Local drug delivery to the entire cochlea without breaching its boundaries

Andrei N. Lukashkin[1,2*], Ildar I. Sadreev[3], Natalia Zakharova[4], Ian J. Russell[1], Yury M. Yarin[5]

[1] Sensory Neuroscience Research Group, School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, UK

[2] Centre for Regenerative Medicine and Devices, University of Brighton, Brighton, UK

[3] Faculty of Medicine, Department of Medicine, Imperial College, London, UK

[4] Otophysica, Uckfield, UK

[5] ENT practice, Dresden, Germany

*Correspondence: Andrei N. Lukashkin, a.lukashkin@brighton.ac.uk

ABSTRACT

The mammalian cochlea is one of the least accessible organs for drug delivery. Systemic administration of many drugs, notably the most frequently used corticosteroids and aminoglycoside antibiotics, is severely limited by the blood-labyrinth barrier. Local intratympanic administration into the middle ear would be a preferable option in this case and the only option for many old and newly emerging classes of drugs and therapies including local anaesthetics, antioxidants, apoptosis inhibitors, neurotransmitters and their antagonists, monoclonal antibodies, growth factors, signalling pathway regulators and genetic material. Intratympanic administration of drugs relies on their remaining in contact with the cochlear round window membrane long enough to allow their diffusion into the cochlea fluid. The ability of drugs to pass through the round window does not, however, lead to their effective distribution along the long and narrow cochlear spiral. This slow technique leads to steady-state, base-to-apex concentration gradients that are orders of magnitude and well outside the therapeutic windows for many drugs. Here we present an efficient, quick, reliable and simple method that can consistently and uniformly deliver drugs along the entire length of the intact cochlea within minutes without disrupting cochlear boundaries. This novel method combines middle ear drug administration and cochlear pumping, through large amplitude, low-frequency reciprocal oscillations of the stapes and round window. Our preliminary experiments using salicylate as a model drug with well-established physiological effect demonstrate the exceptionally high efficiency of the method for drug delivery to the cochlear apex. In the short term, the presented method should lead to significant improvements in the efficacy and reliability of currently employed drugs for the treatment of sensorineural hearing loss,

Menière's disease, noise-induced hearing loss, tinnitus and autoimmune inner ear disease. In the longer term, the outcomes of our research should facilitate new and novel ways of approaching the treatment of inner ear disorders since we have overcome the challenge of delivering of therapeutics along the entire length of the cochlea.

INTRODUCTION

Reliable, efficient and uniform drug delivery into the cochlea remains an unsolved challenge and is a major barrier to the prevention or treatment of inner ear disorders. The mammalian cochlea is one of the least accessible organs for drug delivery (Salt and Plontke, 2009; Hao and Li, 2019). Systemic administration of many drugs, notably the most frequently used corticosteroids and aminoglycoside antibiotics, is severely limited by the blood-labyrinth barrier (Salt and Hirose, 2018; Nyberg et al., 2019). Direct injection into the cochlea is limited by the requirement for surgical interventions, and, significantly, does not guarantee uniform drug delivery along the cochlea.

There has been an increase in the number of therapeutic compounds with potential to treat inner ear disorders. This comprises old and newly emerging classes of drugs and therapies including corticosteroids, local anaesthetics, antioxidants, apoptosis inhibitors, neurotransmitters and their antagonists, monoclonal antibodies, growth factors, signalling pathway regulators and genetic material (Hao and Li, 2019; Devare et al., 2018). A recent review identified 43 biotech companies currently pursuing experimental compounds for inner ear therapy (Schilder et al., 2019). All such efforts depend on a technique allowing reliable delivery of such compounds uniformly along the entire length of the cochlea.

Intratympanic administration of drugs (Schuknecht, 1956; Patel et al., 2019; Rybak et al., 2019) relies on their remaining in contact with the round window membrane (RW, a membranous opening in the bony wall of the cochlear into the middle ear) long enough to enable their diffusion into the perilymph of the scala tympani (ST) (**Figure 1A**). The ability of drugs to pass through the RW does not, however, guarantee their effective distribution along the cochlear spiral. Drug distribution in the ST is limited by the low flow rate of perilymph within the cochlea (Ohyama et al., 1988) and is dominated by passive diffusion which is constrained by cochlear geometry (**Figure 1A**).

Combined direct measurements, morphological studies and computer modelling revealed that the difference in concentration of drugs between the cochlear base and apex was many orders

of magnitude when drugs entered the ST through the RW and diffused passively to the apex (Saijo and Kimura, 1984; Salt and Ma, 2001; Imamura and Adams, 2003; Mynatt et al., 2006; Plontke et al., 2007; Plontke et al., 2008; Grewal et al., 2013; Borkholder et al., 2014; Creber et al., 2018). Retention of drugs near the RW in the middle ear cavity for an arbitrary long time (Piu and Bishop, 2019), once thought to be a means of overcoming the large concentration gradients, does not decrease concentration gradients along the cochlea. It only stabilizes them (Sadreev et al., 2019). Here we show that the same characteristics of the cochlear geometry that restricts passive basal-apical diffusion, together with cochlear hydrodynamics, promotes the even distribution of substances along the entire cochlear length. This enables drugs to reach apical cochlear regions essential for communication and localization (Nuttall et al., 2018).

Methods

Animals

Animal preparation and signal generation and recording have been described elsewhere (Burwood et al., 2017). Briefly, pigmented guinea pigs of similar weight (350-360 g) and both sexes were anaesthetised with the neurolept anaesthetic technique (0.06 mg/kg body weight atropine sulphate s.c., 30 mg/kg pentobarbitone i.p., 500 µl/kg Hypnorm i.m.). Additional injections of Hypnorm were given every 40 minutes. Additional doses of pentobarbitone were administered as needed to maintain a non-reflexive state. The heart rate was monitored with a pair of skin electrodes placed on both sides of the thorax. The animals were tracheotomized and artificially respired with a mixture of O_2/CO_2, and their core temperature was maintained at 38°C with a heating blanket and a heated head holder. All procedures involving animals were performed in accordance with UK Home Office regulations with approval from the University of Brighton Animal Welfare and Ethical Review Body.

Signal generation and recording

The middle ear cavity of the ear used for the measurements and salicylate application was opened to reveal the RW. Compound action potentials (CAPs) of the auditory nerve in response to pure tone stimulation were measured from the cochlear bony ridge in the proximity of the RW membrane using Teflon-coated silver wire coupled to laboratory designed and built extracellular amplifier (James Hartley). Thresholds of the N1 peak of the CAP at different frequencies which corresponds to different distances from the cochlear base (Greenwood, 1990) were estimated visually using 10 ms pure tone stimuli at a repetition rate of 10 Hz.

For acoustic stimulation sound was delivered to the tympanic membrane by a closed acoustic system comprising two Bruel and Kjaer 4134 ½" microphones for delivering tones and a single Bruel and Kjaer 4133 ½" microphone for monitoring sound pressure at the tympanum. The microphones were coupled to the ear canal via 1 cm long, 4 mm diameter tubes to a conical speculum, the 1 mm diameter opening of which was placed about 1 mm from the tympanum. The speculum was sealed in the ear canal. The closed sound system was calibrated in situ for frequencies between 1 and 50 kHz. Known sound pressure levels were expressed in dB SPL re 2×10^{-5} Pa.

All acoustic stimuli in this work were shaped with raised cosines of 0.5 ms duration at the beginning and at the end of stimulation. White noise for acoustical calibration and tone sequences for auditory stimulation were synthesised by a Data Translation 3010 board at 250 kHz and delivered to the microphones through low-pass filters (100 kHz cut-off frequency). Signals from the acoustic measuring amplifier (James Hartley) were digitised at 250 kHz using the same board and averaged in the time domain. Experimental control, data acquisition and data analysis were performed using a PC with programmes written in MATLAB (MathWorks, MA).

Salicylate application

5 µl of 100 mM sodium salicylate solution in Hanks' Balanced Salt Solution were placed on the RW using pipettes. The solution was removed from the RW using paper wicks to observe the wash out effect.

Generation of pressure oscillations in the ear canal

A modified mouse ventilator MiniVent Type 845 (Hugo Sachs Elektronik, March, Germany) was used to generate oscillating air pressure in the ear canal. Output of the ventilator was connected and sealed to the closed acoustic system. The stroke frequency (4 Hz) and stroke volume (150 µl) were the same for the all experiments reported. The stroke volume was maximized to achieve maximum stapes displacement limited only by the crista stapedius.

Recording of stapes vibrations

Stapes vibrations were recorded using a CLV-2534 laser vibrometer (Polytec GmbH, Waldbronn, Germany). The laser beam was focussed onto the stapes head. The output voltage

123 from the vibrometer was low-pass filtered at 100 kHz, with a sensitivity of 5 mm/s/V. Stapes
124 displacement was found by integrating the velocity responses off-line.

125 **RESULTS**

126 When inaudible low-frequency air pressure oscillations are presented at the ear canal, they are
127 transmitted to the stapes which causes back and forth cochlear fluid movements through the
128 scala vestibuli (SV) and ST (red arrows in **Figure 1A**). The RW works as a pressure relief
129 valve during these movements and moves in counter phase with the stapes because the cochlear
130 bony wall and fluid are poorly compressible. The poor compressibility results in the fluid
131 volume velocity along the SV and ST to be the same. Consequently, fluid linear displacement
132 and velocity are much higher in the narrow apical parts of the scalae than at the base.
133 Specifically, the apical ST cross-sectional area in guinea pigs is almost 20 times smaller than
134 in the basal cochlear region (it is almost 6 times smaller in humans) (Thorne et al., 1999). This
135 proportional increase in the fluid displacement and velocity will facilitate distribution of a drug,
136 which originally diffuses through the RW and oval window into the cochlear base, along the
137 entire cochlea. Due to the small diameter of the cochlear scalae, the fluid flow along them is
138 dominated by fluid viscosity (i.e. it occurs at low Reynolds numbers). Thus, the fluid flow is
139 laminar and turbulent mixing will not contribute to uniform drug distribution. Chaotic
140 mixing/advection, both transversal and longitudinal, is, however, observed for laminar fluid
141 flows in helical pipes (Jones et al., 1989; Nguyen, 2011) which can be further facilitated by
142 periodic changes of the flow direction (Ottino and Wiggins, 2004). Therefore, under specific
143 condition of cochlear stimulation, the chaotic advection may well be a major factor contributing
144 to the mixing of drugs along the ST and SV, which resemble helical pipes (**Figure 1A**).

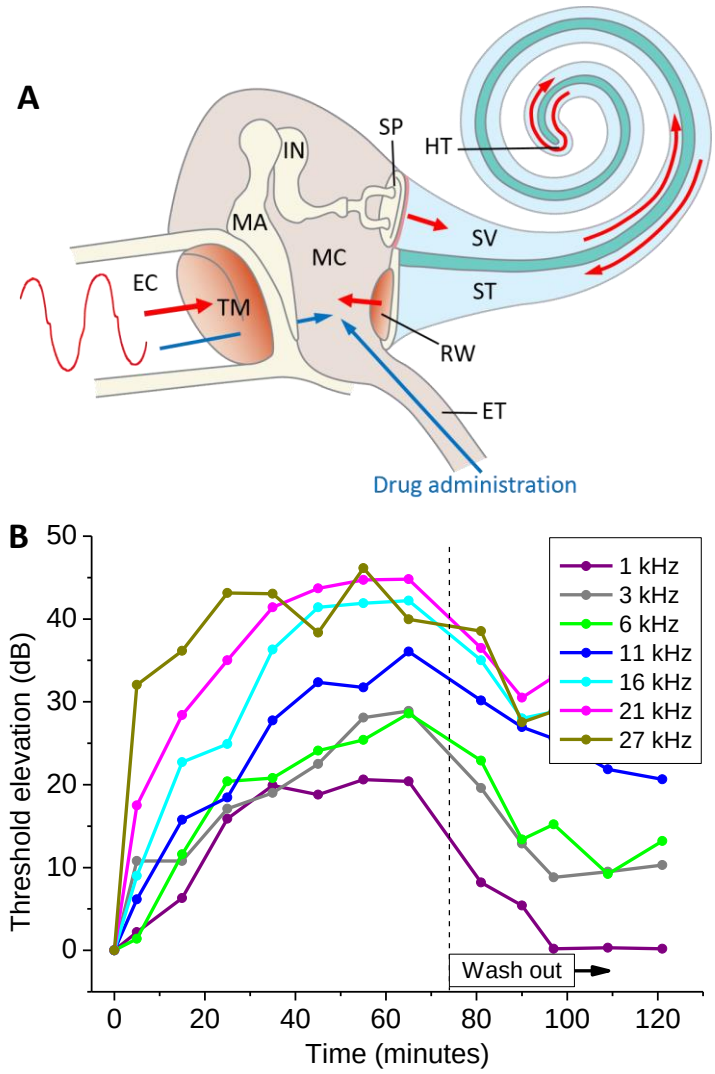

FIGURE 1. The principle of cochlear pumping **(A)** and its efficiency in drug distribution along the cochlea **(B)**. **(A)** Air pressure oscillations in the ear canal (EC) cause oscillations of the tympanic membrane (TM) which are transmitted to fluids of the cochlear spiral through the middle ear bones: maleus (MA), incus (IN) and stapes (SP). Resultant fluid oscillations in the scala vestibuli (SV) and scala tympani (ST) connected through the helicotrema (HT) at the cochlea apex are facilitated by the flexible round window membrane (RW). Blue arrows show two common routes of local drug administration into the middle ear cavity (MC): intratympanic, through the TM, and through the Eustachian tube (ET). Drugs are left in contact with the RW and the oval window below the stapes to diffuse into the ST and SV. **(B)** A representative example of a single preparation showing threshold response of the compound action potential of the auditory nerve after application of 100 mM salicylate solution to the RW at time zero and recovery of the thresholds after washing out of salicylate. Frequency of pure tone acoustic stimulation for eliciting the compound action potential is indicated in the figure legend for each curve.

145

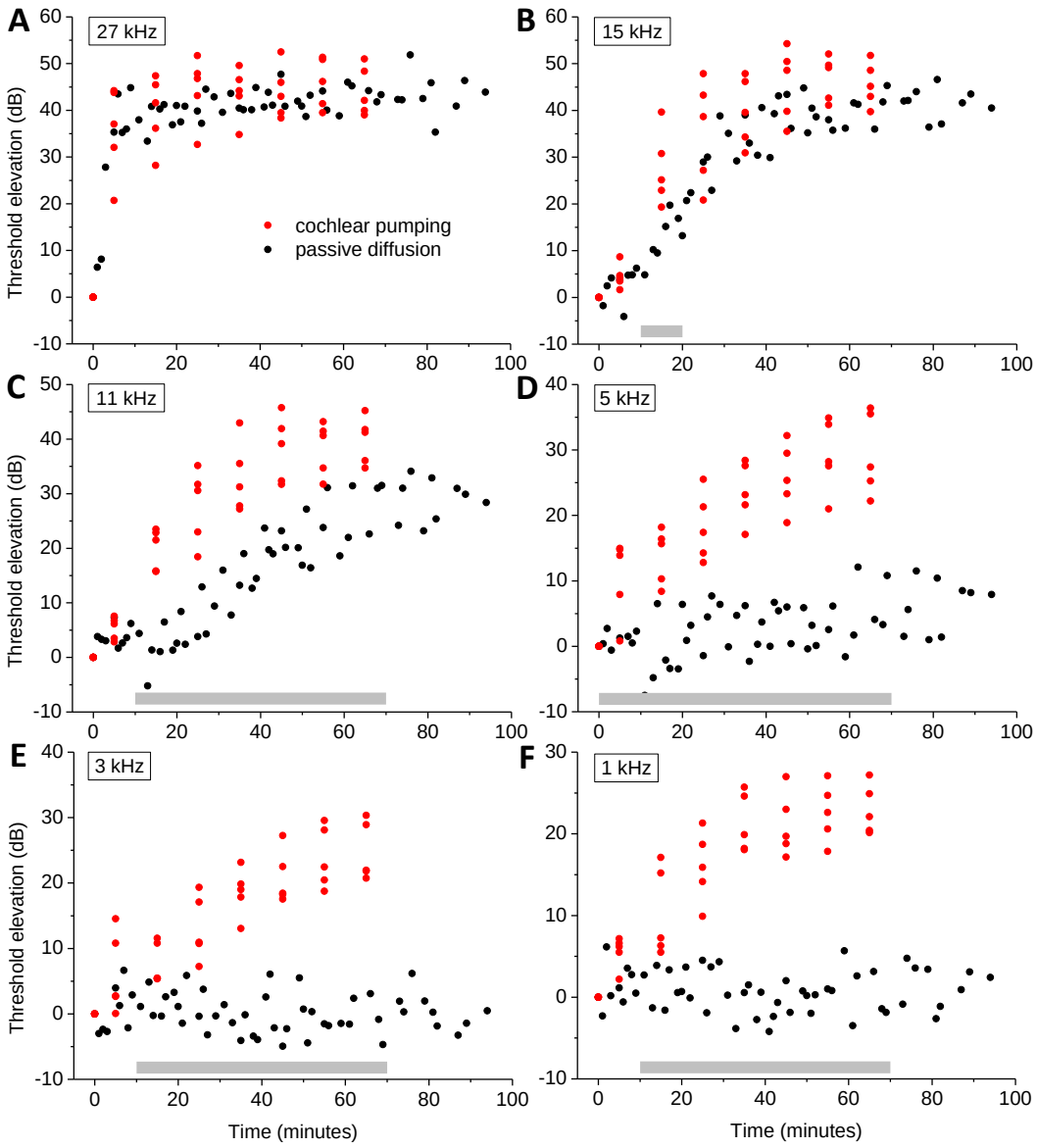

FIGURE 2. Comparison of the efficiency of the cochlear pumping technique and passive diffusion in the distribution of salicylate along the cochlea. Pooled data for experiments with cochlear pumping (red symbols, 5 preparations) and passive diffusion (black symbols, 5 preparations (Sadreev et al., 2019). Pressure oscillations at 4 Hz were applied to the ear canal during 5 minutes before each red non-zero time point plotted to cause large-amplitude (~50 μm) stapes movement and the CAP thresholds were measure during following 5-minute interval without pressure oscillation. Frequency of acoustic stimulation is indicated within each panel. 5 μl of 100 mM salicylate solution were applied to the RW at time zero. Grey lines near the horizontal axis indicate statistically significant ($p < 0.05$, unpaired t-test) differences between data for the cochlear pumping and passive diffusion within consecutive 10-minute intervals. Some of the passive diffusion data have been presented before (Sadreev et al., 2019).

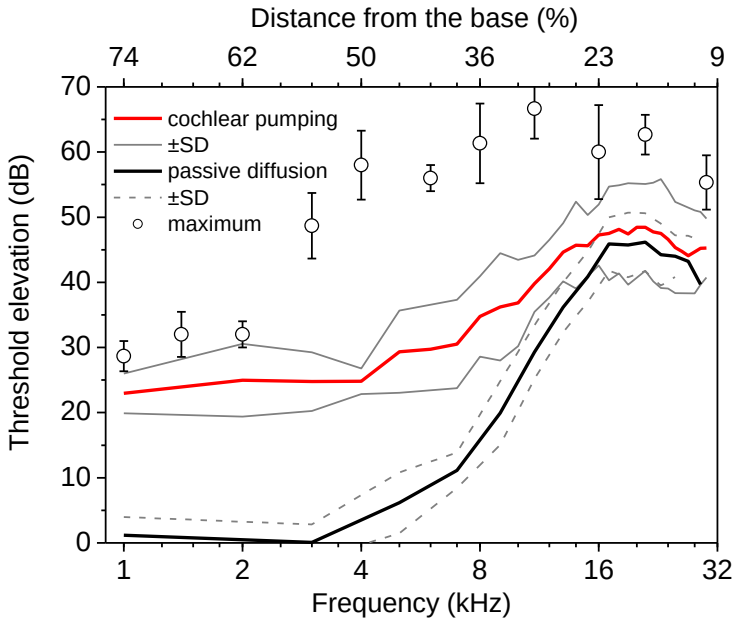

FIGURE 3. Frequency dependence of the CAP threshold elevation after 60 minutes of salicylate application during cochlear pumping (35 minutes of the total pumping time) (mean±SD, n=5) and its comparison with the frequency dependence for passive diffusion (mean±SD, n=5). Open circles show maximal increase of the CAP thresholds after complete block of the cochlear amplifier (Sadreev et al., 2019). Data for passive diffusion have been partially presented before (Sadreev et al., 2019).

The ability to distribute drugs uniformly along the entire cochlea spiral using relatively large, low-frequency periodic displacement of fluid in the ST and SV (**Figure 1A**) was demonstrated in our experiments with application of salicylate to the RW. Salicylate readily passes through the RW (Borkholder et al., 2014; Sadreev et al., 2019). To monitor salicylate diffusion along an intact guinea pig cochlea *in vivo*, we utilized the suppressive effect of salicylate on cochlear amplification via block of the outer hair cell somatic motility (Russell and Schauz, 1995; Hallworth, 1997). We measured elevation of the threshold response of the compound action potential (CAP) of the auditory nerve caused by salicylate at different frequencies, which, due to cochlear tonotopicity, corresponds to different distances from the RW (Greenwood, 1990). Salicylate did not cause elevation of the CAP threshold responses for frequencies below 5 kHz, which corresponds to about 45% of the total cochlear length from the base, when it diffused through the cochlea passively (Sadreev et al., 2019). The calculated gradient of base-to-apex salicylate concentration was about 13 orders of magnitude. When, however, placement of salicylate solution on the RW was followed by cochlear pumping, i.e. by 5-minute cycles of large-amplitude (80 μm peak-to-peak), low-frequency (4 Hz) stapes movements caused by

pressure oscillations in the ear canal, the CAP threshold was elevated throughout the entire 1 kHz - 30 kHz frequency range tested (**Figures 1B, 2A-F, 3**). This corresponds to about 75% of the total cochlear length from the base (Greenwood, 1990). The technique had no observable influence, compared to passive diffusion, on responses to RW salicylate application for locations close to the RW at the base of the cochlea (27 kHz, **Figure 2A**). Cochlear pumping, however, led to more rapid threshold elevation for locations which were distal and apical to the RW, even if the maximal threshold elevations were similar for both experimental paradigms (15 kHz, **Figure 2B**). The threshold elevation saturated after 4-5 cycles of stimulation even for the low frequencies of the most apical locations (**Figure 1B, 2E-F**) where passive diffusion produced no effect. Smaller threshold elevations at low frequencies were due only to the reduced contribution of cochlear amplification to cochlear responses at these frequencies (Sadreev et al., 2019, Robles and Ruggero, 2001) and, in fact, reached almost maximal possible elevations for those frequencies (**Figure 3**).

DISCUSSION

Cochlea pumping at 4 Hz was chosen because the helicotrema (an opening between the ST and SV at the cochlear apex, HT in **Figure 1A**) shunts fluid pressure at 4 Hz preventing overstimulation of the cochlear sensory apparatus. *In our control experiments, 4 Hz large-amplitude stimulation used alone, without salicylate, did not cause any elevation of the CAP threshold during the same protocol of stimulation and, in fact, during continuous CP at 4 kHz for 20 minutes.* Partial recovery of the CAP thresholds after washing out salicylate from the RW (**Figure 1B**) provided confirmation that the integrity of the sensory cells was preserved.

A stapes displacement of 80 μm in our experiments corresponds to 1.6 mm linear displacement of the fluid in the apical parts of ST, because in guinea pigs the apical ST cross-sectional area is almost 20 times smaller (Thorne et al., 1999) than the stapes area (Sim et al., 2013) and both the cochlear bony wall and fluid are poorly compressible. Therefore, while salicylate effect in the most apical 25% of the cochlear length was not measured due to poor hearing sensitivity of guinea pigs below 1 kHz, most of the fluid in this region was replaced by fluid from more basal regions during a single cycle of cochlear pumping. Hence, estimates of salicylate distribution derived for the basal regions are valid for the most apical 25% of the cochlea.

The tentative physical principles which govern the uniform distribution of salicylate along the cochlea are universal and should be valid for the distribution of an arbitrary substance, including nanoparticles, in the human cochlea. Salicylate was used in these initial experiments

because of its well-documented physiological effect which allows estimation of the drug distribution along the intact cochlea. It was also used because it challenged the method of cochlear pumping, being a difficult drug to distribute along the cochlea because it is cleared rapidly from the ST (Sadreev et al., 2019). It is anticipated that drugs, which are better retained in the ST, will be redistributed along the cochlea even more quickly and efficiently (Salt and Ma, 2001; Sadreev et al., 2019).

ETHICS STATEMENT

All procedures involving animals were performed in accordance with UK Home Office regulations with approval from the University of Brighton Animal Welfare and Ethical Review Body.

AUTHOR CONTRIBUTIONS

A.L., N.Z., Y.Y. and I.R. conceived and designed the study. A.L. performed the experiments. A.L. and I.S. analyzed experimental results. All authors contributed to analysis and discussion of the results. A.L. and I.R. wrote the manuscript with contribution from all authors.

FUNDING

The research was funded by a grant from the Medical Research Council (MR/N004299/1).

CONFLICT OF INTEREST STATEMENT

A.L., N.Z. and Y.Y. are inventors on a United Kingdom Patent Application No. 1908260.1 submitted by The University of Brighton that covers method and device for substance delivery to the inner ear. N.Z. is employed by the company Otophysica Ltd, Uckfield, UK. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

Borkholder, D. A., Zhu, X., and Frisina, R. D. (2014). Round window membrane intracochlear drug delivery enhanced by induced advection. *J. Control. Release* 174, 171-176. doi: 10.1016/j.jconrel.2013.11.021

Burwood, G. W., Russell, I. J., and Lukashkin, A. N. (2017). Rippling pattern of distortion product otoacoustic emissions evoked by high-frequency primaries in guinea pigs. *J. Acoust. Soc. Am.* 142, 855-862. doi: 10.1121/1.4998584

Creber, N. J., Eastwood, H. T., Hampson, A. J., Tan, J., and O'Leary, S. J. (2018). A comparison of cochlear distribution and glucocorticoid receptor activation in local and systemic dexamethasone drug delivery regimes. *Hear. Res.* 368, 75-85. doi: 10.1016/j.heares.2018.03.018

Devare, J., Gubbels, S., and Raphael, Y. (2018). Outlook and future of inner ear therapy. *Hear. Res.* 368, 127-135. doi: 10.1016/j.heares.2018.05.009

Greenwood, D. D. (1990). A cochlear frequency-position function for several species--29 years later. *J. Acoust. Soc. Am.* 87, 2592-2605. doi: 10.1121/1.399052

Grewal, A. S., Nedzelski, J. M., Chen, J. M., and Lin, V. Y. (2013). Dexamethasone uptake in the murine organ of Corti with transtympanic versus systemic administration. *Journal of Otolaryngology - Head & Neck Surgery* 42, 19. doi: 10.1186/1916-0216-42-19

Hallworth, R. (1997). Modulation of outer hair cell compliance and force by agents that affect hearing. *Hear. Res.* 114, 204-212. doi: 10.1016/S0378-5955(97)00167-6

Hao, J., and Li, S. K. (2019). Inner ear drug delivery: Recent advances, challenges, and perspective. *Eur. J. Pharm. Sci.* 126, 82-92. doi: 10.1016/j.ejps.2018.05.020

Imamura, S. I., and Adams, J. C. (2003). Distribution of gentamicin in the guinea pig inner ear after local or systemic application. *J. Assoc. Res. Otolaryngol.* 4, 176-195. doi: 10.1007/s10162-002-2036-8

Jones, S. W., Thomas, O. M., and Aref, H. (1989). Chaotic advection by laminar flow in a twisted pipe. *J. Fluid Mech.* 209**,** 335-357. doi: 10.1017/S0022112089003137

Mynatt, R., Hale, S. A., Gill, R. M., Plontke, S. K., and Salt, A. N. (2006). Demonstration of a longitudinal concentration gradient along scala tympani by sequential sampling of perilymph from the cochlear apex. *J. Assoc. Res. Otolaryngol.* 7, 182-193. doi: 10.1007/s10162-006-0034-y

Nguyen, N.-T. (2011). Micromixers: fundamentals, design and fabrication. William Andrew.

Nuttall, A. L., Ricci, A. J., Burwood, G., Harte, J. M., Stenfelt, S., Cayé-Thomasen, P., Ren, T., Ramamoorthy, S., Zhang, Y., Wilson, T. and Lunner, T. (2018). A mechanoelectrical mechanism for detection of sound envelopes in the hearing organ. *Nat. Commun.* 9, 4175. doi: 10.1038/s41467-018-06725-w

Nyberg, S., Abbott, N. J., Shi, X., Steyger, P. S., and Dabdoub, A. (2019). Delivery of therapeutics to the inner ear: The challenge of the blood-labyrinth barrier. *Sci. Transl. Med.* 11**,** eaao0935. doi: 10.1126/scitranslmed.aao0935

Ohyama, K., Salt, A. N., and Thalmann, R. (1988). Volume flow rate of perilymph in the guinea-pig cochlea. *Hear. Res.* 35, 119-129. doi: 10.1016/0378-5955(88)90111-6

Ottino, J. M., and Wiggins, S.R. (2004). Introduction: mixing in microfluidics. *Philos. Trans. A Math. Phys. Eng. Sci.,* 362**,** 923-935. doi: 10.1098/rsta.2003.1355

Patel, J., Szczupak, M., Rajguru, S., Balaban, C., and Hoffer, M. E. (2019). Inner Ear Therapeutics: An Overview of Middle Ear Delivery. *Front. Cell. Neurosci.* 13, 261. doi: 10.3389/fncel.2019.00261

Piu, F., and Bishop, K. M. (2019). Local Drug Delivery for the Treatment of Neurotology Disorders. *Front. Cell. Neurosci.* 13, 238. doi: 10.3389/fncel.2019.00238

Plontke, S. K., Biegner, T., Kammerer, B., Delabar, U., and Salt, A. N. (2008). Dexamethasone concentration gradients along scala tympani after application to the round window membrane. *Otol. Neurotol.* 29, 401-406. doi: 10.1097/MAO.0b013e318161aaae

Plontke, S. K., Mynatt, R., Gill, R. M., Borgmann, S., and Salt, A. N. (2007). Concentration gradient along the scala tympani after local application of gentamicin to the round window membrane. *Laryngoscope*, 117, 1191-1198. doi: 10.1097/MLG.0b013e318058a06b

Robles, L., and Ruggero, M.A. (2001). Mechanics of the Mammalian Cochlea. *Physiol. Rev.* 81**,** 1305-1352. doi: 10.1152/physrev.2001.81.3.1305

Russell, I. J., and Schauz, C. (1995). Salicylate ototoxicity: Effects on stiffness and electromotility of outer hair cells isolated from the guinea pig cochlea. *Auditory Neurosci.* 1, 309-319.

Rybak, L. P., Dhukhwa, A., Mukherjea, D., and Ramkumar, V. (2019). Local Drug Delivery for Prevention of Hearing Loss. *Front. Cell. Neurosci.* 13, 300. doi: 10.3389/fncel.2019.00300

Sadreev, I. I., Burwood, G. W. S., Flaherty, S. M., Kim, J., Russell, I. J., Abdullin, T. I. et al. (2019). Drug Diffusion Along an Intact Mammalian Cochlea. *Front. Cell. Neurosci.* 13, 161. doi: 10.3389/fncel.2019.00161

Saijo, S., and Kimura, R. S. (1984). Distribution of HRP in the inner ear after injection into the middle ear cavity. *Acta Otolaryngol*. 97, 593-610. doi: 10.3109/00016488409132937

Salt, A. N., and Hirose, K. (2018). Communication pathways to and from the inner ear and their contributions to drug delivery. *Hear. Res.* 362, 25-37. doi: 10.1016/j.heares.2017.12.010

Salt, A. N., and Ma, Y. (2001). Quantification of solute entry into cochlear perilymph through the round window membrane. *Hear. Res.* 154, 88-97. doi: 10.1016/S0378-5955(01)00223-4

Salt, A. N., and Plontke, S. K. (2009). Principles of local drug delivery to the inner ear. *Audiol. Neurotol.* 14, 350-360. doi: 10.1159/000241892

Schilder, A. G. M., Su, M. P., Blackshaw, H., Lustig, L., Staecker, H., Lenarz, T., et al. (2019). Hearing Protection, Restoration, and Regeneration: An Overview of Emerging Therapeutics for Inner Ear and Central Hearing Disorders. *Otol. Neurotol.* 40**,** 559-570. doi: 10.1097/MAO.0000000000002194

Schuknecht, H. F. (1956). Ablation therapy for the relief of Meniere's disease. *Laryngoscope* 66, 859-859. doi: 10.1288/00005537-195607000-00005

Sim, J. H., Röösli, C., Chatzimichalis, M., Eiber, A., and Huber, A. M. (2013). Characterization of Stapes Anatomy: Investigation of Human and Guinea Pig. *J. Assoc. Res. Otolaryngol.* 14**,** 159-173. doi: 10.1007/s10162-012-0369-5

Thorne, M., Salt, A. N., DeMott, J. E., Henson, M. M., Henson, O. W. Jr., and Gewalt, S. L. (1999). Cochlear fluid space dimensions for six species derived from reconstructions of three-dimensional magnetic resonance images. *Laryngoscope* 109, 1661–1668. doi: 10.1097/00005537-199910000-00021