk et al., 2016). In **chapter 4** we found no significant protective effect of intracochlear corticosteroid treatment using the electrode array as carrier device on hearing preservation (only a trend of an effect after 4 weeks at 16 kHz). Further, we found a suggestion for less fibrosis in the most basal region of the cochlea in the dexamethasone treated animals, however not significant. Therefore, we cannot conclude that dexamethasone treatment was effective in this study. Importantly, dexamethasone was safe when administered directly into the cochlea, especially of importance in the cases with severe destruction of cochlear structures. Other animal studies share this last conclu-

sion that direct intracochlear delivery is safe, with a follow-up up to 1 year postoperatively (Douchement et al., 2014; Stathophoulos et al., 2014; Liu et al., 2015; Astolfi et al., 2016; Bas et al., 2016; Wilk et al., 2016). Also, most studies agree that animals implanted with a dexamethasone coated device showed less fibrosis (Statophoulos et al., 2014; Astolfi et al., 2016; Wilk et al., 2016), and one study describe a correlation with lower impedances in these animals (Wilk et al., 2016).

In patients less fibrosis (and consequently lower electrode impedances) is thought to be favorable for cochlear implant hearing performance due to a more favorable electrode-tissue interface. There are however no clinical studies that described a positive correlation between lower impedance measurements and improved cochlear implant hearing performance. Also, recent temporal bone studies on cochlear implant recipients showed that word recognition scores were not significantly correlated with fibrous tissue alone (Kamakura and Nadol, 2016; Ishai et al., 2017). There was no correlation either of fibrous tissue formation with the degree of intracochlear insertion trauma (Kamakura and Nadol, 2016). Finally, the majority of the animal studies report better functional hearing preservation (4 out of 6 studies) at least at the higher frequencies. We only found a trend of this effect at 16 kHz.

In conclusion, glucocorticoid (dexamethasone) treatment in both animal and clinical studies for hearing preservation purpose in cochlear implantation has been shown to be safe. However, a placebo controlled clinical trial is necessary in the future since its effectivity is yet to be proven. Preserving residual hearing in patients has not yet been proven to improve speech perception performance in CI patients. Helbig et al., (2016) and O'Connell et al., (2017) both described successful speech recognition scores also in cases of minimal hearing preservation. Therefore, such studies should report on both residual hearing preservation and correlate this to the actual hearing performance with a CI (e.g. speech perception scores). Also, because preserving low-frequency residual hearing may be related to improved prosody elements of speech and/or music appreciation due to better pitch perception abilities (e.g. Gantz et al., 2005; Gfeller et al., 2006; Golub et al., 2012), these quality of hearing aspects are also clinically relevant to investigate.

2.3. Partial deafness animal model

As mentioned before, hearing preservation studies are especially of interest for patients with low-frequency residual hearing. Most animals studies on hearing preservation strategies are performed in normal-hearing or deafened animals. **Chapter 5** presents a guinea pig model of partial deafness, resembling cochlear implant candidates with low-frequency residual hearing. This may contribute in the future development of optimal electrical stimulation strategies in a partially degenerated nerve in animal studies. Further, neurotrophic treatment in the partially degenerated nerve may have a better structural effect (preventing SGC degeneration) or functional improvement compared with animal studies performed in deafened animals (as described in this thesis in chapter 2 and 3). Finally, intracochlear delivery of dexamethasone

in the case of both severe structural damage and an already partially degenerated nerve may induce infection to the cochlea.

2.4. Future research and the translation to the clinical practice

Glucocorticoids are already used in patients to reduce electrode insertion trauma caused by damaging intracochlear structures and/or preventing a foreign body reaction to the electrode. Glucocorticoids are thought to preserve residual hearing and reduce fibrous tissue formation with as goal to improve cochlear implant hearing performance. Clinical pilot studies have been already performed investigating the effect of glucocorticoid eluting devices on residual hearing and impedance measurements. The actual effect of this treatment on hearing performance with a CI would be very interesting and should be addressed in future clinical trials.

Neurotrophic factors have shown to prevent the degeneration process of SGCs in animal studies and also may improve the functionality of SGCs which may result in better cochlear implant performance. Future clinical application is of special interest in patients with poor CI hearing performance. Further research in identifying this patient group would give the opportunity to start neurotrophic factor treatment preoperatively to optimize the auditory nerve condition. Neurotrophic factors may also contribute to improving speech in noise, prosody elements of speech and music appreciation, of importance for all CI patients. Also, local application of neurotrophic factors as described in this thesis may reduce the auditory nerve degeneration process in other acquired sensorineural hearing losses (noise or aging induced hearing loss, sudden sensorineural hearing loss or ototoxicity) (Liberman and Kujawa, 2017). Since the prevalence of deafness induced by noise and/or aging is expected to grow, treatment with neurotrophic factors may be of great importance and can be part of the treatment in specific subpopulations with a high risk of developing severe to profound hearing disability.

For cochlear implant patients it could also be interesting to combine both drugs. Future research can be performed on delivering a combination of these otoprotective drugs to further optimize their effects. In vitro studies have already shown that neurite outgrowth by neurotrophic factors was not reduced when dexamethasone was given at the same time (Stöver et al., 2007).

3. Hearing preservation strategies: is current clinical practice best clinical practice?

In developing and applying novel strategies (e.g. otoprotective drugs, surgical techniques) one of the main goals is improving cochlear implant hearing performance in patients. Since implantation criteria are expanding due to improved hearing with a cochlear implant, these

strategies have shown to be effective. Up till now many aspects regarding the principles of the surgical technique used are thought to be of influence on residual hearing preservation: type of electrode, round window or cochleostomy insertion, the speed of insertion, the use of dexamethasone per(i)operatively. Regarding cochlear implantation each surgeon is trained to perform a specific technique, and due to individual experience and (technical) developments these are often adapted and refined over time. One of these developments is that round window insertion is thought to be less traumatic than inserting the electrode via a cochleostomy approach. In **chapter 6** we reviewed the evidence on the round window and the cochleostomy insertion techniques and compared their effects on postoperative residual hearing. The available data did not show benefit of one surgical approach over the other regarding the preservation of residual hearing. Two other meta-analysis on this subject showed conflicting results with either a benefit for hearing preservation for the cochleostomy (Santa Maria et al., 2014) or the round window group (Causon et al., 2015). The inclusion of a different subsets of patients (i.e. the inclusion of children and/or adults, electrode types) and other definitions of hearing preservation can partially explain these differences in outcome. The results also show that each surgeon has a strong preference for a certain type of insertion method over the other. It seems logical that performing this technique regularly and refining it can be of benefit for the results. However, it remains important for an otologist to be trained to master both techniques since the round window technique is not always possible in patients because of anatomical variations. The conflicting results confirm that randomized clinical trials, in which possible confounding factors, such as the surgeon, electrode array and the use of corticosteroids, are properly controlled for, are required to determine the actual difference in clinical outcome between both surgical approaches. As yet, there are no valid data to support any statement on the clinical superiority of either approach. As discussed above, also for the treatment with glucocorticoids in these patients, which are already common practice, there are conflicting results on their effectivity.

In **Chapter 7** we provided a descriptive overview of the residual hearing outcome and performance status of a cohort of thirteen patients with low-frequency hearing implanted with one specific electrode by one surgeon. Hearing outcome results were comparable to literature with mean low-frequency threshold shifts of 20 to 27 dB at respectively 2 months and the last follow-up moment (2 months to 5 years after implantation). Besides a single poor performer with speech perception scores of 15%, the other twelve patients had an excellent mean speech perception word scores at the individual last follow-up moment of 79%. The mean speech perception word score for the implanted postlingually profoundly deaf adult is~60% (e.g. Kraaijenga et al., 2015). Further, the speech perception performance did not correlate to the level of postoperative residual hearing, which is also described in other clinical studies (e.g. Helbig et al., 2016; O'Connell et al., 2017). This raises the question of the actual role of residual hearing preservation for better cochlear implant hearing performance. On the one hand, further refinement of more atraumatic surgical techniques and residual

hearing preservation are highly important since the SGCs of the auditory nerve are the direct target of possible otoprotective drug therapies (i.e. neurotrophic factors) in the future. Also, it may improve the electrode-nerve interface and together with technical developments may provide better cochlear implant hearing performance. On the other hand, there are probably other factors of influence on CI hearing performance in these patients (e.g. the etiology of deafness). It is of great importance in the future to be able to identify and predict the small group of poor performers and develop strategies to improve the preoperative counselling for and/or the hearing performance in these patients.

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Chapter 9

Summary in Dutch - Nederlandse samenvatting

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Curriculum Vitae

Slechthorendheid is een serieus gezondheidsprobleem wereldwijd. Verschillende factoren zoals toenemende blootstelling aan harde muziek en vergrijzing van de bevolking voorspellen een groei van het aantal mensen met invaliderend gehoorverlies. De meest voorkomende vorm van ernstig gehoorverlies wordt veroorzaakt door schade aan de zintuigcellen, de haarcellen, in de cochlea (het slakkenhuis). In het gezonde oor zetten deze haarcellen de geluidstrillingen die de cochlea bereiken om in elektrische signalen. Een aangeboren dysfunctie of secundaire schade van deze haarcellen (door bijvoorbeeld lawaai, schadelijke medicatie, infecties of veroudering) leidt tot ernstig gehoorverlies of zelfs volledige doofheid.

Een cochleair implantaat (CI) bevat een elektrodenbundel die in het slakkenhuis wordt aangebracht en rechtstreeks de gehoorzenuwcellen (zogenaamde spirale ganglion cellen, SGC's) elektrisch stimuleert. De conditie van de gehoorzenuw (het aantal overlevende SGC's) is cruciaal voor het functioneren van het CI; patiënten met meer SGC's lijken beter te functioneren met hun CI. In een gezonde cochlea produceren de haarcellen zogenaamde neurotrofe factoren welke deze SGC's in leven houden. Bij afwezigheid van of ernstige schade aan de haarcellen sterven de SGC's af. Het toedienen van deze neurotrofe factoren (bijvoorbeeld ***brain-derived neurotrophic factor***, BDNF) in het binnenoor van doofgemaakte cavia's beschermt de SGC's tegen afsterven. In de **hoofdstukken 2 en 3** wordt een mogelijk klinisch toepasbare manier beschreven om de neurotrofe factor BDNF toe te dienen. BDNF heeft een beschermend effect op een deel van de SGC populatie basaal in het slakkenhuis wanneer dit opgelost in gelfoam op het ronde venster van het slakkenhuis van doofgemaakte cavia's wordt geplaatst. Perforeren van het ronde venster voorafgaand aan de plaatsing van gelfoam met BDNF geeft naast het gehoorzenuw beschermende effect (behoud van SGC's) ook een functioneel beschermend effect, zoals de elektrofysiologische metingen aan de gehoorzenuw laten zien.

De gunstige gehoorresultaten met een CI hebben geleid tot een versoepeling van de indicaties voor het plaatsen van een CI. Ook patiënten met een nog aanwezig restgehoor voor lage tonen kunnen in aanmerking komen voor een CI. Voor deze patiënten is een strategie ontwikkeld waarbij de lage tonen aangeboden worden met een hoortoestel en de hoge tonen met het CI (gecombineerde elektrische en akoestische stimulatie*,* EAS). **Hoofdstuk 5** beschrijft een diermodel van de partieel gedegenereerde gehoorzenuw; cavia's met restgehoor voor lage tonen en ernstig gehoorverlies voor hoge tonen. Dit diermodel lijkt op de situatie bij patiënten met restgehoor in de lage tonen en kan gebruikt worden in toekomstig dierexperimenteel onderzoek naar het verder optimaliseren van de EAS strategie.

Het probleem is dat dit restgehoor (gedeeltelijk) verloren kan gaan na cochleaire implantatie chirurgie. Er wordt gedacht dat verschillende factoren een invloed kunnen hebben op het behoud van dit restgehoor: het type CI, de methode van insertie, de snelheid van insertie

en het gebruik van glucocorticoïden rondom de operatie. In **Hoofdstuk 4** wordt beschreven dat intracochleaire toediening van dexamethason (een glucocorticoïd) in normaalhorende cavia's, verwerkt als coating op het te implanteren gedeelte van de CI elektrode, veilig is maar geen significant gehoor beschermend effect heeft. Resultaten van glucocorticoïden behandeling in vergelijkbare dierexperimentele studies laten tegenstrijdige resultaten zien, welke variëren van geen tot een significant aanwezig gehoor beschermend effect. Deze toedieningsvorm is wel veilig gebleken in dierexperimenteel onderzoek. Klinische studies met deze met glucocorticoïd gecoate elektrodes toegepast in patiënten met restgehoor zijn al opgestart en zullen het mogelijk gehoor sparende en gehoorfunctie verbeterende effect met het CI aan kunnen tonen.

In **Hoofdstuk 6** wordt een literatuurstudie beschreven naar de resultaten van twee verschillende gangbare CI operatietechnieken en het effect hiervan op het postoperatieve restgehoor. Het direct inbrengen van de elektrodenbundel via het ronde venster van het slakkenhuis en het inbrengen via een cochleostomie (een gaatje dat in het slakkenhuis wordt geboord) werden vergeleken. De belangrijkste conclusie is dat beide insertietechnieken een vergelijkbare mate van verlies van het postoperatief restgehoor lieten zien. Echter, door de aanwezigheid van mogelijke uitkomst beïnvloedende variabelen in de geïncludeerde individuele studies, kan geen uitspraak gedaan worden over de eventuele superioriteit van een van de twee beschreven operatietechnieken.

Tot slot is **Hoofdstuk 7** een retrospectieve studie waarin de gehoorresultaten na cochleaire implantatie van 13 patiënten met een preoperatief restgehoor worden beschreven. Deze patiëntengroep functioneert goed met het CI en heeft een gemiddeld verlies van het restgehoor in de lage tonen van 20 tot 25 dB. Er was geen relatie tussen spraakverstaan met het CI en de mate van het verlies van het postoperatieve restgehoor. Concluderend is dit een patiëntengroep die goede gehoorresultaten laat zien met het CI, ook in de gevallen waarbij het restgehoor nagenoeg verloren gaat na de operatie.

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Curriculum Vitae

Sarah Havenith was born in Maastricht, the Netherlands, on May 20th, 1984. After graduating from high school (VWO, Thij College, Oldenzaal) in 2002, she started her studies in Pharmacy at Utrecht University. In 2003, she started Medical School at Utrecht University. In her final year of medical school, she spent six months at the Otorhinolaryngology, Head and Neck department of the Utrecht University Medical Center, supervised by Prof. Dr. W. Grolman. She graduated from medical school in 2009 and started her Ph.D project in 2010 at the Utrecht University Medical Center. Her Ph.D, that led to this thesis, was supervised by Prof. Dr. W. Grolman, Dr. S.F.L. Klis and Dr. H. Versnel. Sarah is a resident in Otorhinolaryngology, Head and Neck surgery, from February 2012, finishing in November 2017. During this residency, she also worked at the St. Antonius Hospital, Nieuwegein (supervised by Dr. M. Copper), Gelderse Vallei Hospital, Ede (supervised by Dr. M.H.J.M. Majoor) and Deventer Ziekenhuis (supervised by Dr. J. Buwalda).