

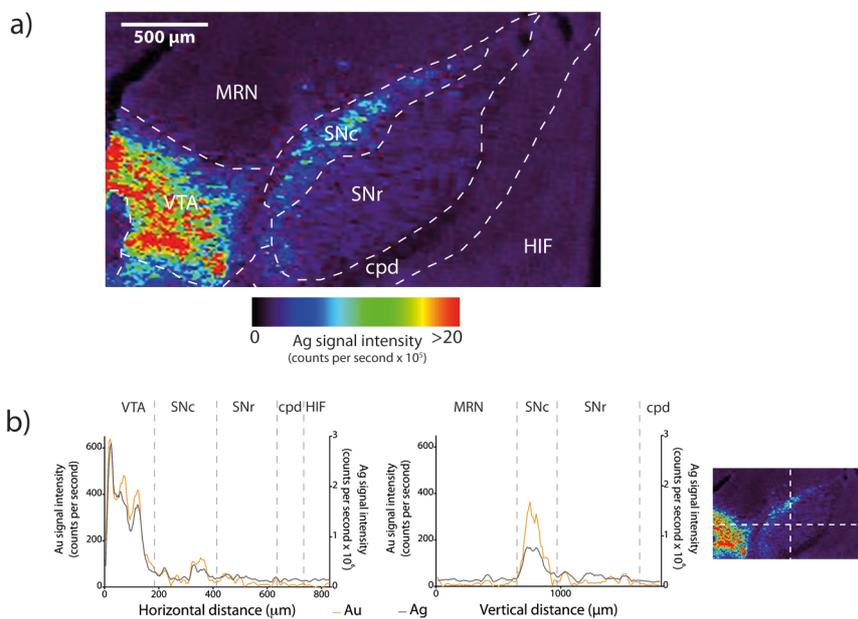
Supporting Information for:

An iron-dopamine index predicts risk of parkinsonian neurodegeneration in the substantia nigra pars compacta.

Dominic J. Hare, Peng Lei, Scott Ayton, Blaine R. Roberts, Rudolf Grimm, Jessica L. George, David P. Bishop, Alison D. Beavis, Sarah J. Donovan, Gawain McColl, Irene Volitakis, Colin L. Masters, Paul A. Adlard, Robert A. Cherny, Ashley I. Bush, David I. Finkelstien and Philip A. Doble.

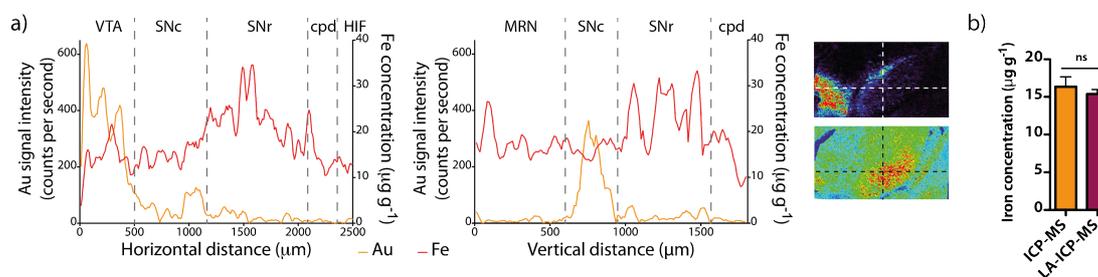
SUPPLEMENTARY FIGURES

Supplementary Figure 1: Ag enhanced TH immunolabeling



(a) Ag enhancement kit was used to reduce ionic Ag ions to metallic Ag on the Au nanoparticle surface, confirming the presence of Au-labeled TH positive neurons. (b) Overlaid time resolved analysis plots of Au ($m/z = 197$) and Ag ($m/z = 107$) in both horizontal and vertical planes.

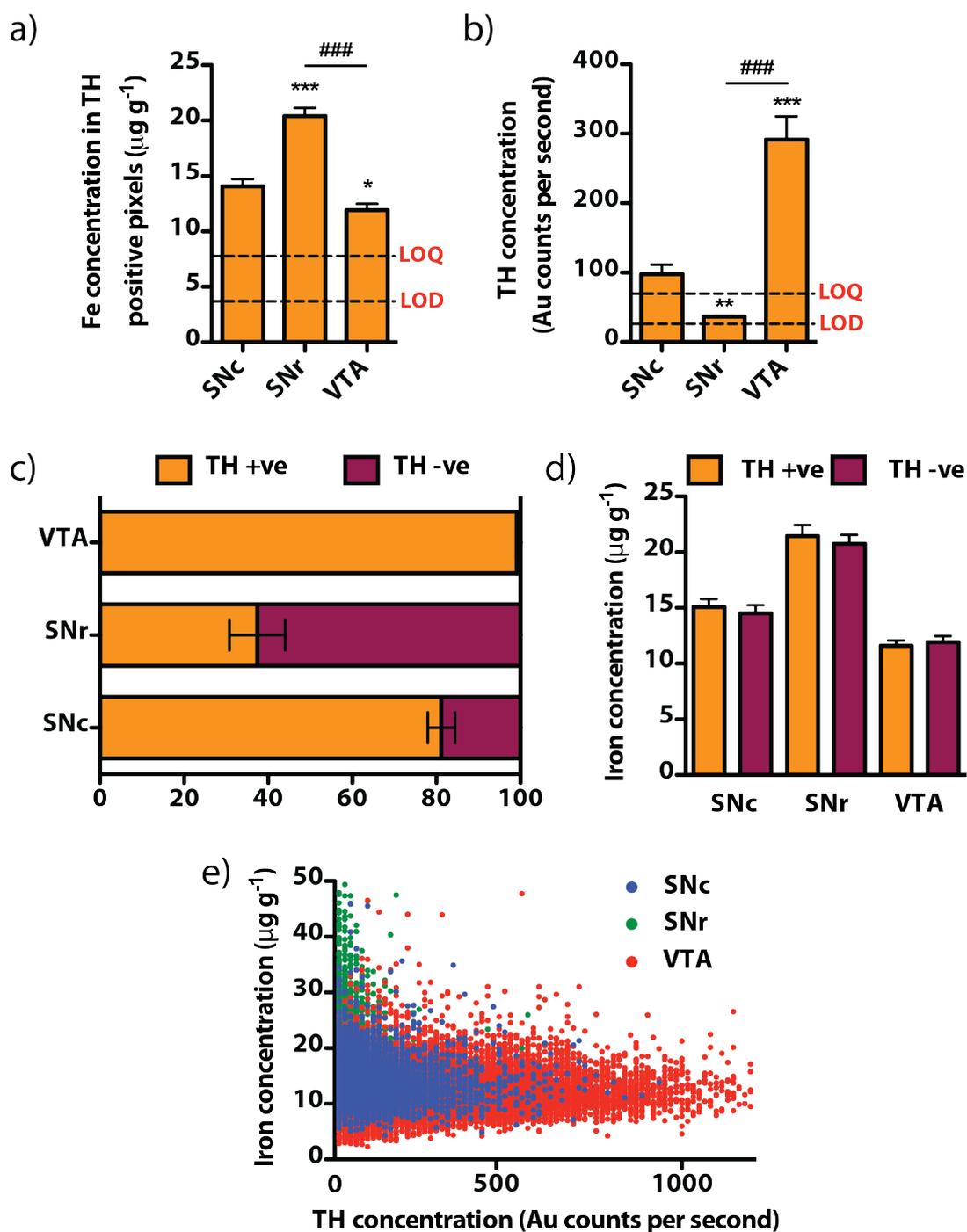
Supplementary Figure 2: Fe is distributed throughout the midbrain according to granular nuclei and is analytically valid



a) Horizontal and vertical line profiles demonstrates the distribution of Fe within the midbrain varies depending on nuclei. b) No significant deviation in Fe content was

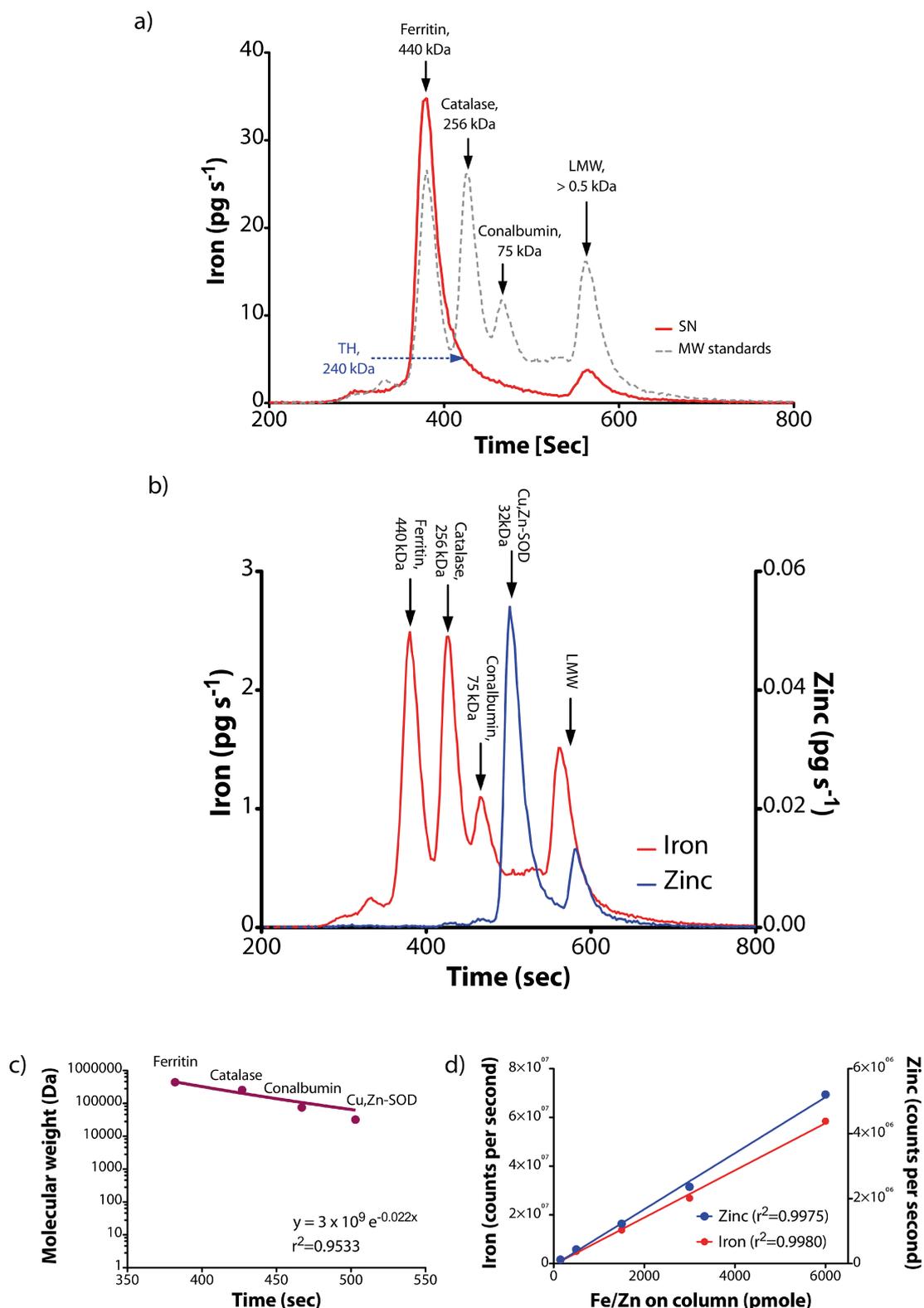
observed in each alternate hemisphere ($n = 4$, $P = 0.5$) when analyzed by bulk dissection, digestion and solution nebulization ICP-MS compared to mean Fe concentration in the midbrain determined by LA-ICP-MS.

Supplementary Figure 3: Fe in TH-positive and -negative pixels



(a) and (b) The distribution of Fe and TH in the dopaminergic neurons of the mesencephalon mirrored that of the whole nuclei. (c) The percentage area of each nuclei containing TH also reflected the TH concentration per TH-positive pixel. (d) No significant variation between TH-positive and negative areas was identified. (e) A small, yet significant negative correlation ($r_s = -0.199$, $p < 0.001$) was observed between Fe and TH.

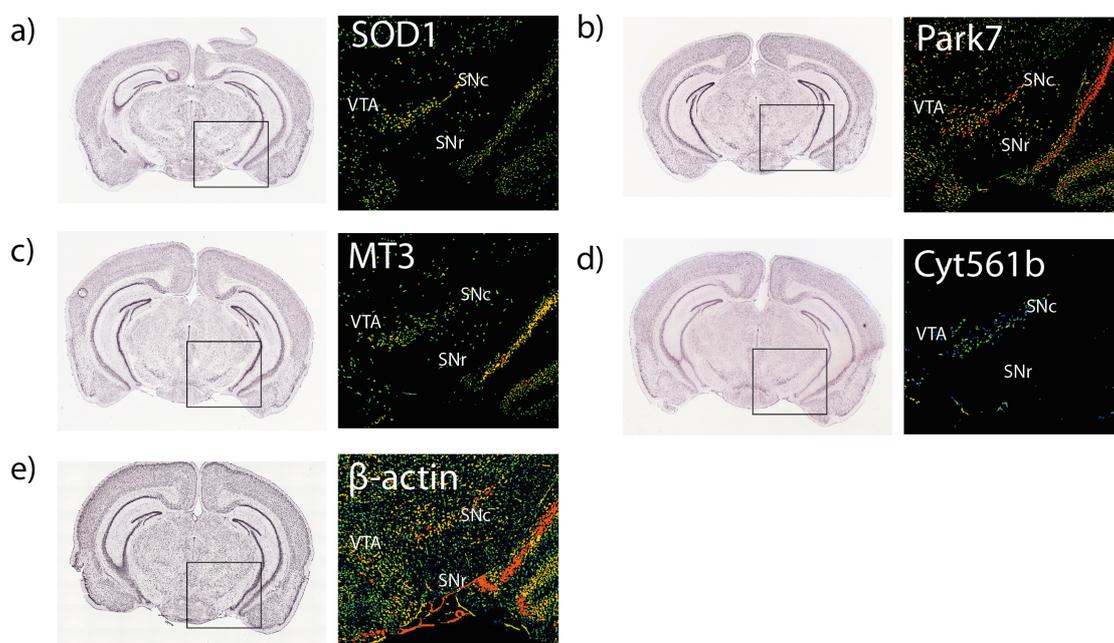
Supplementary Figure 4: SEC-ICP-MS analysis of Fe binding proteins



(a) SEC-ICP-MS analysis of soluble Fe-binding proteins in the mesencephalon by peak area showed that 83.7% of soluble-phase Fe could be attributed to the major Fe

storage protein ferritin (MW = 440 kDa), with the other major component of soluble Fe (10.1%) bound to low molecular weight (LMW) species, such as APT and citrate ligands. The approximate retention time for tetrameric TH (MW = 240 kDa) is marked in blue. (b) and (c) The SEC column was calibrated for molecular weight range using standard solutions of Fe-containing proteins ferritin (440 kDa), catalase (256 kDa) and conalbumin (75 kDa), and the zinc-containing copper/zinc superoxide dismutase (Cu,Zn-SOD; 32 kDa). (d) Chromatograms are displayed in pg s^{-1} following calibration against increasing injected amounts of Fe (as ferritin) and zinc (as Cu,Zn-SOD). LMW = low molecular weight species.

Supplementary Figure 6: mRNA expression of antioxidant proteins in the mouse brain



(a) Superoxide dismutase-1, (b) Park7 (DJ-1), (c) metallothionein-3, (d) cytochrome 561b and (e) β -actin mRNA ISH and expression masks from the Allen Reference Atlas¹.

SUPPLEMENTARY TABLES

Supplementary Table 1: Limits of analysis.

Limits of detection (3σ) and quantification (10σ) for Fe ($\mu\text{g g}^{-1}$) Au and Ag (counts per second). Reported as average \pm 95% confidence interval ($n = 6$).

	Fe ($\mu\text{g g}^{-1}$)	Au (counts per second)	Ag (counts per second $\times 10^3$)
Limit of detection (LOD)	4.11 ± 1.88	31.6 ± 4.1	2.67 ± 2.21
Limit of quantification (LOQ)	7.92 ± 3.25	65.9 ± 12.4	5.49 ± 5.37

SUPPLEMENTARY METHODS

Solution nebulization ICP-MS. Lyophilized homogenates of mouse midbrains were digested in 50 μL Suprapur 65% HNO_3 (Millipore) at room temperature overnight, then at 90°C for 20 minutes. An equivalent volume of 30% Aristar H_2O_2 (BDH) was added and samples were further digested at 70°C for 30 minutes. Samples were diluted to volume in 1% HNO_3 and analysed using an Agilent Technologies 7700x ICP-MS.

Size exclusion chromatography ICP-MS. Excised mouse mesencephalon was added to 40 μL Tris buffered saline (TBS, 50 mM Tris pH 8.0, 150 mM NaCl) containing EDTA free protease inhibitors (Roche) (40 μL) and homogenized using polypropylene pestle designed for 1.5mL centrifuge tubes. After homogenization the sample was centrifuged for 5 min at 16,500 g at room temperature. The supernatant was recovered and used for analysis by size exclusion ICP-MS. Separations were performed on an Agilent 1200 Series liquid chromatography system hyphenated to an Agilent 7700x ICP-MS. Helium was used as a collision gas for interference removal. An Agilent Technologies BioSEC 5 size exclusion column (5 μm particle size, 300 \AA pore structure; 4.6 mm i.d.) was used with a 50 mM ammonium nitrate (pH 8)

buffer. The column was calibrated using a mixed standard solution of ferritin, catalase, conalbumin (GE) and bovine superoxide dismutase-1 (Sigma).²

Statistics

Statistical unpaired *t*-tests were performed using Prism (GraphPad). All tests were two-tailed, with the level of significance set at $P = 0.05$. Analysis of variance (ANOVA) was performed using SPSS (IBM). Levene's test was used to determine homogeneity of variance, which determined the post hoc test used (Tukey HSD or Games-Howell). The level of significance was set at $P = 0.05$. All data values are reported as \pm SEM. Limit of detection was calculated using the blank signal (S_{blank}) and standard deviation (σ_{blank}) of the blank signal, according to:

$$LOD = S_{blank} + 3\sigma_{blank}$$

Limit of quantification was calculated according to:

$$LOQ = S_{blank} + 10\sigma_{blank}$$

Limits of analysis are given in **Supplementary Table 1**

SUPPLEMENTARY REFERENCES

1. E. S. Lein, M. J. Hawrylycz, N. Ao, M. Ayres, A. Bensinger, A. Bernard, A. F. Boe, M. S. Boguski, K. S. Brockway and E. J. Byrnes, *Nature*, 2006, 445, 168-176.
2. D. J. Hare, A. Grubman, T. M. Ryan, A. Lothian, J. R. Liddell, R. Grimm, T. Matsuda, P. A. Doble, R. A. Cherny, A. I. Bush, A. R. White, C. L. Masters and B. R. Roberts, *Metallomics*, 2013, 5, 1656-1662.