

# CHARACTERIZATION OF INNER EAR INFLAMMATION IN RODENTS USING *IN VIVO* DYNAMIC CONTRAST ENHANCED MAGNETIC RESONANCE IMAGING AND *EX VIVO* LIGHT MICROSCOPY

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## Introduction

The inflammation of inner ear tissues is thought to be a major contributor to the development of hearing loss and balance disorders [1]. However, because of the inner ear location deep within the temporal bone it is difficult to determine the occurrence and pathological influence of dynamic inflammatory disease, without invasive techniques. Recent advances in magnetic resonance imaging (MRI) offer innovative opportunities for studying function and metabolism of the intact and damaged cochlea [2]. Here, we report the detection of cochlear inflammation *in vivo* on MR images using dynamic contrast enhanced technique and the distribution of inflammatory cells in the cochlea.

## Methods

**Animal models of inflammation:** To induce cochlear inflammation, guinea pigs (GP; n=6) and rats (n=7) were either exposed to acute broadband noise (110dB SPL for 4 hours) in a sound-attenuating booth or were sensitised by bacterial lipopolysaccharide (LPS; 0.01% weight/volume; 0.8mg/kg) followed 24 hours later by bilateral intra-tympanic injection of LPS (1% weight/volume; 30µl/tympanum). Two rodent species were used to study cochlear response to proinflammatory signals.

**Tissue preparation and light microscopy:** Rats were euthanized at 1, 3, 5 and 7 days post-LPS inoculation by intraperitoneal injection of sodium pentobarbitone (90 mg/Kg) followed by intracardiac perfusion with saline and fixative (4%PFA) solution. Cochleae were then extracted for immunoperoxidase histochemical analysis of infiltrating inflammatory cells (with the F4/80 cell surface marker) and expression of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$  and IL-6). Cochlear sections were viewed under a digital light microscope (Axioskop 2 Mot Plus, Carl Zeiss, Inc., Germany).

**MR sequence protocol:** MR experiments were performed on a UnityNova 4.7T MRI system (Varian Inc.) using gradients with a maximum gradient strength of 20G.cm<sup>-1</sup> with a rise time of 375µs. Images were acquired using a 72mm ID birdcage-design quadrature RF coil (m2m Imaging). During the scans, rodents were anaesthetized with isoflurane (3%-4% induction and 1.8%-2.5% maintenance) with heart rate, respiration and temperature monitored throughout the scan (SA Instruments). Body temperature was maintained at 37°C by circulating warm air through the magnet bore. Either immediately or up to 3 days after inducing inflammation anaesthetised animals were scanned. Scout images were acquired for each animal to aid in positioning the animal and cochlea in the magnet. All subsequent scans were based on a slab-selective 3D Gradient Recalled echo sequence used with the following parameters: TR/TE/spoiling gradient strength/pulse width = 20ms/4.5ms/2G.cm<sup>-1</sup>/1000µs, acquisition matrix=512x256x16 and FOV=80mm\*50mm\*12mm(rats)/15mm(GP). To localize the inner ear tissues, a proton density (PD) weighted volume set of MR images (flip angle = 10°, 4 averages) was used. A single T1-weighted set was acquired prior to injection of a contrast agent (Gadodiamide, Omniscan, 500mmol/L, Gd) to use as a reference for post-image analysis. Rodents were then removed from the MR system and Gd was injected intravenously either in the femoral vein (GPs; [Gd]=1.5mmol/kg) after a small surgery or in the tail vein (rats; [Gd]=0.7mmol/kg). Rodents were rapidly re-positioned in the scanner and a scout acquired to confirm the appropriate positioning of the rodents and comparing the SNR with the original scout. The same T1-weighted sequence was run approximately every 1.5min post-injection over a maximum of 70min to detect and track the presence of Gd in the inner ear tissues. Changes in signal intensity in the cochleae were measured relative to a tube of 5mM Gadodiamide solution placed adjacent to the rodent to allow for comparison between MR acquisitions. At the end of the MR session, the rodents were euthanized and the cochlear tissues were collected as described above.

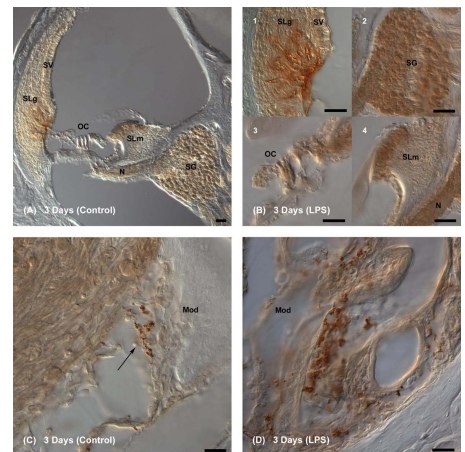
**Image analysis:** Region of interests (ROIs) covering inner ear tissues were selected on both sides. The signal intensity was estimated by calculating the average of the pixel greyscale values within the ROIs (ImageJ), ensuring identical gray level scale between images and normalised against the signal intensity measured in the Gd solution. Signal enhancement was defined as the ratio between relative signal intensities after and before injection of Gd.

## Results and discussion

**Immunohistochemistry:** Cochlea mounts a substantial inflammatory response following LPS-induced middle ear inflammation, with recruitment of F4/80+ cells (Fig. 1). Cochlear structures immunoreactive to F4/80 included the spiral ligament, spiral limbus, and spiral ganglion. These structures, along with the stria vascularis, also showed expression of TNF- $\alpha$ , which was most prominent at early stages of inflammation. In contrast to TNF- $\alpha$ , IL-1 $\beta$  and IL-6 immunolabelling was mainly restricted to inflammatory cells in the perivascular spaces of the modiolus.

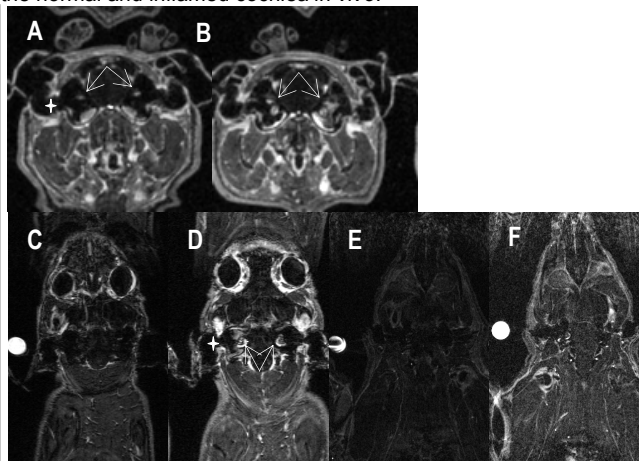
## MR findings

In the LPS case, there was evidence of signal enhancement in the cochlear tissues in rats and guinea pigs (Fig. 2) which increased over time and was greater than the signal enhancement found in the controls. In the acute noise-exposed case, rats did not show any signal enhancement whereas guinea-pigs did (results not shown). The signal enhancement seemed to be more important in the basal turn in both animals (Fig. 2 D and F). First, MR and immunohistochemistry results suggest that Gd uptake increase correlated to cochlear inflammation. We suggest that increase in Gd uptake occurred as a consequence of increased vascular permeability. Second, these results demonstrate that MRI can monitor longitudinally the normal and inflamed cochlea *in vivo*.



**Fig 1: F4/80 immunolabelling in cochlea (A) Control; (B) F4/80+ staining found in spiral ligament (SLg) (1), spiral ganglion (SG) (2), and interdental cells of spiral limbus (SLm) (4). No staining was observed in the organ of Corti (OC) (3). (D) F4/80+ inflammatory cells in the perivascular spaces of the modiolus (Mod), which was much greater in number in the LPS-treated cochlea than the Control (C). Scale bar = 50µm.**

**Fig.2 Signal enhancement in GP and rat cochlear tissues (white arrows). Inner ear canals (4 point star). Images (16bit) have been interpolated to maintain anatomical proportion. A-B) GP T1 weighted coronal images 13min after Gd injection A) Control; B) LPS case. C-F) Axial images 50min and 36min after injection of Gd in GP and rat, respectively: C) GP Control; D) GP LPS case; E) rat control and F) rat LPS case.**



of cochlear blood flow. Brain Research Reviews, 43, 2003, 17-28. [2] Zou J *et al.* Differential passage of gadolinium through the mouse inner ear barriers evaluated with 4.7T MRI. 46<sup>th</sup> Workshop on Inner Ear Biology, O11, 2009. **Acknowledgements:** We thank Dr. R.S. Telang and Ms V. Paramanathasivam for GP surgery and iv injections. The work was supported by the Faculty Research Development Fund, The University of Auckland.