# Mn-Enhanced MRI (MEMRI) of Auditory Brain Activity in Unilaterally Deafened Mice

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## Introduction and Significance

We recently reported that MEMRI can be used to detect neural activity in the mouse auditory midbrain, showing significant MEMRI enhancement in normal mice compared to deafened controls after IP injection of $MnCl_2$ and prolonged periods of auditory stimulation [1]. The main goal of the current study was to determine whether MEMRI could be used to provide *in vivo* data on the development of auditory brain function. Although there is currently debate over the interpretation of Hubel & Wiesel's results, it has been long been known that visual experience during development plays a critical role in the maintenance and confinement of patterned visual brain activity [2], [3]. Currently it is unclear whether hearing experience during early periods of development also influences patterned auditory brain activity. Previous studies used 2-deoxyglucose (2-DG), an *ex vivo* auto-radiography method, to demonstrate developmental changes in midbrain metabolic activity in unilaterally deafened gerbils [4]. We investigated whether MEMRI could be used to provide similar data, with the important advantage of enabling *in vivo* longitudinal studies, examining differences in sound-evoked activity in auditory brain nuclei in the ascending projection pathways of mice experiencing conductive hearing loss (CHL) at different developmental stages.

## Methods

Unilateral CHL was produced in Swiss-Webster mice by puncturing the tympanic membrane and surgically removing the malleus of one ear. Surgery was performed at postnatal day 10 (P10), before onset of hearing, and at P21, after the establishment of an adult-like auditory system. For MEMRI, mice were injected IP with 0.4 mM/kg body weight of $MnCl_2$ in saline. No behavioral abnormalities were observed over more than 2 months in mice injected with this dose of $MnCl_2$. After injection of $MnCl_2$, mice were exposed to sound stimulation for 24 hr, and were then anesthetized with isoflurane (1-1.5% in air) during MRI. Sound stimulation consisted of frequency- and amplitude-modulated broadband pulses (1-59 kHz, 95 dB peak SPL). Images were acquired at P21 and at 6-weeks of age (P6w). Normal (non-deafened) control mice underwent the same experimental protocol as the mice with unilateral CHL. MRI was performed on a SMIS console interfaced to a 7T horizontal bore magnet with 250-mT/m actively shielded gradients (Magnex), using a custom mouse head holder and 22-mm (ID) saddle coil. MR images were acquired with a 3D T1-weighted gradient echo sequence (TE/TR=5/50ms) with 100-µm isotropic spatial resolution and an acquisition time of 2 hours.

## Results and Conclusion

As expected from the known ascending axonal projections, mice with unilateral CHL showed MEMRI enhancement in reference to the functional ear, in the ispilateral (ipsi-) cochlear nucleus (CN) and the contralateral (contra-) inferior colliculus (IC). In contrast, there was no difference in MEMRI intensity between the two sides of CN and IC in control (non-deaf) mice (Fig 1A, D, I). In mice experiencing CHL at P21 (Fig 1B, E), MEMRI enhancement in ipsi-CN was significantly increased at both P21 and P6w (Fig 1H; *p<0.01) compared to both (non-deaf) control CN/IC and caudate putamen (CPu), a non-auditory brain region, whereas enhancement in contra-CN was only significantly increased at P21 (Fig 1H; *p<0.01). These in vivo MEMRI results are consistent with data from ex vivo 2-DG in gerbils with unilateral CHL [4]. Furthermore, in mice imaged at both P21 and P6w, the MEMRI results (at P6w) were the same as in mice imaged only once at P6w (n=7; data not shown), showing the feasibility of longitudinal MEMRI experiments. Finally, in mice experiencing CHL at P10 (Fig 1C, F), MEMRI enhancement was significantly increased in ipsi-CN and contra-IC at both P21 and P6w (Fig 1G; *p<0.01). Moreover, at both P21 and P6w MEMRI enhancement in ipsi-CN and contra-IC was significantly greater in mice deafened at P10 compared to mice deafened at P21 (Fig 1G, H; *p<0.01). These results indicate that larger and more persistent changes in auditory brain activity result when hearing loss occurs at a critical early stage of development. We conclude that this easily-implemented MEMRI protocol enables longitudinal studies of neuronal plasticity in the early developing auditory system, allowing future functional neuroimaging studies of mutant mice with abnormal auditory brain development.

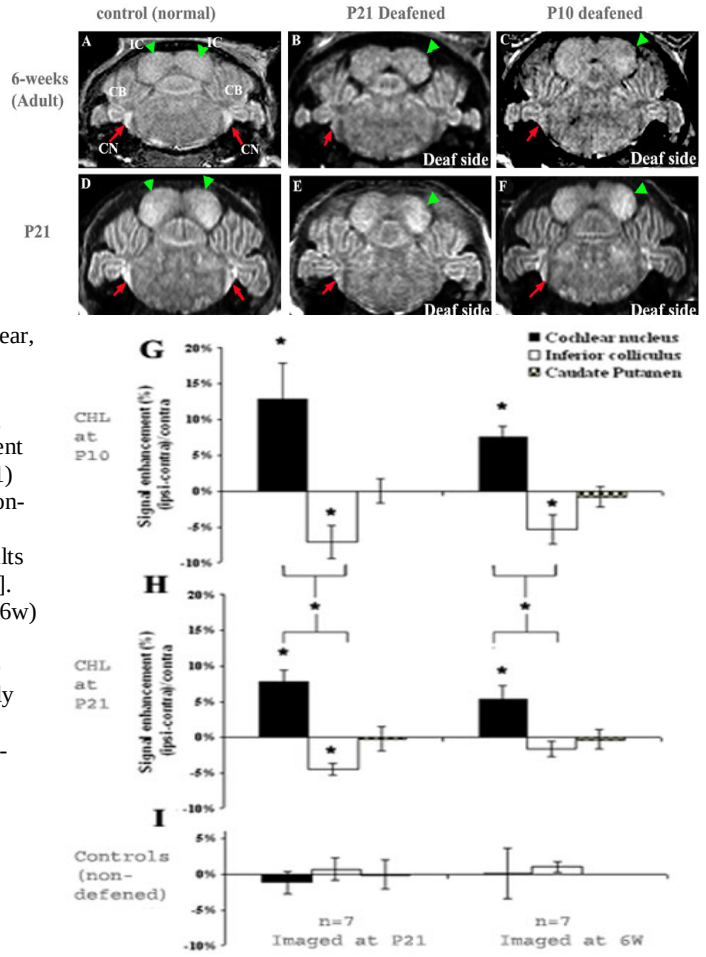


Fig1. MEMRI enhancement was observed in ipsi-CN (red arrow) and contra-IC (green arrow) to the functional ear in mice with unilateral CHL. Mice were separated into three groups: normal (non-deaf) controls (A, D); CHL at P21 (B, E); and CHL at P10 (C, F). MEMRI was performed at P6w (A, B, C) and P21 (D, E, F). Quantitative ROI analyses of MEMRI signal differences are shown fo CHL at P10 (G) and P21 (H), as well as for (non-deaf) controls (I); n=7 for each result; *T-test, compared to both CPu and non-deaf controls, p<0.01. Note the MEMRI enhancement in cerebellum (CB) independent of auditory activity, as described previously [5].

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## References

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