Age-related increased R2 and R2* correlates with increased brain iron in a normal ageing mouse model

Thomas Walker[1], Christos Michaelides[1], Harry Parkes[2], William Crum[1], Tina Geraki[3], Amy Herlihy[4], and Po-Wah So[1]

[1]Department of Neuroimaging, Institute Of Psychiatry, King's College London, London, United Kingdom, [2]CR-UK, Clinical MR Research Group, Institute of Cancer Research, Sutton, Surrey, United Kingdom, [3]Diamond Light Source, Harwell Science and Innovation Campus, Didcot, Oxfordshire, United Kingdom, [4]Agilent Technologies, Yarnton, Oxfordshire, United Kingdom

Introduction: Impaired cognitive and motor abilities are hallmarks of ageing, resulting from a multitude of perturbed molecular and structural mechanisms[1, 2]. Dysregulated iron homeostasis[3] has been postulated as one potential key mechanism, involving dysfunctional mitochondria and induction of oxidative stress. Application of MR relaxometry in ageing studies suggests age-related elevations in iron, most notably in the basal ganglia[5-7]. To date, no study has demonstrated correlation of age-related changes in MR relaxometry with direct measures of brain iron levels. The purpose of this research was therefore to measure age-related changes in R2 and R2* in the basal ganglia and correlate these changes to direct iron levels by synchrotron-based X-ray fluorescence (SR-XRF) in a normal ageing mouse model. This study is thus targeted to an audience interested in age and age-related disease and the use of animal models of ageing to determine the biological substrates underlying age-related changes in MRI metrics.

Methods: Paraformaldehyde-fixed brains from male C57BL/6J mice aged 2 (n= 11), 6 (n= 8), 19 (n= 11) and 27 months (n= 8) underwent MRI at 7T. R2 relaxometry was performed with a multi-echo fast spin-echo sequence with a TR of 7600ms and 16 echo times with variable TE (11-186ms). R2* relaxometry was performed with a multi-echo gradient echo sequence with a TR of 2000ms and 5 echo times with variable TE (2.5-26.5ms). For all relaxometry sequences, the field of view = 30mm x 30mm, matrix size = 256 x 256 and 40 contiguous axial slices were collected at 0.5mm thickness for whole brain coverage. Following MRI, brain samples were cryoprotected and sectioned at 40µm SR-XRF elemental iron mapping at Diamond, at 100µm in-plane resolution. Values of R2, R2* and iron were obtained from regions of interest (ROIs) located in the basal ganglia (striatum, STR; globus pallidus, GP and substantia nigra, SN) using Image J (NIH) from the relaxometry and SR-XRF elemental iron maps, respectively.

Results and Discussion: Significantly higher R2 (data not shown) and R2* was observed with advancing age in the STR, GP and the SN, with a greater increase observed with R2* (P<0.001, Figure 1A-C). These findings are consistent with that seen from human studies of ageing[5-7].

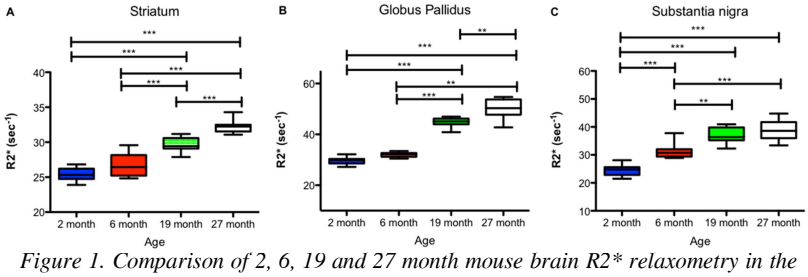

Figure 1. Comparison of 2, 6, 19 and 27 month mouse brain R2 relaxometry in the (A) striatum, (B) globus pallidus and (C) substantia nigra.*

Significant age-related increases in iron were observed in the STR (P<0.001), GP (P<0.001) and SN (P=0.0029), (Figures 2 and 3). To our current knowledge, this study is the first to show that the increased R2 and R2* in the basal ganglia during normal ageing are concurrent with increased iron within these regions. The data here substantiates the use of R2 and R2* MR relaxometry for detecting dysfunctional iron homeostasis within the basal ganglia in normal ageing. Histopathological investigations are underway to determine the age-related changes in ferritin and the potential immune and neurodegenerative consequences of the aforementioned iron increase.

Conclusion: Age-related increases in R2 and R2* found here in the aged C57BL/6J mouse brain are consistent with those reported in human ageing[5-7] and we have shown this arises, at least partially, from increased iron. Furthermore, normally ageing C57BL/6J mice may be a test-bed for exploring age-related mechanisms and testing future therapies to attenuate the detrimental effects of ageing.

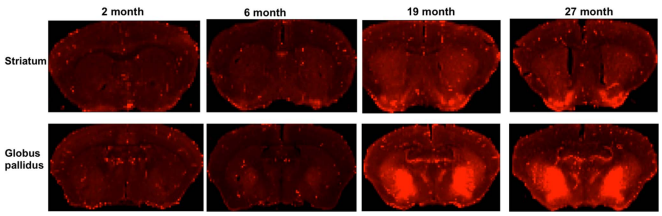

Figure 2. SR-XRF elemental iron maps showing elevated iron in the STR and GP from 2 to 27 months of age

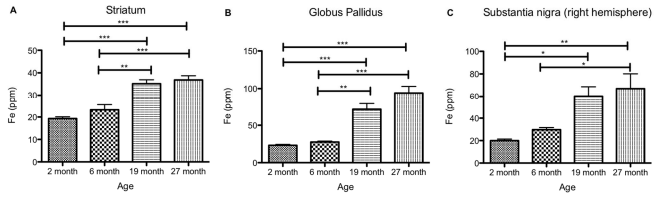

Figure 3. Comparison of 2, 6, 19 and 27 month mouse brain iron content in the (A) striatum, (B) globus pallidus and (C) substantia nigra.

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