MEMRI reveals functional alterations in the auditory midbrain of Fgf17 knockout mice

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Introduction and Significance

Fibroblast growth factor 17 (*Fgf17*) acts in coordination with *Fgf8* to regulate the early patterning of the mammalian midbrain and cerebellum [1]. Previous studies have demonstrated a reduced midbrain, and misfolding of the anterior cerebellum in *Fgf17* knockout mice. However, nothing is known about the functional phenotypes in these anatomically altered brain regions. In our study, Mn-Enhanced MRI (MEMRI) was applied to detect patterns of sound-evoked activity within the inferior colliculus (IC), the auditory midbrain of *Fgf17* mutant mice. Using broadband (1-59 kHz) sound stimuli to cover the audible frequency range of mice, we analyzed the anatomical and functional IC reduction of *Fgf17* $^{-/-}$ homozygous mice compared to *Fgf17*$^{+/-}$ heterozygous littermates [1]. We also observed an altered tonotopic organization in the IC, consisting of a large overlap of the 16 and 40 kHz activity patterns of *Fgf17* $^{-/-}$ mice compared to *Fgf17*$^{+/-}$ mice. These results clearly demonstrate that the loss of *Fgf17* induced both anatomic and functional alterations in the central auditory system, and further provide a novel MRI imaging model to study functional neurological phenotypes of genetically engineered mouse models.

Methods

We imaged *Fgf17* mutant mice with a similar MEMRI protocol to previously described [2]. Briefly, mice were injected IP with 0.4 mmol/kg body weight of $MnCl_2$ in saline at postnatal 19 to 24 days, exposed to 24 hr of defined sound stimulation, and then anesthetized with isoflurane (1-1.5% in air) during MRI. Mice were exposed to broadband (1-59 kHz) and pure tone (16 and 40 kHz) sound stimuli, with amplitude modulated between 65 and 89 dB sound pressure level (SPL). For tonotopic analyses, mice were first tested at 40 kHz at P19, and after 2 days for the washout of Mn from the activated IC regions, mice were tested again at 16 kHz. Mice were maintained in a free field inside an acoustic isolation chamber during sound exposure or quiet control condition (Mac-1; Industrial Acoustics). 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 volume coil. MR images were acquired using a 3D T1-weighted gradient echo sequence (TE/TR = 4/50ms, flip angle = 65°) with 100-μm isotropic spatial resolution and an acquisition time of 2 hours. 3D images of the IC were analyzed with Amira (Mercury Computer Systems). The volumetric MRI data from each mouse brain were extracted, co-registered and normalized to establish a uniform template of the whole brain. The IC was then segmented from 3D brain images using an interactive threshold-based region-growing algorithm. Voxel-by-voxel 3D T-test statistical analysis protocols (Matlab) were used to analyze the activated IC neuronal populations [3].

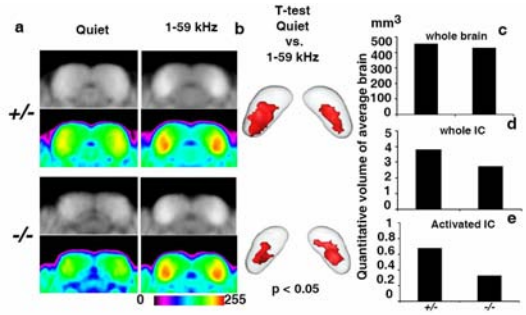

Fig 1. Auditory midbrain anatomy and function are altered in the *Fgf17*$^{-/-}$ mice. Broadband stimulation induced different activity patterns in *Fgf17*$^{-/-}$ and *Fgf17*$^{+/-}$ mice (a). 3D p-maps showed a larger enhanced region in *Fgf17*$^{+/-}$ than in *Fgf17*$^{-/-}$ mice (b) ($p<0.05$; $n>7$ in each group). Quantitative analysis showed that there was a 6% reduction in the whole brain size of *Fgf17*$^{-/-}$ mice (c) and a 27% reduction in the whole IC size (d), while the reduction in the activated IC was 39% (e).

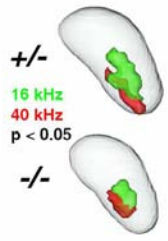

Fig 2. Activity patterns of 16 and 40 kHz were largely overlapped in the *Fgf17*$^{-/-}$ mice. The statistical 3D p-maps showed a normal tonotopic organization in the *Fgf17*$^{+/-}$ mice. In *Fgf17*$^{-/-}$ mice, 16 and 40 kHz activity patterns were reduced and overlapping. Note that the two lobes of the IC in each mouse were registered together before the voxel based cross-subject comparisons (n>14).

Results

To characterize the functional neuronal population in the IC of *Fgf17* $^{-/-}$ and *Fgf17*$^{+/-}$ mice, we exposed mice to broadband sound stimuli (1-59 kHz) to activate a large population of IC neurons responsive to frequencies covering the audible range for mice. We compared the 3D IC images of mice exposed to 1-59 kHz broadband to those kept in quiet environment (Fig 1a). The regions of statistical significance ($p<0.05$) were color-coded in red and defined as the active IC regions (Fig 1a, b). Quantitative analysis of *Fgf17* mutant mice showed that the reduction of active IC region was much greater than the anatomic reduction in *Fgf17*$^{-/-}$ mice (Fig 1c-e). We also analyzed the tonotopic organization of the *Fgf17* $^{-/-}$ and *Fgf17*$^{+/-}$ IC. The 16 and 40 kHz IC activity patterns in *Fgf17*$^{-/-}$ mice were smaller in extent along the axis of the iso-frequency band and were largely overlapped compared to *Fgf17*$^{+/-}$ littermates (Fig. 2). Our results demonstrate clear functional alterations in the auditory central system of *Fgf17* knockout mice.

Conclusions

Fgf17 growth factor is critical for the normal development of the mid-hindbrain region, regulating cell proliferation and differentiation in the midbrain and cerebellum. Using MEMRI the anatomical and functional phenotypes of the *Fgf17* knockout mouse was analyzed quantitatively. These results provide the first example of MEMRI analysis to characterize the functional pathology of a genetically engineered mutant mouse model with an auditory brain phenotype. (This research was supported by NIH grants NS038461 and DC006892)

References

1. Xu J, Liu Z, Ornitz DM. *Development*. **127**, 1833-43, 2000 2. Yu X et al. *Nature Neuroscience*. **8**, 961-8, 2005
3. Yu X et al. *Proc. Intl. Soc. Mag. Reson. Med.* 229, 2006