SUPPLEMENTARY NOTE

Effects of scanner noise on MEMRI results

MRI scanner noise, which presents a unique problem for fMRI studies of the auditory brain[S1], should not contribute significantly to the MEMRI signals in mice for several reasons. First, mice are more sensitive to higher than lower frequency sound from the onset of hearing[S2,S3], and the noise characteristics of our MRI protocol has no significant frequency components above 5-kHz while the sound stimuli used for these studies covered the major audible range of mice, up to 60-kHz (**Supplementary Fig. 1**). Furthermore, the MEMRI signal reflects the accumulative effect of sound stimulation over the 24-h exposure period, which should dominate any effects due to MRI noise over the 2-h imaging period. Finally, the mice in these studies were anesthetized during imaging which is expected to significantly decrease brain metabolism and activity during the acquisition of MRI data[S4]. The excellent agreement of our results with those produced by other non-MRI mapping methods confirms that MEMRI provides an accurate measure of sound-evoked activity, independent of scanner noise.

Effects of hearing loss during development

Previous studies have shown that unilateral sound deprivation or cochlear ablation during early stages of development can induce cellular alterations in the auditory brainstem. Unilateral sound deprivation during early stages of ferret development was reported to induce an increase in the number of ipsilateral projections from the functional CN to IC[S5], similar to the effects seen after cochlear ablation in neonatal gerbils and ferrets[S6,S7]. In the case of cochlear ablation during early postnatal development, the resulting sensorineural hearing loss is associated with more profound neuroplasticity

changes than CHL, including an increased sensitivity and discharge level in response to stimulation of IC neurons ipsilateral to the functional ear[S8]. At this point, there are few data on the neuroplasticity effects of CHL during early postnatal brain development. Future studies, combining MEMRI and histological analyses, should provide important insights into the cellular alterations and subsequent changes in auditory activity in a variety of mouse models of hearing loss.

Transport of systemically administered Mn into neurons

Relevant to our studies are the mechanisms of transport of the IP injected Mn into the brain. It is known that Mn can bind transferrin (Tf-Mn) and then be transported across the BBB *via* receptor-mediated endocytosis[S9]. Nevertheless, the same level of injected Mn is taken up in normal and hypotransferrinemic mice[S10], demonstrating that other transport systems also mediate Mn uptake. Furthermore, free Mn is transported into the brain more rapidly than Tf-Mn[S11], again suggesting that Tf-mediated transport is not the primary system for Mn uptake. At the level of MRI detection, previous studies have shown that injected Mn rapidly accumulates in the ventricular cerebral spinal fluid, and is taken up in the brain parenchyma more slowly over a period of hours unless the BBB is disrupted[S12-S15], suggesting that Mn uptake is *via* absorption through the ependymal surfaces. Additionally, there may be an axonal tract-tracing component to the Mn uptake, which can cross multiple synapses as demonstrated in the olfactory system[S16].

A number of studies indicate that a primary mechanism for transporting divalent Mn^{2+} into neurons is through calcium channels[S17,S18], which provided the original motivation to develop MEMRI approaches[S12]. Indeed, Mn^{2+} entry through calcium channels is used to quench the fluorescence of fura-2 in a standard, widely applied assay to measure cellular Ca^{2+} influx[S19]. Taken together, these data indicate that systemically administered Mn

diffuses into the brain in free ionic form, entering cells either directly through calcium channels, or indirectly through tract tracing, providing an excellent opportunity for apping brain activity with MRI.

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