

Background

- The Locus Coeruleus (LC) is a small brainstem structure (~1 mm width, ~15 mm length [1]).
- Responsible for memory and emotion processes, rich in iron, copper and neuromelanin (NM) [2].
- Early affected in Alzheimer's disease (AD), undergoing demyelination and increased iron accumulation [1].
- Imaging the LC is very difficult, especially in-vivo and in-situ acquisitions [1,2].
- Quantitative Susceptibility Mapping (QSM) is a quantitative MRI (qMRI) technique sensible to iron [3].
- Electron Paramagnetic Resonance (EPR) is a spectroscopic technique sensible to paramagnetic ions [4].

Main objective

- Assess the main **biophysical sources** of contrast within the LC in 7T MRI of ex-situ brains.

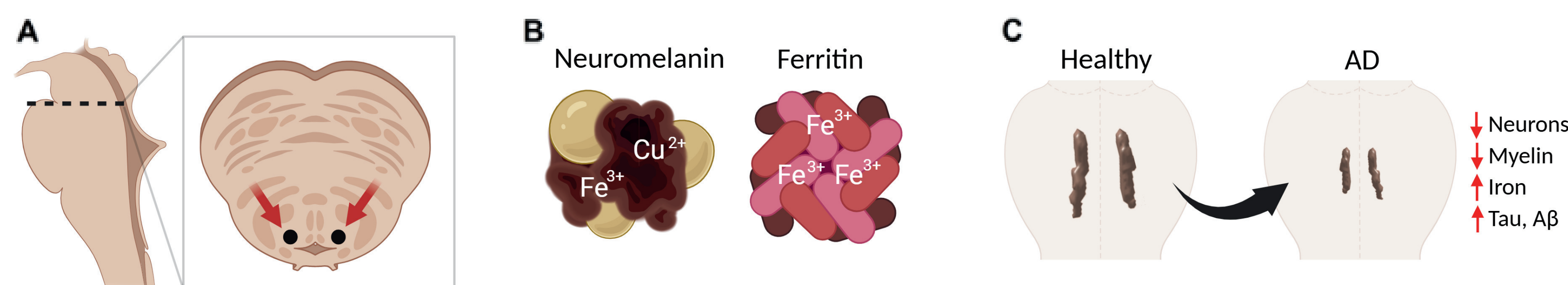


Figure 1. (A) Schematic view of the LC (red arrows), including (B) the main macromolecules present in this structure, and (C) the effect of neurodegeneration, especially in AD.

Methodology

Samples

- 20 post-mortem subjects (healthy, 66±15 years old, 2 female).
- Brain extraction and formalin fixation (>30 days).
- LC extraction after ex-situ MRI.

7T MRI (SIEMENS) of Ex-situ brains

- 3D gradient echo (GRE) sequence (Flip angle = 20°, TR/TE = 42 ms/3 ms, 13 echoes, 0.5x0.5x0.5 mm³).

Spectroscopy

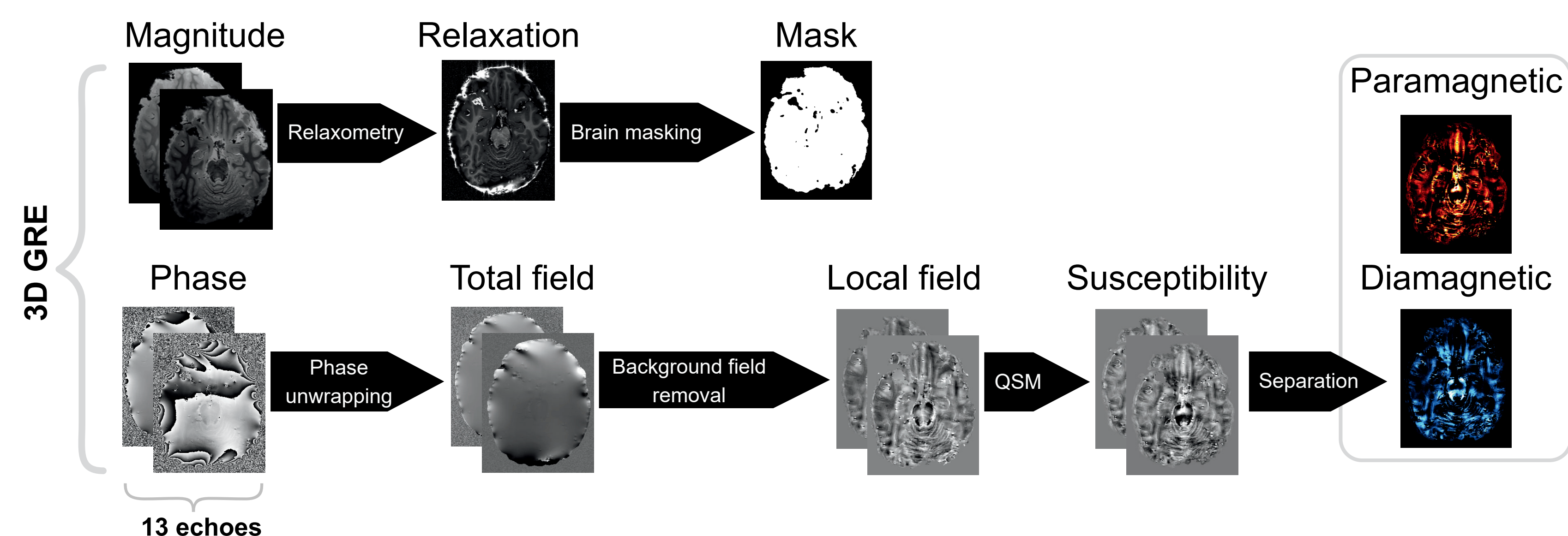
- Electron Paramagnetic Resonance (9 GHz EPR, JEOL) for paramagnetic ions relative concentrations.
- Mass spectrometry (ICP-MS, PerkinElmer) for iron and copper absolute concentrations.

Statistical analysis

- Manual segmentation of the LC region-of-interest (ROI) to extract qMRI values.
- Linear fitting and Pearson's correlation between qMRI values and spectroscopic measurements (p<0.05).

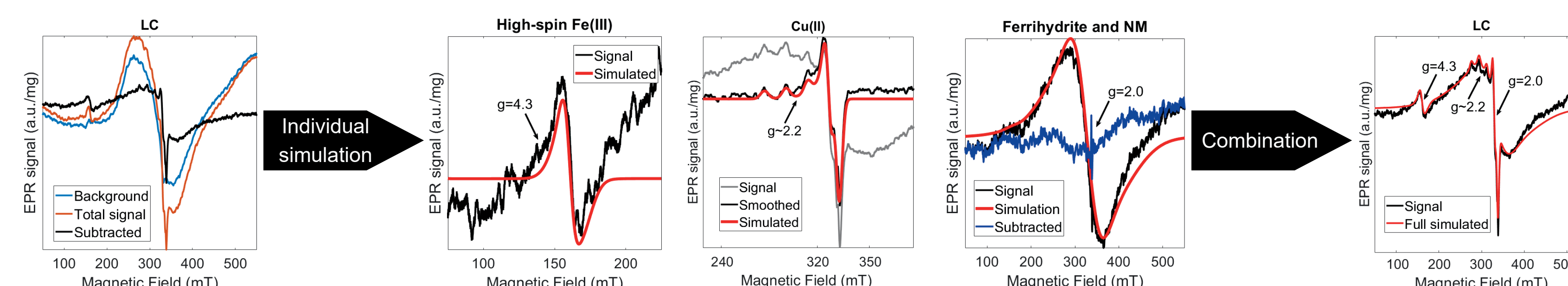
Image processing (relaxometry and susceptibility mapping)

- Exponential fitting of T2*w image to calculate R2* map (ARLO [5]).
- Brain masking (BET [6]) from R2* map and refining to remove air bubbles (ITK-SNAP [7]).
- Phase unwrapping (ROMEO [8]) and background field removal (LBV [9] and V-SHARP [10]) before susceptibility mapping (STAR-QSM [11]). Paramagnetic and diamagnetic components of susceptibility are separated from QSM (DECOMPOSE [12]).



Spectral processing (EPR)

- Pre-processing steps: background signal subtraction and baseline correction.
- Individual simulations (EasySpin [13]): High-spin Fe(III) (g=4.30), Cu(II) (g~2.22), ferrihydrite (g=2) and organic NM radical (blue, g=2).
- Quantification: 2nd integral over simulated signal, NM radical is made by peak-to-peak amplitude.



Main findings

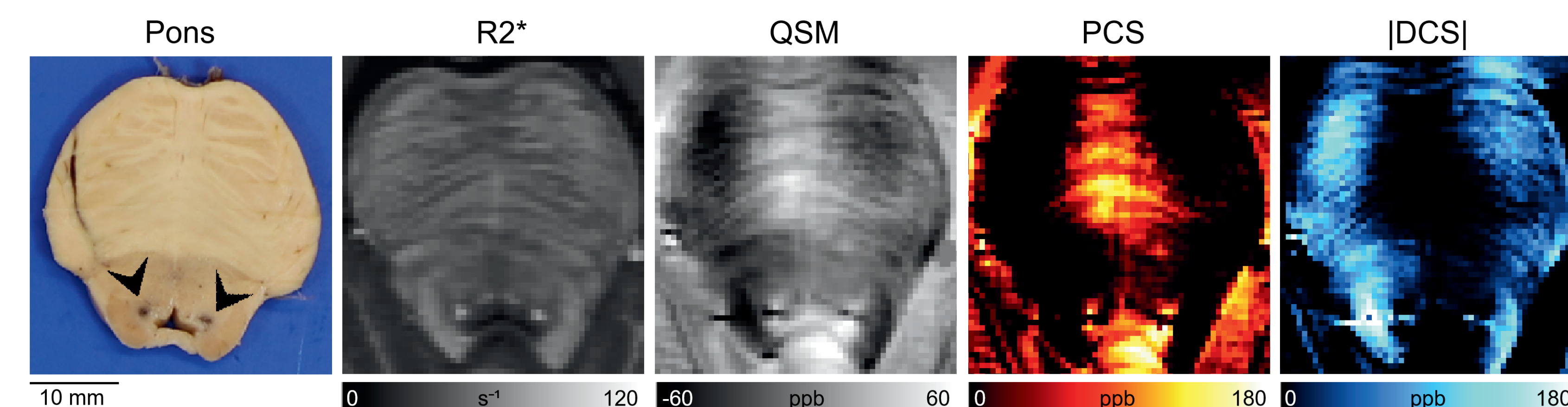


Figure 2. qMRI of the LC from a representative post-mortem subject of this study. From left to right: pons sample including the LC (black arrowheads), relaxation rate map (R2*), susceptibility map (QSM), paramagnetic component of susceptibility (PCS) and diamagnetic component of susceptibility (DCS) from DECOMPOSE.

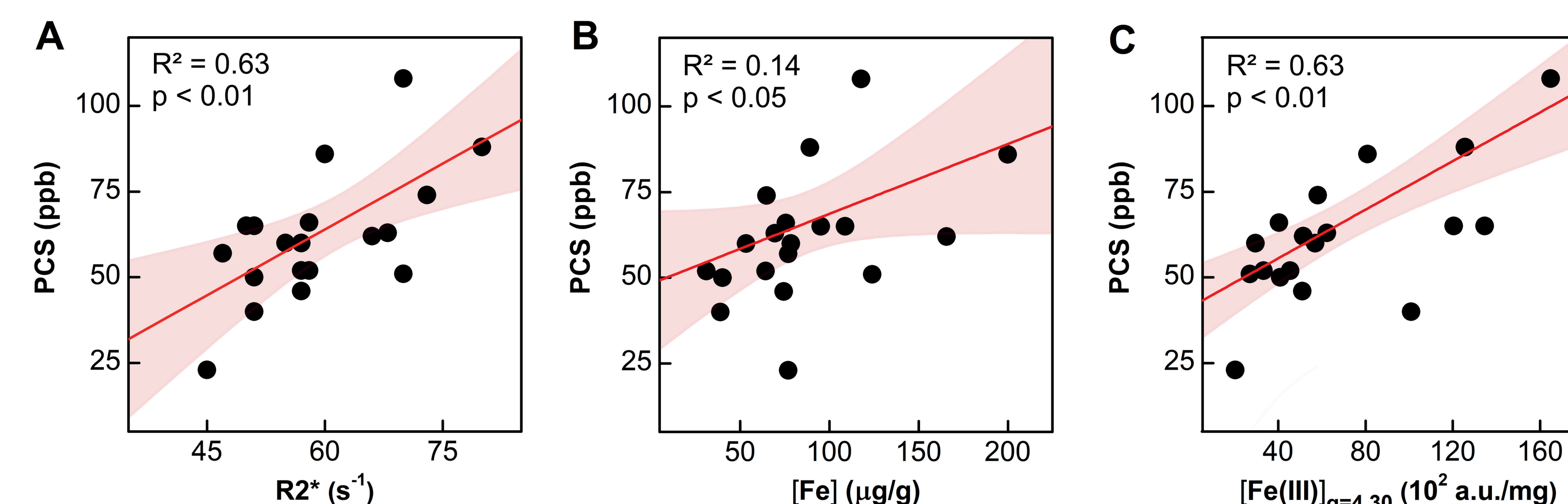


Figure 3. Correlation analysis between qMRI values and spectroscopic measurements, with 95% confidence interval. (A) Paramagnetic component of susceptibility (PCS) vs relaxation rate (R2*), slope is 1.3 ppb/s⁻¹. (B) PCS vs absolute iron concentration ([Fe]) from ICP-MS, slope is 0.2 ppb/μg/g. (C) PCS vs relative concentration of high-spin Fe(III) (g=4.3) from EPR, slope is 0.04 ppb/a.u./mg.

- The LC is well visualized in qMRI of the ex-situ brain.
- There is no correlation between Cu(II), ferrihydrite and organic NM radical with qMRI of the LC.
- Iron is the main responsible for the LC contrast in R2* and paramagnetic maps.
- Fe(III) (g=4.30) has stronger correlation with qMRI values rather than the absolute iron concentration.

Conclusions

- Iron is the principal biomarker in 7T MRI of the LC in the ex-situ brain, especially the high-spin Fe(III).