# Effects of systemic iron and/or inflammation on mouse brain R2*

***Azhaar Ahmad Ashraf*** [1], ***Chantal Hubens*** [1], ***Harry G. Parkes*** [1,2], ***Xinwei Chen*** [1], ***Manal Aljuhani*** [1], ***Po-Wah So*** [1].

[1]*Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom.* [2]*Institute of Cancer Research, Sutton, Surrey.*

## Introduction

Iron dyshomeostasis, accompanied by progressive cognitive decline, is associated with brain ageing[1,2]. Iron stimulate microglia, increase NFκB activation and pro-inflammatory cytokine production[3]. Conversely, lipopolysaccharide (LPS)-induced inflammation increased expression of the iron storage protein, ferritin[1,3]. The aim of this study was to determine if brain R2* (as a measure of iron) differs in saline or iron-treated mice, both with and without systemic LPS-induced neuroinflammation.

## Methods and Materials

C57Bl/6J adult mice (8 weeks, male) were treated with intraperitoneally (IP) with saline (n = 15) or iron (II) sulphate (3mg/kg, n = 16) daily for five consecutive days and then given saline or LPS (0.25mg/kg) IP at 13 weeks of age yielding four groups: Saline (n=7), Iron+saline (n=8), saline+LPS (n=8) and Iron+LPS (n=8). At 15 weeks of age, mice were anesthetized, transcardially perfused with phosphate-buffered saline (PBS) and brains rapidly removed. The left hemisphere was fixed in 4% paraformaldehyde in PBS for 48 hours before storage in PBS-sodium azide (at 4ºC) until MRI. *Ex vivo* T2* was performed on a 7T MRI scanner, using a multi-echo, gradient echo sequence with 7 TEs (2.8 - 25.6 ms), echo spacing = 3.8 ms; TR = 60ms, 6 averages; flip angle 15º; bandwidth = 46296.3; field of view (FOV), 19.2 x 19.2 x 19.2 mm and matrix size, 128 x 128 x 64. R2* maps were calculated from T2* maps, the latter generated by pixel-by-pixel fitting to $y = M_0 \exp^{(-TE/T2^*)}$ and region of interests manually drawn in the substantia nigra (SN), striatum, hippocampus and cortex on the R2* maps using JIM (Xinapse Systems, UK). One-way analysis of variance (ANOVA) with post-hoc Tukey's correction was used to test for R2* differences between treatment groups with $p<0.05$ considered significant.

## Results and Discussion

R2* was significantly higher in the SN, striatum, hippocampus and the cortex of all groups compared to the saline group ($p<0.0001$, Figure), suggesting iron injections and/or inflammation increases iron content in these brain regions. Iron and inflammation appears to synergistically increase iron accumulation in the SN as R2* in the iron+LPS group compared to the LPS-only group, highlighting the vulnerability of nigral neurons to iron and inflammation-induced oxidative stress.

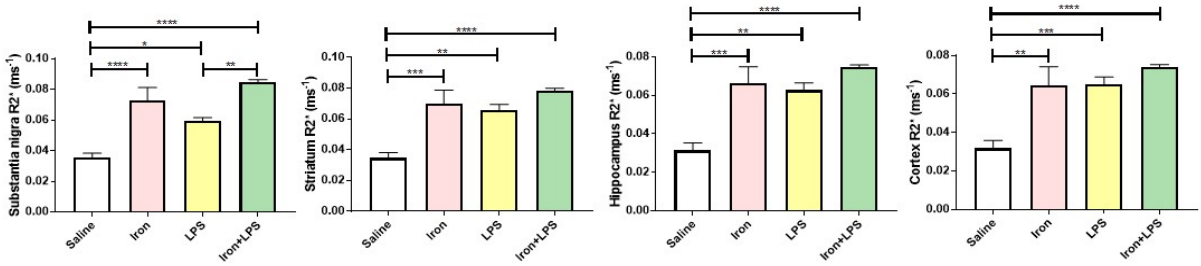


Figure: R2* from various regions of the brain in mice treated with saline, iron, LPS and iron+LPS. Values are mean ± SEM. P<0.05*, P<0.01**, P<0.001***, P<0.0001****.

## Conclusion

Our data strongly suggests that systemic administrations of iron and/or LPS increases the brain R2* signal, particularly in the substantia nigra, suggesting increased brain iron in these areas.

## References

[1]Ashraf et al (2018). Front Aging Neurosci 10:65. [2]Walker et al (2016). Aging (Albany NY) 8(10):2488-2508. [3]Li et al (2016). J Neuroinflammation 13(1):268.