Mouse Brain Iron Distribution: Histochemical and Quantitative MRI (7T) Assessment

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Introduction: Iron plays an essential role in most of the critical metabolic functions of the brain and its regulation is altered in a variety of neurodegenerative diseases. Since NMR signal decay is influenced by the presence of iron, MRI has been frequently used as a non-invasive means to study brain iron. Most studies using MRI to assess brain iron have relied upon measurements of the transverse relaxation rates (R_2, R_2* and R_2') [1], but these parameters can also be affected by iron-independent factors. Recently, we have developed a new MRI-based technique referred to as Magnetic Field Correlation (MFC) Imaging [2;3] for measuring microscopic magnetic field inhomogeneities, such as those generated by iron-rich cells. MFC has the advantage that it is not influenced by molecular relaxation mechanisms, such as dipolar interactions, and consequently, may be a more specific measure of brain iron providing information complementary to that given by other MR parameters. In this study, we investigated the relationship between quantitative MFC, R_2, R_2* and R_2' values and iron distribution (determined from published histochemical analyses [4]) in normal mouse brain at 7Tesla.

Methods: A total of 7 (C57Bl/6 x DBA_2) x SW mice (7-month old) were perfused with phosphate-buffered saline (PBS) (pH 7.4) through the left cardiac ventricle, followed by 10% buffered formalin. After perfusion fixation, the brains were removed, embedded in agar (0.4%), positioned at the center of a plastic tube and tightly sealed for MR measurements. All experiments were performed on a 7 T MR system (SMIS, Guilford, UK). R_2 was acquired using a multi-slice single spin-echo sequence with the following imaging parameters: 2 averages, 25 slices, FOV=12.8mm, matrix=64 x 64, echo times (TE) of 15,25,40,60 ms, and repetition time (TR) of 3000 ms. R_2* was acquired using a gradient echo sequence with the following imaging parameters: 4 averages, 25 slices, FOV=12.8mm, matrix=64 x 64, TE of 4,8,13,20 ms, and (TR) of 750 ms. For the calculation of R_2 and R_2*, parametric maps were generated using exponential fits to multi-echo data using MEDx software (Sensor Systems Inc., Sterling, VA). R_2' was calculated as (R_2*- R_2). MFC images were acquired using an asymmetric dual spin-echo pulse sequence. Imaging parameters were TE/TR = 30/3000 ms, FOV=12.8mm, slice thickness 200 μm, matrix=64 x 64, and echo time shifts of 0, ±3, ±6, ±7.8. MFC values were calculated by non-linear least square fit of signal intensities on echo time shifts, based on the relationship between MFC and asymmetric echo time shift [2]. Regions of interest (ROIs) were manually drawn in the cortex (Ctx), caudate and putamen (Cpu) region, globus pallidus (Gpa) and substantia nigra (SN). R_2 and R_2* were estimated, for each ROI, by computing the mean values from the respective maps. To estimate MFC values, the signal intensity averages were computed prior to fitting.

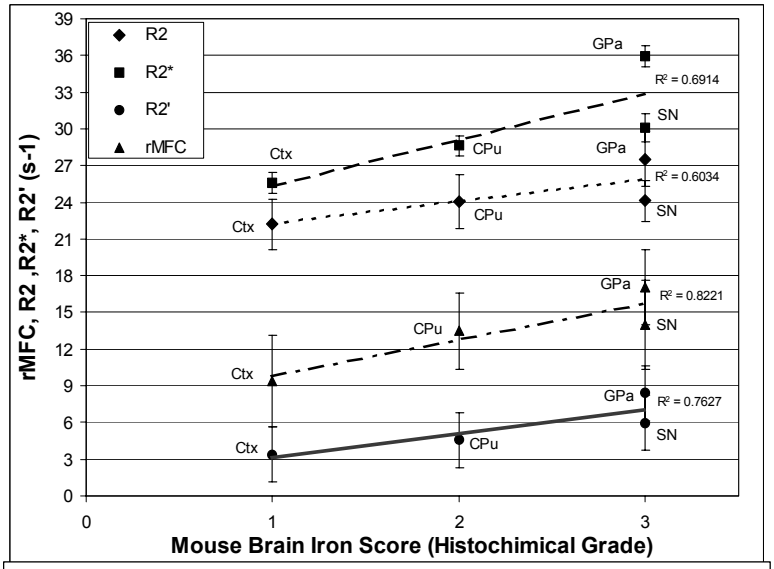

Figure 1: Regional rMFC, R2 , R2* and R2' versus mouse brain iron score based on histochemical grade (1= low; 2= moderate; 3= high) as reported on Hill, 1988. Brain regions are: cortex (Ctx), Caudate/Putamen (Cpu), Globus Pallidus (Gpa) and Substantia Nigra (SN).

Results and Discussion: Figure 1 shows a graph of rMFC, R_2, R_2* and R_2' values versus mouse brain iron graded histochemically, determined from published histochemical analyse [4] for various brain regions. Here, $rMFC=(MFC)^{-1/2}$. While all MRI parameters showed a positive correlation with iron grade in mouse brain areas known to have high iron concentration and distribution, such as globus pallidus and substantia nigra, the rMFC demonstrated a stronger linear correlation than either R_2, R_2* or R_2', reflecting the fact that these quantities are physically distinct. We also measured these MR parameters in two 20-months old mice to assess the age-dependence of these parameters. Although the number of mice is not sufficient for a meaningful statistical analysis (data are part of an ongoing project), the data clearly shows that all of these MR parameters correlate with the known increase of iron content occurring with age in the globus pallidus. Furthermore, the data indicate that the regional variation in MFC values is higher than that for the other MR parameters (Gpa rMFC mean value for 7-month old group (n=7) = 16.93 and for 20-months old group (n=2) = 22.53) suggesting that MFC imaging is more sensitive to iron-induced differences in brain tissue susceptibility. In conclusion, our data suggest that MFC imaging provides a distinct MR measure and can be a potentially useful tool for assessing brain iron and the role of brain iron disruption in the pathogenesis of many neurodegenerative diseases.

References: **1**. Haacke EM, et al. Magn Reson Imaging. 2005 Jan;23(1):1-25. **2**. Jensen JH and Chandra R. Proc. Intl. Soc. Magn. Reson. Med. 2002b;10:2297. **3**. Lee SP et al. Proc. Intl. Soc. Mag. Reson. Med. 2005;13 :421 **4**. Hill JM, The distribution of iron in the brain. In: Brain Iron: Neurochemistry and Behavioural Aspects, Youdim MBH, ed., Taylor & Francis, London, pp.1-24, 1988.

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