

MEMRI Atlas of Neonatal Mouse Brain Development

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Introduction: In the past decade significant progress has been made in the field of mouse genetics making the mouse the preferred model for studies of mammalian brain development and neurodevelopmental disorders. In these studies, micro-MRI approaches offer the potential for rapid detection and analysis of brain phenotypes that could be then further investigated with more traditional methods such as histology. Manganese (Mn)-enhanced MRI (MEMRI) approach is particularly well suited to visualize brain anatomy and it has been successfully used to do so in mice *in vivo* at embryonic to adult stages [1-4]. Here, we have extended MEMRI for longitudinal studies of brain development in individual mice during the critical early postnatal period. Based on these data, a brain atlas was created, consisting of individual and average brains at 11 developmental stages, from postnatal day 1 (P1) to P11. The database generated in this project will serve as an important resource for future phenotypic MEMRI analyses of mutant mice with brain defects. This database will allow for voxel-by-voxel analysis of differences in 2D and 3D brain morphology (Figs. 1, 2), rate of growth (Fig. 3), and variability in brain anatomy at different developmental stages, comparing wildtype and mutant mice.

Methods: Mn was delivered to neonates through the milk after maternal intraperitoneal (IP) injection of MnCl₂ in isotonic saline (0.4 mM/kg). Neonatal Fvb/N mice were imaged in two groups at odd and even stages 24h after the maternal injection of MnCl₂ solution. The first group (N=6) was imaged at 6 stages, every other day from postnatal day P1 to P11, and the second group (N=6) at 5 stages from P2 to P10. A 7T Bruker Biospec system with 750mT/m actively shielded gradients and a 25-mm (ID) quadrature Litz coil (Doty) was used to acquire 3D T1-weighted gradient echo images (TE/TR=3.6/50 ms, FA=40°, FOV=(2.56 cm)³, matrix=256³, NEX=2, time~2h) using a self-gated sequence [5]. The neonates were positioned in a custom built holder, including isoflurane anesthesia delivery and warm air to maintain homothermic body temperatures. Registered and averaged images (N=6) were generated at each developmental stage using MICE software following previously described methods [6, 7, 8]. 3D renderings and color coded overlays were produced using Amira (v4.2, Mercury Computer Systems-TGS).

Results and Discussion: Representative mid-sagittal images from P3, P7 and P11 from one individual mouse (Fig. 1a) compared to the corresponding sections from the averaged images (Figs 1b, c). As expected, averaged MEMRI images are characterized by higher SNR and better visibility of different brain regions. Specifically, improvement in visibility of cerebellum (Cb) foliation patterns on the averaged sections allowed for visualization and analysis of changes in Cb morphology that take place within the first week and a half of early postnatal development in 2D sections (Fig. 1 b, c) and 3D renderings (Fig. 1d). Fig. 1 also demonstrates the differential enhancement in different brain regions, and the striking enhancement in many nuclei throughout the neonatal brain (arrows). In addition to brain nuclei, several fiber tracts connecting them could also be visualized (Fig. 2), including the Cb peduncles (not shown) and the fasciculus retroflexus (fr) connecting habenula (Hb) with the interpeduncular (IP) nuclei. Voxelwise analysis of the rate of growth over entire brain was performed based on the brain atlas, showing obvious neonatal growth of the Cb and selected cortical regions (Fig. 3). In summary, the MEMRI Atlas of Neonatal Mouse Brain Development will be an important resource, especially after full annotation, for detection and analysis of brain abnormalities in mutant mice. The ability to analyze changes in brain circuitry and defects in regional growth rates from MEMRI data will provide critical insights into the roles of different genes in brain development and disease.

References: [1] Deans et al. (2008). *MRM* 59(6): 1320-8; [2] Wadghiri YZ et al. (2004). *NMR Biomed* 17(8): 613-9; [3] Koretsky AP, Silva AC (2004). *NMR Biomed* 17: 527-31; [4] Silva AC et al. (2004). *NMR Biomed* 17: 532-43; [5] Nieman BJ et al. (2009). *MRM* 61(5): 1148-57; [6] Kovacevic N et al. (2005). *Cereb Cortex* 15: 639-45; [7] Nieman BJ et al. (2007). *Human Brain Mapp* 28: 567-75; [8] Lerch JP et al. (2008). *Neuroimage* 39: 32-9.

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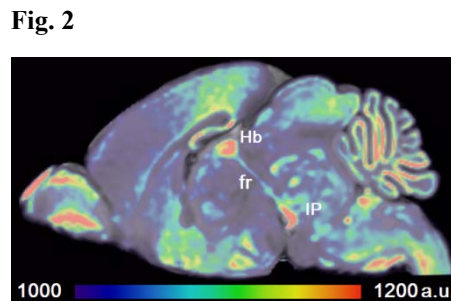
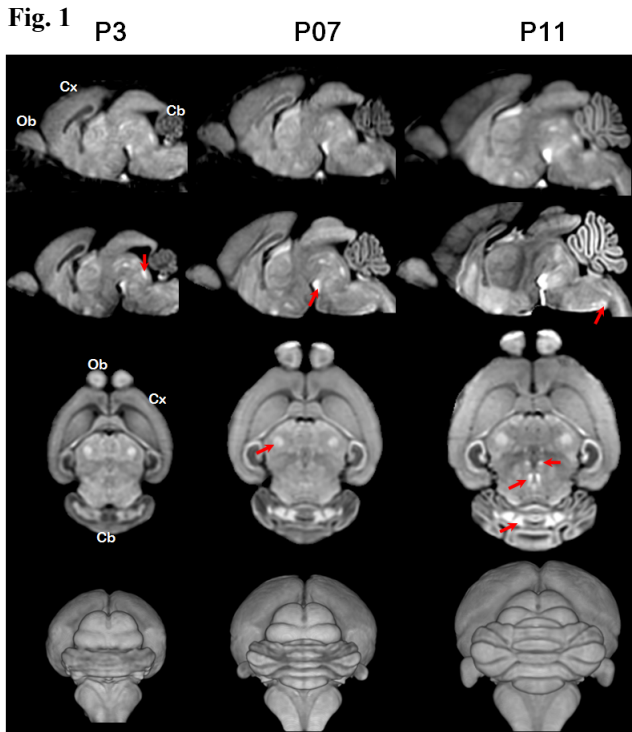


Fig. 2 Color coded 2D sagittal section through the P11 average image. Enhancement of numerous brain nuclei and white matter tracts was apparent on average images at all stages (Hb - habenula, fr - fasciculus retroflexus, IP - interpeduncular nucleus).

