Supplementary Material

Methods: Blood Samples

The complete blood count samples were collected in a 0.5-mL EDTA (ethylenediaminetetraacetic acid) tube (BD Microtainer, Franklin Lakes, NJ) and then analyzed using an automated hematology analyzer (Sysmex XN 3000, Sysmex America Inc, Lincolnshire, IL). The iron indices and C-reactive protein were collected in a 0.5-mL serum separator tube (BD Microtainer, Franklin Lakes, NJ) and analyzed using a clinical chemistry analyzer (Architect ci4100, Abbott Laboratories, Abbott Park, IL). All samples, except ferritin, were processed at Women & Infants Hospital. Ferritin was processed at the Mayo Medical Laboratories (Rochester, NY) using an immunoassay system (Beckman Coulter Unicel DXL 800, Beckman Coulter Inc., Brea, CA).

MRI Data Acquisition and Processing

Parents were contacted to schedule the 12-month magnetic resonance imaging (MRI). Children were brought to the MRI center either at nap or bedtime. Special sleep rooms were provided for parents to get the infant to sleep. When sleeping, the infant was securely placed on an MRI-compatible cart and transported to the MRI scanner. Parents (after appropriate screening) were invited to stay with the infant during the MRI. The MRI scan lasted approximately 30 to 45 minutes once the infant was asleep. If an infant was unable to fall asleep or to return to sleep after waking, the MRI exam was rescheduled. Within 1 week after a successful MRI scan, children were scheduled for developmental testing.

Measures of brain myelin content were acquired using the mcDESPOT MRI technique. mcDESPOT is a multicomponent relaxometry technique that decomposes the measured MRI signal into contributions from myelin and nonmyelin water-based on the unique relaxation properties of each of these water pools.[1–4] Unlike traditional multicomponent relaxometry techniques,[1,2] mcDESPOT utilizes rapid and time efficient gradient echo sequences, acquired over a range of flip angles, to quantify the relaxation characteristics of multicompartment water pools.[5,6] Specifically, the mcDESPOT protocol included eight T_1-weighted spoiled gradient-recalled echo (SPGR) and 16 T_1/T_2-weighted balanced steady-state free precession (bSSFP) images acquired over multiple flip angles.[5,6] Two inversion-prepared (IR or inversion recovery)-SPGR images were additionally acquired for correction of radiofrequency or RF (B_1) inhomogeneities and bSSFP images were acquired with two phase cycling patterns ($\phi = 180°$ and 0°) for correction of main magnetic field (B0) inhomogeneities.[7] Choice of scan acquisition parameters for the mcDESPOT protocol has been optimized according to the relaxation characteristics at various stages of infancy and early childhood.[8] Specific acquisition parameters of the SPGR, bSSFP, and IR-SPGR scans used in the current study are:

SPGR: repetition time (TR) = 12 ms; echo time (TE) = 5.8 ms; flip angles (α) = (2, 3, 4, 5, 7, 9, 11, 14) degrees; receiver bandwidth (BW) = 350 Hz/voxel, and 6/8 partial *k*-space in the phase and slice-encode directions.

bSSFP: TR = 10 ms; TE = 5 ms; α = (9, 14, 20, 27, 34, 41, 56, 70); BW = 350 Hz/voxel; 6/8 partial *k*-space in the phase, and slice-encode directions.

IR-SPGR: TR = 12 ms; TE = 5.8 ms; inversion times (TI) = (600, 950) ms; $\alpha = 5$ degrees; 6/8 partial *k*-space in the phase-encode directions. Half the resolution in the slice direction.

All data were acquired from each participating 12-month-old infant on a Siemens Tim Trio 3 Tesla scanner with a 12-channel head RF array. To help the children sleep during the scan, acoustic noise levels were minimized by reducing imaging gradient slew rates and peak values. Additional passive sound attenuation was achieved using a sound-insulating bore liner (Ultra Barrier HD Composite; American Micro Industries, Chambersberg, PA) and MiniMuff ear pads. Electrodynamic and sound-attenuating headphones (MR Confon GmbH, Magdeburg, Germany) were also used and provided constant white noise throughout the duration of the scan.[9]

Following successful acquisition, image data were visually inspected for motion-related image artifacts (e.g., edge blurring and ghosting). Each participant's SPGR, bSSFP, and IR-SPGR images were then linearly co-registered to account for subtle head movement[10] and nonbrain (i.e., skull) signal was removed.[11] SPGR and IR-SPGR images were used to estimate the flip angle correction map.[12] Myelin water volume fraction (VFm) values were calculated at each image voxel by fitting the SPGR and bSSFP data to a multicomponent relaxometry model of three microstructural water compartments: intra/extra-axonal water, myelin-associated water, and nonexchanging free water.[6]

For group comparisons, individual VFm maps were nonlinearly aligned to a common study template[8] using a fully three-dimensional image registration approach.[13] Prior to statistical analyses, aligned VFm data were smoothed with a modest 4-mm full-width-at-half-maximum three-dimensional Gaussian kernel to account for residual registration inaccuracies.[14]

References

1 MacKay A, Whittall K, Adler J, Li D, Paty D, Graeb D. In vivo visualization of myelin water in brain by magnetic resonance. Magn Reson Med 1994;31(06):673–677

2 MacKay A, Laule C, Vavasour I, Bjarnason T, Kolind S, Mädler B. Insights into brain microstructure from the T2 distribution. Magn Reson Imaging 2006;24(04):515–525

3 Deoni SC. Quantitative relaxometry of the brain. Top Magn Reson Imaging 2010;21(02):101–113

4 Alexander AL, Hurley SA, Samsonov AA, et al. Characterization of cerebral white matter properties using quantitative magnetic resonance imaging stains. Brain Connect 2011;1(06):423–446

5 Deoni SC, Rutt BK, Jones DK. Investigating exchange and multi-component relaxation in fully-balanced steady-state free precession imaging. J Magn Reson Imaging 2008;27(06): 1421–1429

6 Deoni SC, O'Muircheartaigh J, Elison JT, et al. White matter maturation profiles through early childhood predict general cognitive ability. Brain Struct Funct 2016;221(02):1189–1203

7 Deoni SC, Mercure E, Blasi A, et al. Mapping infant brain myelination with magnetic resonance imaging. J Neurosci 2011;31(02): 784–791

8 Deoni SC, Dean DC III, O'Muircheartaigh J, Dirks H, Jerskey BA. Investigating white matter development in infancy and early childhood using myelin water faction and relaxation time mapping. Neuroimage 2012;63(03):1038–1053

9 Dean DC III, Dirks H, O'Muircheartaigh J, et al. Pediatric neuroimaging using magnetic resonance imaging during non-sedated sleep. Pediatr Radiol 2014;44(01):64–72

10 Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 2002;17(02):825–841

11 Smith SM. Fast robust automated brain extraction. Hum Brain Mapp 2002;17(03):143–155

12 Deoni SC. High-resolution T1 mapping of the brain at 3T with driven equilibrium single pulse observation of T1 with high-speed incorporation of RF field inhomogeneities (DESPOT1-HIFI). J Magn Reson Imaging 2007;26(04):1106–1111

13 Avants BB, Tustison NJ, Song G, Cook PA, Klein A, Gee JC. A reproducible evaluation of ANTs similarity metric performance in brain image registration. Neuroimage 2011;54(03):2033–2044

14 Mechelli A, Price CJ, Friston KJ, Ashburner J. Voxel-based morphometry of the human brain: methods and applications. Curr Med Imaging Rev 2005;1:105–113