Phase Imaging: a novel tool for myelin quantification.

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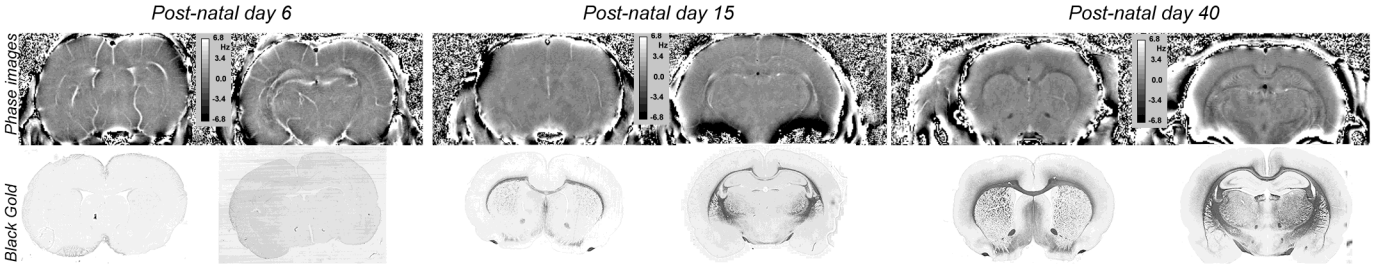
Introduction: Accurate myelin quantification is of prime importance not only in animal models of adult cerebral white matter disease but also in neonatal models of brain injury. Its quantification by MRI would provide a non-invasive tool to evaluate the impact of injury on myelination and the efficacy of neuroprotective treatments. To date, there is no readily available fast MRI-technique for myelin quantification at high field using high resolution multiple slices. Although diffusion anisotropy has been shown to reflect myelin integrity in adult animal models, it is not the case in the developing brain where significant anisotropy can be found in non-myelinated tracks. It is known that phase images acquired with gradient-recalled echo is greatly enhanced at high field and is affected mainly by tissue lipid, iron and microstructure[1], but not by tissue cerebral blood flow as initially postulated[2, 3]. The purpose of this study was to investigate the relevance of phase imaging in detecting and quantifying myelination in developing rats at 9.4 Tesla.

Methods: Post-natal day 4 (P4), P6, P15, P40 Wistar rats were anesthetized with isoflurane (0.5-2 %) and placed in a costumed built MRI holder in ertalyte. Wistar rat were imaged on a 9.4 T scanner (Varian/Magnex Scientific) with a birdcage of 2.5 or 3.5 cm diameter (Stark Contrast, Erlangen, Germany) depending on their age. Magnetic field homogeneity was adjusted using FASTMAP. A standard gradient-echo sequence was used with TR/TE = 900/14 ms, matrix size 256 x 256 with a square FOV of 16 to 27 mm depending on the rat age, a slice thickness of 0.5 mm, an optimized flip angle and bandwidth, and 8 averages. Large-scale phase shifts across the phase images were removed with 2D Gaussian high-pass filter with a kernel size 63 and a width of 5 voxels. Regions of interest were placed on the corpus callosum and on the anterior commissure and frequency shifts differences between those white matter structures and surrounding grey matter were calculated. After MR imaging, the rat received a lethal injection of ketamine and lidocaine and were transcardially perfused with saline followed by 4% paraformaldehyde. Brains were extracted and immersion fixed in 4% paraformaldehyde for 24 hours at 4° C and then cryoprotected by immersion in 30% sucrose. 50 µm thick coronal sections were cut by a cryostat and conserved in a cryoprotectant solution until staining was done. Corresponding brain sections were later stained with Black Gold II myelin staining kit (Millipore) and scanned at 20 X with the Mirax Midi (Zeiss). A mean optical densitometry was calculated on same regions of interest with the software Mercator (Explora Nova).

Figure 1:



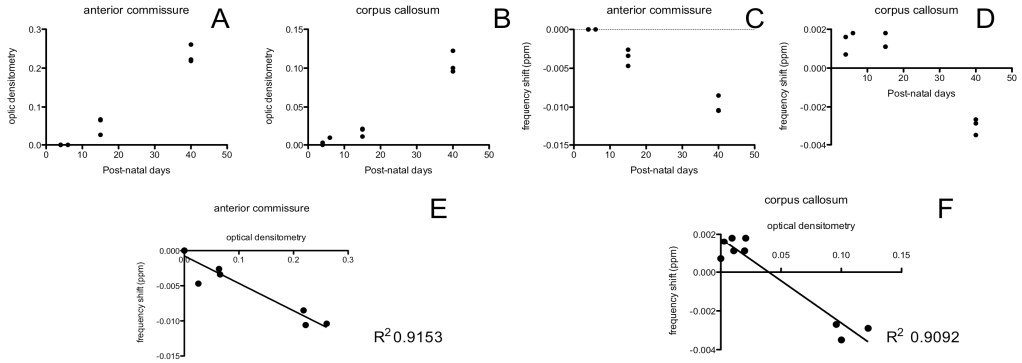


Figure2:

Results and discussion: Phase imaging was able to distinguish myelinated from non-myelinated white matter (Figure 1) in contrast to DTI. Phase imaging contrast distribution at P40 (Figure 1) correlated anatomically with myelinated tracks stained by Black Gold. The linear evolution of myelination assessed by Black gold staining in the anterior commissure and the corpus callosum (Figure 1, 2 A-B) was in agreement with the literature[4]. Initially having the same contrast as the surrounding regions at a time when myelination is not yet present, phase contrast progressed over time (Figure 2C-D) and correlated significantly with myelination (Figure 2 E-F). Early myelination in the corpus callosum seen at P15 on histology was not as evident on Phase imaging (Figure 1, 2D and F), possibly due to partial volume effect or insufficient SNR. These preliminary results indicate that contrast seen in phase imaging in these regions is correlated to myelination. To quantify the myelination level from the frequency shift maps, it would be necessary to back calculate susceptibility maps from the measured frequency shifts[5,6]. The fact that such calculation was not performed explains the different slopes of Fig. 2E and 2F. Nevertheless we can state that the measurement of the local frequency shift is a measure of the local myelination process, given that the geometry of cerebral white matter structures is conserved throughout development.

Acknowlegment: This study was supported by SNF 31003A-112233, by CIBM of the UNIL, UNIGE, HUG,CHUV, EPFL and the Leenaards, Boninchi, De Reuter and Jeantet Foundations.

References: [**1**]. Duyn JH, van Gelderen P, Li TQ, de Zwart JA, Koretsky AP, Fukunaga M. Proc Natl Acad Sci U S A 2007;104:11796-801. [**2**]. Marques JP, et al. Neuroimage 2009; 46:345-52. [**3**]. Lee J et al. Neuroimage 2010;49:193-8. [**4**]. Hamano K, Iwasaki N, Takeya T, Takita H. Brain Res Dev Brain Res 1996;93:18-22. [**5**]. J.P. Marques, R. Bowtell. Concepts in Magnetic Resonance Part B 2005; Vol. 25B(1):65–78. [**6**]. T. Liu et al. Magn. Reson. in Med. 2009, 61 (1):196-204.