

# Manganese-enhanced MRI combined with acoustic startle reflex testing would be useful as an imaging biomarker for tinnitus

Mun Han<sup>1</sup>, Da Jung Jung<sup>2</sup>, Kyu Yup Lee<sup>2</sup>, Yongmin Chang<sup>\*1,3</sup>, and Hui Joong Lee<sup>\*4</sup>

<sup>1</sup>Department of Medical & Biological Engineering, Kyungpook National University, Daegu, Korea, <sup>2</sup>Department of Otorhinolaryngology & Head and Neck surgery, Kyungpook National University, Daegu, Korea, <sup>3</sup>Department of Radiology & Molecular Medicine, Kyungpook National University, Daegu, Korea, <sup>4</sup>Department of Radiology, Kyungpook National University, Daegu, Korea

## Introduction

Tinnitus, the perception of a ringing, buzzing, or hissing in the absence of an external stimulus due to noise, drugs, or traumatic brain injury, is a major health concern affecting populations<sup>1-3</sup>. The evidence suggests that tinnitus can be linked with increased neuronal activity<sup>4,5</sup>. MEMRI, which makes use of the fact that paramagnetic manganese ions ( $Mn^{2+}$ ) enter synaptically activated neurons through voltage-gated calcium channels and results in signal enhancement on T1-weighted MRI images, is increasingly used for the functional characterization of the auditory system<sup>6</sup>. In this study, we established tinnitus-animal model to check neural activity directly linked to tinnitus and explored the possibility of MEMRI combined with acoustic startle reflex (ASR) measurement as a valuable imaging biomarker for tinnitus.

## Material & Methods

Ten male Sprague-Dawley rats (six weeks old and 170-230g) were maintained according to protocols approved by the Animal Care and Use Committee of Kyungpook National University. The animals were divided into two groups: study group (sodium salicylate, N = 5) and control group (normal saline, N = 5). Animals in study group received a daily intraperitoneal injection of salicylate at a dose of 350g/kg/day for 4 days. For ASR testing, the startle stimulus was embedded in a continuous background noise (60 dB SPL); the center frequency of the narrow band was varied across ASR test conditions. On half the trials, the continuous noise contained a silent gap (50 ms). The onset of the gap preceded the onset of the startle stimulus by 100 ms. The RMS amplitude of the acoustic startle response was measured in the presence of continuous noise with “no gap” or with a “gap”. When the gap preceded the startle stimulus, there was a reduction in the amplitude of the startle response in normal hearing rats, that is the gap serves as a pre-pulse and inhibits the startle response<sup>7,8</sup>. The significant ASR indicated that the rat was able to detect the silent gap. In contrast, gap detection and ASR were expected to be greatly reduced in rats with severe tinnitus since the phantom sound of tinnitus presumably fills in the silent gap. Therefore in rats with tinnitus, the startle amplitude would no longer differ significantly between the “gap” and “no-gap” conditions. To estimate the pitch of the tinnitus, ASR tests were run with narrow bands of noise (1000 Hz bandwidth) centered at 6, 12, 16, 20 or 24 kHz. For each frequency the test set contained 20 pairs of “no-gap” and “gap” trials, with the test order randomized. The animals were anesthetized using Zoletil (Virbac Laboratories, Carros, France) and were injected with manganese chloride (0.125 mmol/kg body weight) dissolved in distilled water via application on round window approach. Imaging was performed using a 1.5 MR scanner (Signa elite GE Medical) with self-manufactured animal receive-only coil. MR imaging was acquired 2D spin-echo T1-weighted image and sequence were as follows: field of view, 50×50 mm matrix size, 192×192 coronal slices, 0.9 mm slice thickness, no gap, repetition time (TR) = 500 ms, echo time (TE) = 15 ms, number of acquisition (NEX) = 15, scan time = 36m 16s. The animals were placed in the magnet in a prone position with the head first. During MRI measurements, each animal was anesthetized.

## Results & Discussion

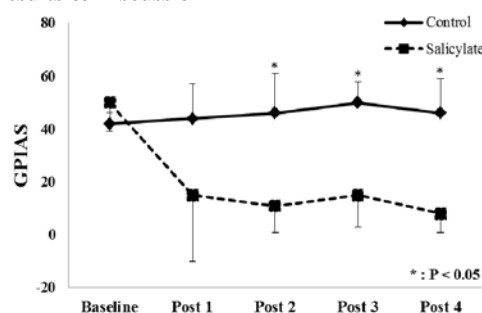


Figure 1. The ASR result.

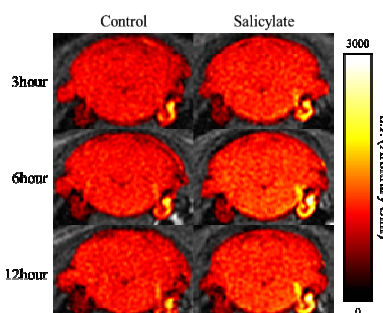


Figure 2. Salicylate group increases MEMRI measured signal intensity in the cochlea and cochlear nucleus.

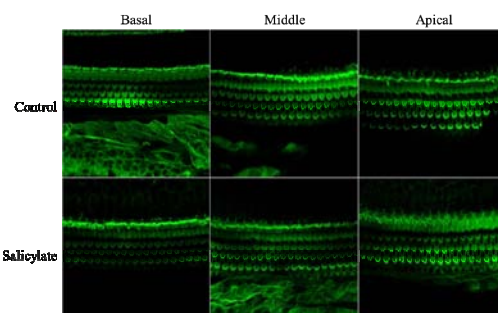


Figure 3. The cochlea images of histological sections.

The ASR data showed a significant difference in acoustic startle reflex between tinnitus group and normal group ( $p < 0.05$ , Figure 1). This ASR data therefore demonstrated that salicylate-treated animal experienced tinnitus. Figure 2 shows MEMRI images at six hour after manganese application through round window. The MEMRI images showed that animal with salicylate-induced tinnitus demonstrated higher signal enhancement in the areas of the cochlea and cochlear nucleus compared to normal hearing control animal ( $p < 0.05$ ). Figure 3 shows the histology images of the cochlea. From Figure 3, no morphological difference was found between tinnitus group and normal group suggesting that the hair cells of the cochlea were not damaged by salicylate-induced tinnitus. However, MEMRI evidenced excessive synaptic activity of the cochlea and cochlear nucleus in animal with salicylate-induced tinnitus compared to control animals. Taken together, our results, for the first time, demonstrated that salicylate-induced tinnitus is not associated with hair cell damage of the cochlea but associated with excessive neuronal activity of hair cell of the cochlea. In conclusion, we established tinnitus-animal model to evaluate auditory neural activity directly linked to tinnitus and demonstrated that MEMRI combined with ASR measurement could be used as a non-invasive valuable imaging biomarker for various studies on tinnitus including new therapeutical methods.

**References:** 1. Cave KM, et al, *Mil Med* 172: 726–730 (2007). 2. Fagelson MA, *Am J Audiol* 16: 107–117 (2007). 3. Lew HL, et al, *J Rehabil Res Dev* 44: 921–928 (2007). 4. Bauer CA, et al, *J Neurosci Res* 86: 2564–2578 (2008). 5. Brozoski TJ, et al, *J Neurosci* 22: 2383–2390 (2002). 6. Watanabe T, et al, *NMR in Biomedicine* 17: 554–568 (2004). 7. Ison JR, et al, *J Acoust Soc Am* 112:740–747 (2002). 8. Yang G, et al, *Hear Res* 226:244–253 (2007).