

PHARMACOKINETIC AND TOXICITY PROFILE OF OTO-104 : A SUSTAINED RELEASE DEXAMETHASONE HYDROGEL FOR INNER EAR DELIVERY

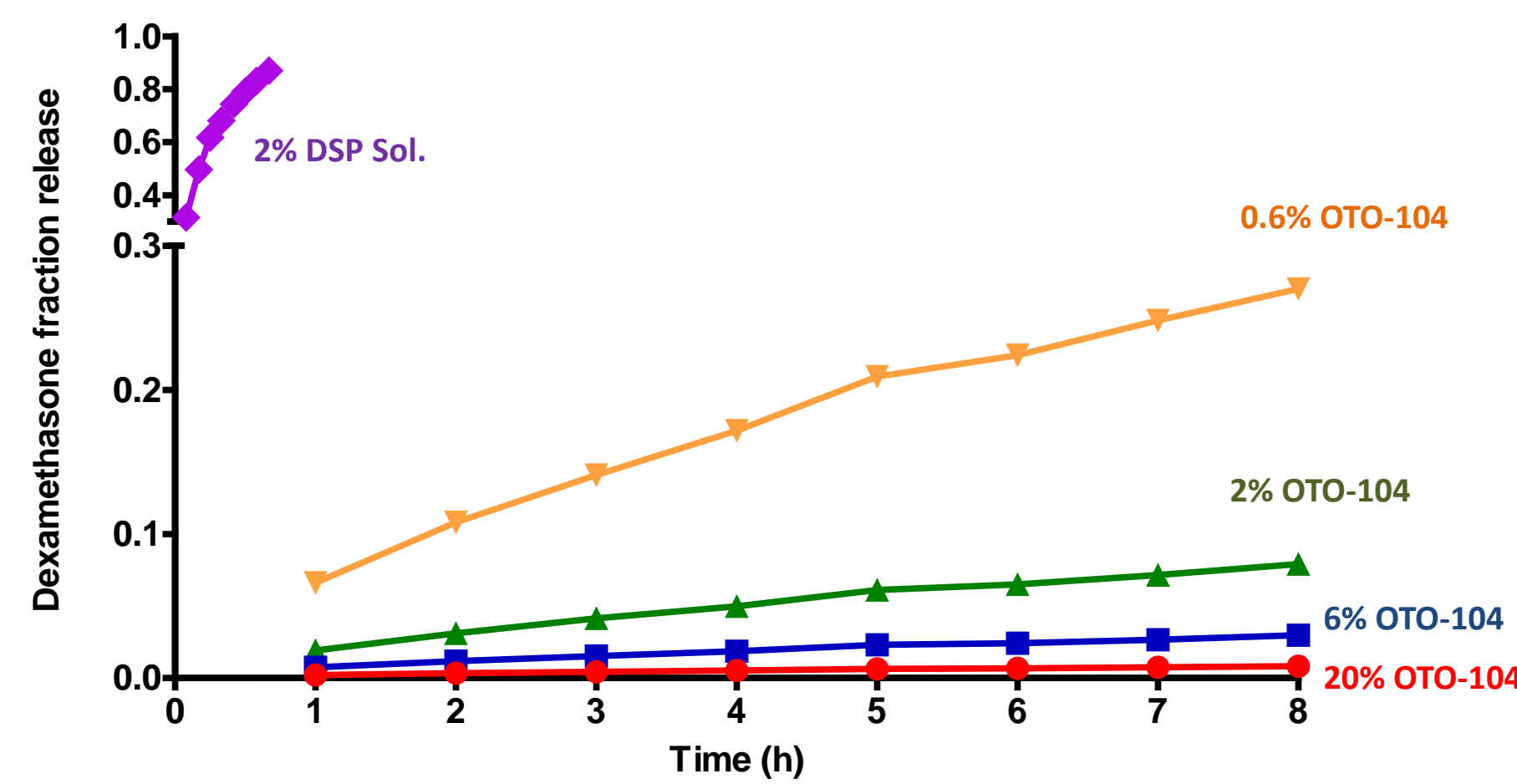


Xiaobo Wang*, Rayne Fernandez, Anne Harrop, Luis Dellamary, Qiang Ye, Elizabeth M. Keithley, Jeffrey P. Harris, Jay Lichter, Carl LeBel and Fabrice Piu.
Otonomy Inc., San Diego, CA

INTRODUCTION

In recent years, intratympanic drug delivery has been investigated as a route of administration to treat a variety of inner ear disorders, such as Meniere's disease and Sudden Sensorineural Hearing Loss. While constituting an improvement in safety and efficacy over the traditional systemic dosing approach (oral, intravenous), multiple issues still remain to be addressed: large differences in dosing schedules and regimen, as well as high variability in clinical outcomes and patient acceptance. These disparities are primarily the result of the nature of the current formulations, namely drug solutions with short residence time and rapid elimination from the middle and inner ear.
OTO-104, a dexamethasone suspension in a poloxamer-based hydrogel, was developed. Poloxamers are tri-block co-polymers (PEO-PPO-PEO) with mucoadhesive and thermoreversible properties that behave as sustained release drug delivery vehicles. OTO-104 was administered to guinea pigs via intratympanic injection and its pharmacokinetic and toxicity profile was examined.

IN VITRO RELEASE PROFILE



Metric	Guinea pig				
	DSP	OTO-104			
	2.0%	0.6%	2.0%	6.0%	20.0%
MDT (h)	0.3	17	57	161	581

Dissolution rate experiments were performed at 37 °C in snapwells of 6.5 mm diameter polycarbonate membrane with a pore size of 0.4 μm. Briefly, 0.2 ml of the test article was deposited into the snapwell; 0.5 ml of the buffer solution (10mM PBS buffer) was placed into the reservoir and shaken at 70 rpm. Samples were taken at indicated times, where 0.1 ml was withdrawn and replaced with the equivalent volume of pre-warmed buffer. Samples analysis was performed by UV/Vis spectrophotometry (at 245 nm) for dexamethasone. The release profile was fitted to the Korsmeyer-Peppas equation:

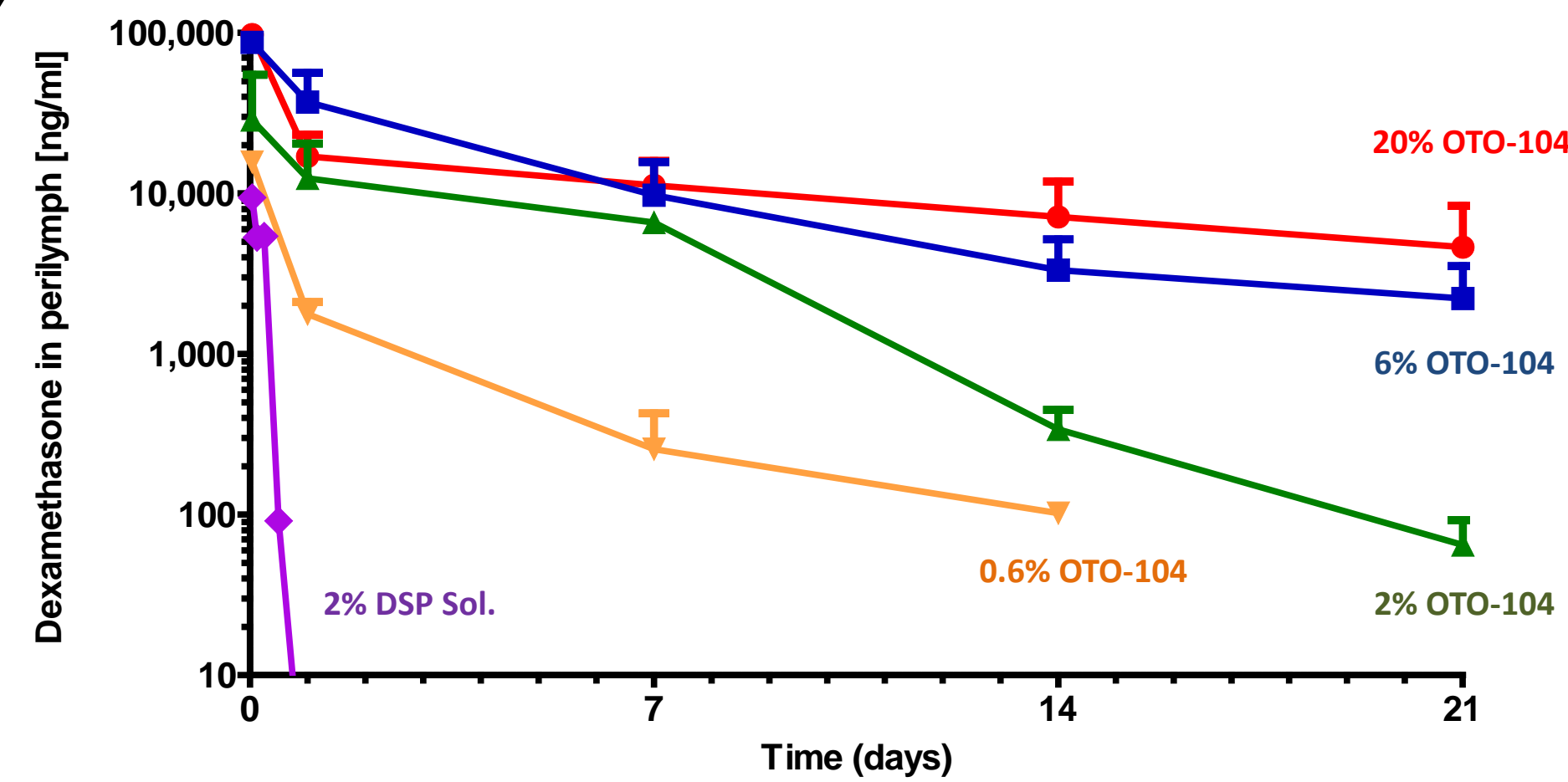
$$Q_t / Q_\infty = kt^n + b$$

where Q_t is the amount released at time t , Q_∞ is the overall released amount, k is a release constant of the n th order, n is a dimensionless number related to the dissolution mechanism and b is the axis intercept, characterizing the initial burst release mechanism. While $n=1$ characterizes and erosion controlled mechanism.

The mean dissolution time (MDT) is the sum of different periods of time the drug molecules stay in the matrix before release, divided by the total number of molecules and is calculated by:

$$MDT = nk^{-1/n} / n + 1$$

INNER EAR PHARMACOKINETICS



Metric	Guinea pig				
	DSP	OTO-104			
	2.0%	0.6%	2.0%	6.0%	20.0%
Cmax (μg/mL)	9.4	15.7	28.7	87.3	97.5
AUC (μg.h/mL)	57	506	2492	6979	7763
MRT (h)	4	72	90	136	302
TET (d)	0.3	4	19	43	103

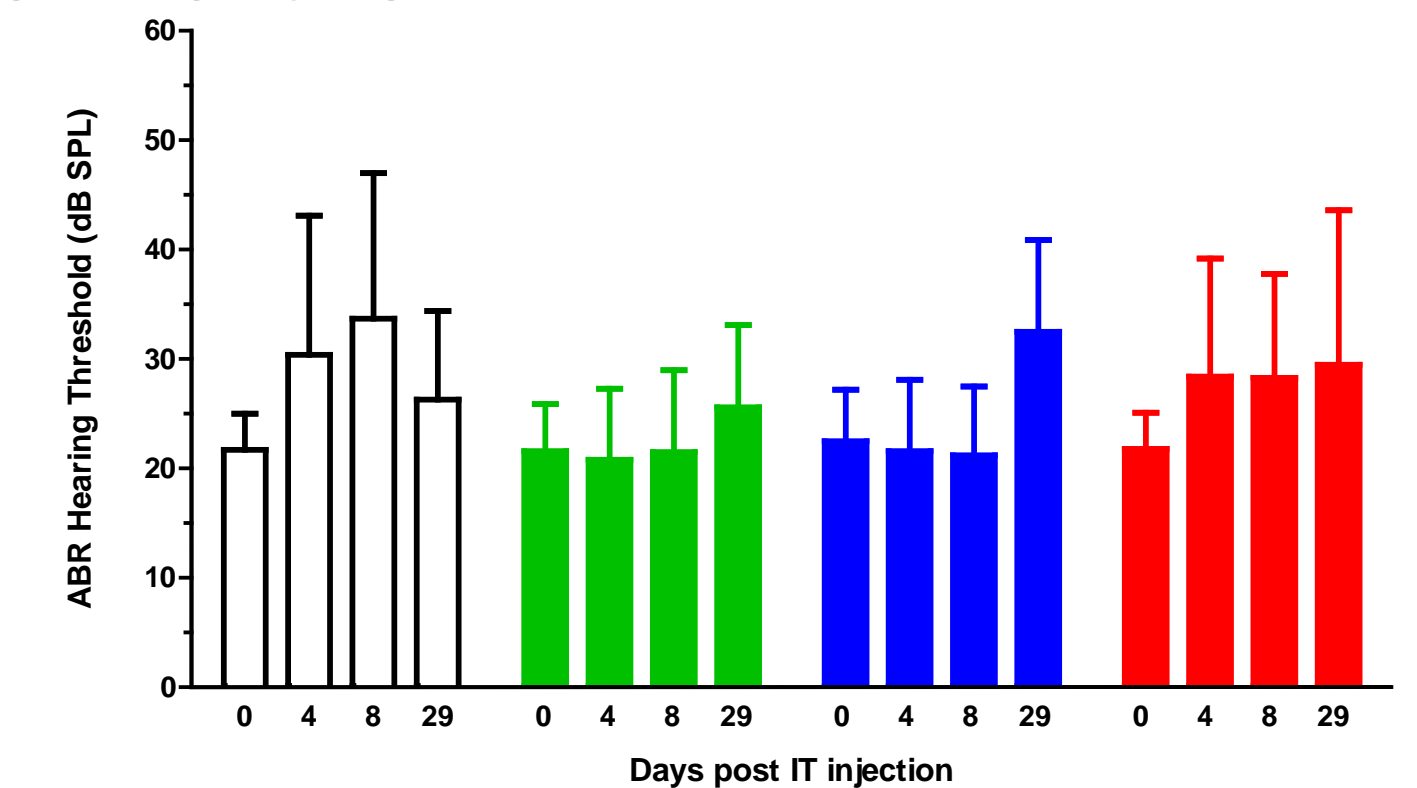
Female guinea pigs (n=4 per group) received a single 50μl intratympanic injection directed towards the round window niche. At the indicated timepoints, perilymph from the base of the cochlea was collected and dexamethasone quantified by LC-MS. AUC: Area Under the Curve, MRT: Mean Residence Time; TET: Terminal Exposure Time (time during which therapeutic drug levels (>40 ng/ml) are present in the inner ear).

CONCLUSIONS

- * In vitro, OTO-104 displays a significantly longer residence time than a 2% DSP solution, up to several orders of magnitude.
- * In guinea pigs, OTO-104 achieves therapeutic levels of dexamethasone in the inner ear for up to 3 months following a single intratympanic injection.
- * Administration of OTO-104 is associated with a small and transient shift in hearing threshold, probably of conductive nature.
- * As determined by histological assessment of middle ear inner tissues, the minimal histological changes observed were no different in the saline, vehicle and OTO-104 treated groups.
- * No hair cell loss was observed following administration of OTO-104.

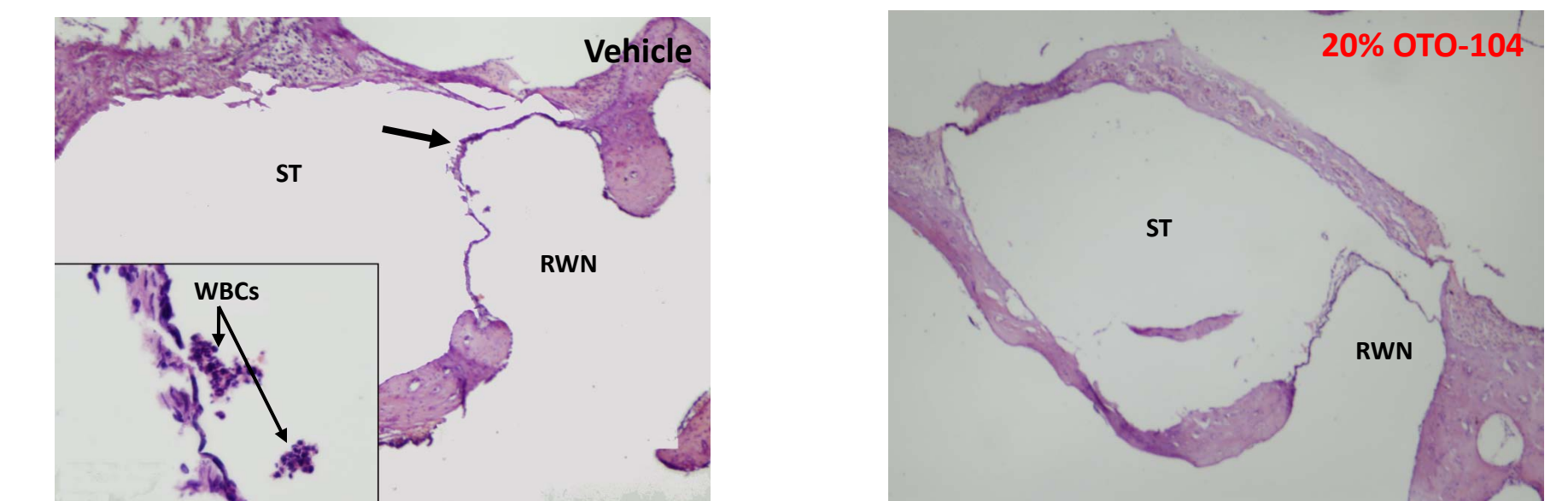
TOXICITY PROFILE

AUDITORY FUNCTION



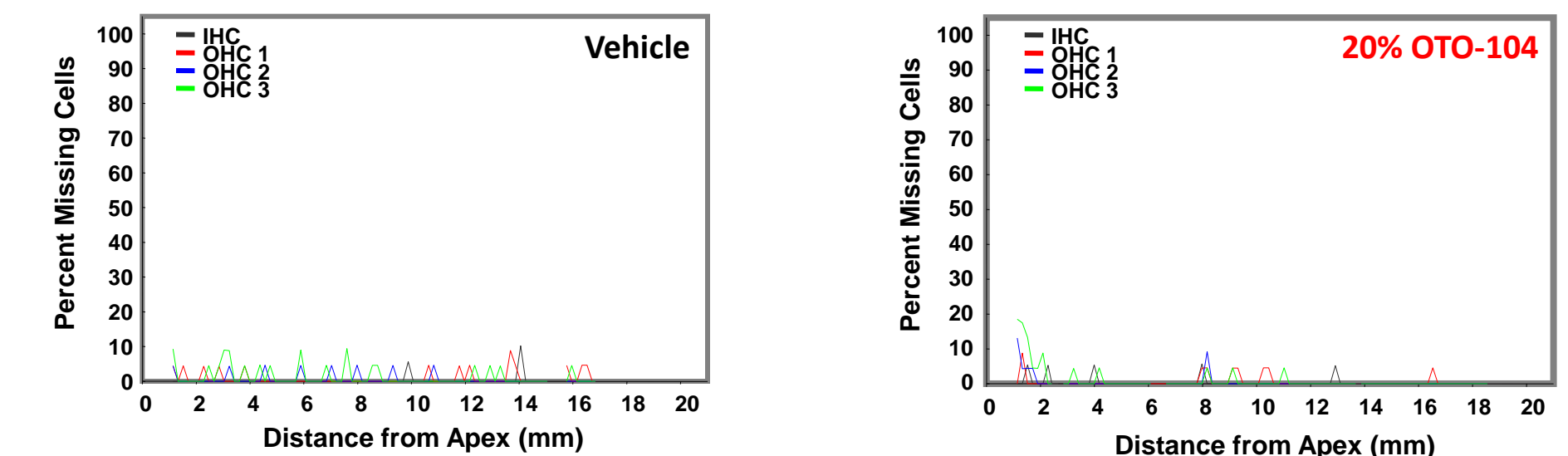
Hearing was tested by recording the brainstem activity in response to a known auditory stimulus, under general anesthesia, in a sound isolation booth. An earphone (EC1, Tucker Davis Technologies) was fitted into the ear just above the external auditory canal orifice. Three subcutaneous needle electrodes were used to measure the brainstem activity, placed in the postauricular area of the ear (reference), on the vertex of the skull (active) and in the hind leg (ground). The acoustic stimulus was generated using the SigGen system (Tucker Davis Technologies) and consisted of 10ms auditory clicks (frequency range 100Hz - 30 KHz). Responses were averaged from 512 presentations with sound level up to 90 dB SPL with increments of 5 dB SPL. Responses were acquired using BioSig (Tucker Davis Technologies) and threshold was determined as the average between the non observable and smallest observable intensity.

HISTOLOGY



Harvested cochleae were decalcified in EDTA for 2 weeks. Following thorough rinsing with water, cochleae were further dissected, trimmed and balanced on their ventral surface. Embedding was performed in a Autotechnicon using the following cycle: ethanol 70% 90min, ethanol 95% 120 min repeated 3 times (3X), ethanol 100% 120min (3X), histoclear 30min (3X), paraffin 120min (2X), paraffin 12-18h in a vacuum oven. Sectioning of the cochlear preparations were carried out taking 7 μm sections. Slides were stained using a standard hematoxylin / eosin protocol before being mounted in Permount.

CYTOCOCHLEOGRAMS



Hair cell status was quantitatively assessed in phalloidin stained surface preparations of the cochlear spiral and plotted from apex to base as cytochleograms. Normal: Scattered hair cell loss under 10% and occasional scattered larger spikes of hair cell loss. Minimal: few regions and a few rows of OHCs with 10-25% loss. Moderate: many regions of loss over 25%. Severe: many regions of loss over 60. Data was generated by R. Altschuler, Kresge Institute, Ann Arbor, Michigan.