Anatomical Phenotyping of Cerebellum and Vestibulo-Cochlear Organ in Mice Using Contrast Enhanced Micro-MRI

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Introduction: Micro-MRI imaging has become an important tool for anatomical phenotyping of genetically engineered mutant mice with brain defects [1,2]. Micro-MRI enables three-dimensional (3D) analysis of central nervous system (CNS) anatomy, yielding information that is complementary to that derived through histology and immunohistochemistry. By imaging samples prior to their processing for frozen or paraffin sections it is possible to obtain 3D images of anatomical structures that are not deformed by the process of cryoprotection in sucrose or dehydration in ethanol. In addition, imaging intact brains inside the skull avoids potential damage to delicate brain structures like the olfactory bulbs and the flocculus-paraflocculus complex, and enables analysis of CNS anatomy within the context of the tissues that surround it, including organs that project to the CNS. In this study we demonstrate a potential of contrast enhanced micro-MRI approach [3,4] for simultaneous anatomical phenotyping of the cerebellum (Cb) and the vestibulo-cochlear organ in wild type (WT) mice and *Gbx2*-CKO mutant mice, which have severe defects in the Cb in the form of deletion of its central part [8]. Additionally, these mice display abnormalities in the anatomy of flocculus-paraflocculus complex, a region of the Cb that receives projections from the vestibular organs and is critical for normal vestibular function. It was therefore of interest to determine whether the Cb defects were accompanied by additional, previously overlooked abnormalities in the vestibulo-cochlear organ (VCO).

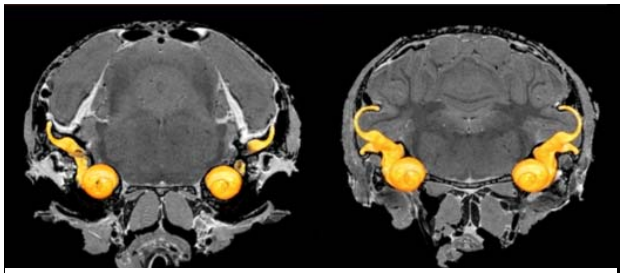

Figure 1. Examples of 2D coronal sections in anterior to posterior direction (A-P) through 3D T1-weighted gradient echo (GRE) micro-MRI image of isotropic resolution of 50µm used for anatomical phenotyping illustrating contrast in the cerebellum (Cb) and surrounding tissues including the VCO (highlighted).

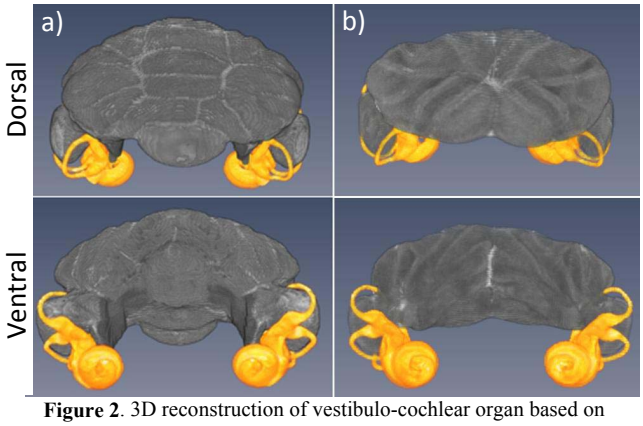

Figure 2. 3D reconstruction of vestibulo-cochlear organ based on micro-MRI images in (a) wt and in (b) *Gbx-2*-CKO mice. Regions of the cochlea and semicircular canals can be seen in detail in the context of the Cb.

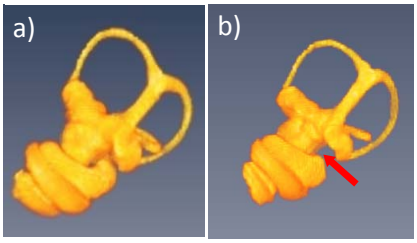

Figure 3. Comparison of 3D reconstructions of VCO of (a) WT and (b) *Gbx2*-CKO mice. An arrow indicates a partial deletion of the *Gbx2*-CKO cochlear region.

Methods: Mice were anesthetized with pentobarbital (0.2 ml/30g) and perfused transcardially with a 10mM solution of gadopenetate dimeglumine (Magnevist, Bayer HealthCare Pharmaceuticals) in 0.9% PBS mixed with heparin (5000 u/L). The initial flush was followed by 4% paraformaldehyde (PFA) at 4ºC mixed with 10mM Magnevist. The brains were left in the skulls and kept in 1mM solution of contrast agent in 4%, 4ºC PFA until imaging 24-48h after perfusion. Heads were transferred to a custom holder and immersed in perfluoropolyether (Fomblin, Solvay Solexis) for the duration of the scan. The MRI data was collected on a 7T micro-MRI (Bruker Biospec), using 750mT/m actively shielded gradients (BGA 09S, Bruker) and a 25-mm (ID) quadrature Litz coil (Doty Scientific). The sequence parameters were: TE/TR=6.26/50ms, FA=40°, FOV=2.56 cm^3, matrix=512^3, NEX=2 and imaging time ~7h 15 min. 3D renderings of the Cb and the VCO were produced in AMIRA (Visage Imaging) using semi-automated segmentation option that was corrected manually. Volumes of structures of interest were calculated by multiplying the number of voxels in the ROI by the volume of individual voxel.

Results and Discussion: Contrast-enhanced micro-MRI has been extensively used in the past to visualize brain anatomy [3, 4] as well as the anatomy of the cochlea [5, 6, 7] in mice and other mammalian species. In this study we demonstrated that it is possible to visualize these structures simultaneously (Figs 1,2). We also showed that in addition to the cochlea, the connecting vestibular system, including the semi-circular canals and otolith organs (utricule and saccule), was enhanced on T1-weighted images, providing sufficient contrast for 3D reconstructions. Volumetric analysis of the vestibulo-cochlear organ in the *Gbx2*-CKO mice revealed a smaller size of this organ in *Gbx2*-CKO compared to WT mice (12% reduction, compared to a 20% reduction of the overall brain volume in *Gbx2*-CKO mice). In summary, the ability to illustrate not only brain anatomy but the anatomy of structures that project to it is of great importance, aiding our understanding of the complex interplay between brain and sensory organs as they develop and providing insights into the function of defined genes in this process.

References: [1] Turnbull DH and Mori S. NMR Biomed; 20(3):265-74 (2007), [2] Nieman BJ et al. NMR Biomed; 18(7):447-68 (2005), [3] Johnson GA et al. J. Magn. Reson Imaging; 16(4):423-429 (2002), [4] Johnson GA et al. Radiology; 222(3):789-793, [5] Henson MM et al. Hearing Research; 75:75-80 (1994); [6] Thorne M. et al. Laryngoscope; 109:1661-68 (1999), [7] Wilson JL et al. Am J Otol; 17(2):347-53 (1996), [8] Li JY et al. Neuron; 36(1):31-43 (2002), [9] Zhengshi L et al. Development; 132(10):2309-2318

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