Age-related increased R2 and R2* in the C57BL/6J mouse Basal Ganglia correlated with elevated iron levels measured by synchrotron-radiation X-ray fluorescence

Thomas Walker[1], Christos Michaelides[1], Harry Parkes[2], William Crum[1], Amy Herlihy[3], 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]*Agilent technologies, Yarnton, Oxfordshire, United Kingdom*

Introduction: Brain senescence is a complex process affecting a multitude of molecular and structural mechanisms culminating in impaired cognitive and motor abilities[1, 2]. Dysfunctional mitochondria driven by oxidative stress mechanisms have been postulated as potential key mediators of senescent mechanisms[3], with a possible role for dysregulated iron homeostasis[4]. MR relaxometry, a well-established iron-sensitive technique, 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 study changes in MR relaxometry and iron levels within the basal ganglia of young and aged mouse brains, to establish if changes in iron underlie age-related changes in MR relaxometry previously observed. This study is thus targeted to an audience interested in age and age-related disease and the use of animal models of ageing to discover the underlying biology of age-related changes in MRI metrics.

Methods: Paraformaldehyde-fixed brains from male C57BL/6J mice aged 2 and 19 months (n= 11/age group) 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. Values of R2 and R2* were obtained from regions of interest (ROIs) located in the basal ganglia (caudate, globus pallidus and substantia nigra) using Image J (NIH) from the relaxometry maps. Following MRI, brain samples were cryoprotected and sectioned at 40µm for synchrotron radiation X-ray fluorescence (SR-XRF) elemental iron mapping at ANKA, at 100µm in-plane resolution.

Results and Discussion: Aged mice had significantly higher R2 (data not shown) and R2* values in the caudate, globus pallidus and the substantia nigra ($P<0.0001$, Figure 1A-C) than young mice, consistent with that seen in human studies[5-7]. Preliminary semi-quantitative (normalised to the cortex) SR-XRF elemental iron maps showed relatively higher levels of iron in the basal ganglia, e.g. caudate, globus pallidus and substantia nigra, compared to cortical iron levels in aged mice: this readily observable regional difference in iron levels was not apparent in the young mice (Figures 2 and 3).

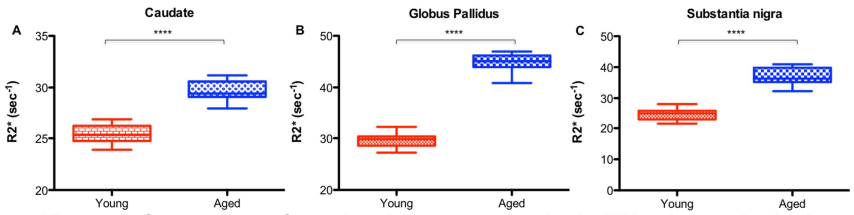

Figure 1. Comparison of aged and young mouse brain R2 relaxometry in the (A) caudate, (B) globus pallidus and (C) substantia nigra.*

Increased iron is therefore a likely source of the aforementioned relaxometry changes in these regions. To our current knowledge, this study is the first to show that the lengthening of R2 and R2* values in normal ageing in the basal ganglia is concurrent with direct and semi-quantitative measured increases in iron within these regions. The data here substantiates the use of R2 and R2* MR relaxometry for detecting perturbed iron homeostasis within the basal ganglia in both normal ageing and neurodegenerative disease.

Conclusion: MR relaxometry changes in the basal ganglia of the aged human brain is also evident in the aged C57BL/6J mouse brain, suggesting C57BL/6J mice may be a test-bed for exploring age-related mechanisms and for testing future therapies to attenuate the detrimental effects of ageing. Further investigations are needed to determine if the increased iron, observed in aged brains in this study plays a crucial role in unhealthy ageing processes.

References: (1) Cass WA, et al. *Neurobiology of Aging.* 2007; 28: 258-71. (2) Clausen A, et al. *Neurobiology of Aging.* 2010; 31: 425-33. (3) Schipper HM, et al. *Ageing research reviews.* 2004; 3: 265-301. (4) Zecca L, et al. *Nature Reviews: Neuroscience.* 2004; 5: 863-873. (5) Bartzokis G, et al. *Magnetic Resonance Imaging.* 1997; 15: 29-35. (6) Bilgic B, et al. *Neuroimage.* 2012; 59: 2625-2635. (7) Penke L, et al. *Neurobiology of Aging.* 2012; 33: 510-517.

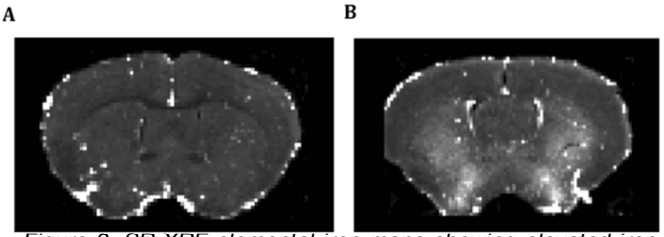

Figure 2. SR-XRF elemental iron maps showing elevated iron in the caudate region relative to the cortex in the (A) young and (B) aged mouse brain.

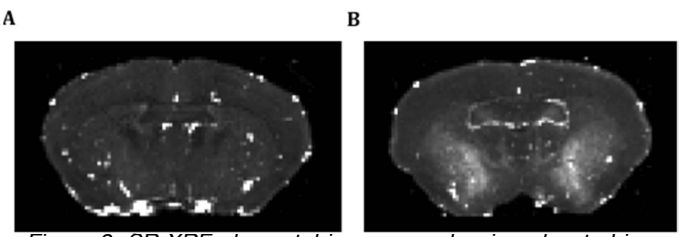

Figure 3. SR-XRF elemental iron maps showing elevated iron in the globus pallidus region relative to the cortex in the (A) young and (B) aged mouse brain.