Supplement for:

## A combined MR and CT study for precise quantitative analysis of the avian brain.

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## Additional information for Results and Discussion



**Supplementary Figure 1 | Comparative effects of SPT treatment for improved CT contrast of intracranial structures in adult** *Phasianus colchicus*. (a) Visualization of a male specimen with SPT showing enhanced contrast between major brain tissue regions; (b) female specimen with no internal structures detected when SPT was withheld. Arrow indicates internal structures visible only after SPT treatment.



**Supplementary Figure 2** | **MR imaging (2D RARE) of a control sample** (*Opisthocomus hoazin*) **to test the effects of prolonged scan time.** (a) Visualization immediately following removal from medium; (b) visualization after 13 hours prolonged scanning. Highlighted area (dashed line) was targeted for comparative T<sub>2</sub> calculations.



**Supplementary Figure 3** | **Co-registration of CT and 3D RARE MR images of the cranium from an adult** (male) *Phasianus colchicus*. (a) CT image; (b) checkerboard mosaic image (from CT and MR) registered by mutual information minimization in 3D; (c) MR image.



**Supplementary Figure 4 | Delineation of brain compartments in 3D RARE MR images of the cranium from an adult (male)** *Phasianus colchicus*. (a) Saggital middle plain; (b) saggital plane 2.7 mm from the middle plain. Colour demarcation: red = prosencephalon; yellow = mesencephalon; purple = cerebellum and hindbrain (rhombencephalon). Compartmental areas were delineated manually based on Karten *et al.*<sup>1</sup>

## References

Karten, H. J., Brzozowska-Prechtl, A., Lovell, P. V., Tang, D. D., Mello, C. V., Wang, H., & Mitra, P. P. Digital atlas of the zebra finch (*Taeniopygia guttata*) brain: A high-resolution photo atlas. *J. Comp. Neurol.* 521, 3702–3715 (2013).

## Additional information for Methods

Supplementary Table 1 | Parameters for relaxometry measurements of  $T_1/T_2$  versus SPT. Abbreviations: • $T_1$ , utilizes saturation recovery sequence; •• $T_2$ , utilizes CPMG sequence.

Parameters	•T <sub>1</sub>	••T <sub>2</sub>
Recycle delay (s)	10	5
Number of points	10	2000
Duration factor	1.85	-
Pulse separation (ms)	-	1
Echo number	-	2000



Supplementary Figure 5 | Screen capture of the Fakir probe compartmental brain volume measurements from an adult (male) *Phasianus colchicus*. Orthoslice includes one of seven parallel line sets. Colour demarcation: green = non-brain tissues or space; purple = prosencephalon; red = mesencephalon; yellow = cerebellum and hindbrain (rhombencephalon); cyan = spinal cord. Filled squares delimit the brain surface.