

Optimised μ MRI for Phenotyping the *Tc1* Model of Down Syndrome

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Introduction Active staining of *ex-vivo* tissues, where MR contrast agents are used to shorten T_1 during the fixation process, is now a key part of MR microscopy. Recently it has been shown that using higher contrast agent concentrations ($>5\text{mM}$ Gd-DTPA) when preparing excised mouse brains can lead to enhanced delineation of structures[1]. Although excised brains allow full contrast agent penetration into tissue in as little as 5 days, brains left in the skull are less susceptible to damage and preparation effects during tissue extraction and subsequent imaging. Resulting volume measurements and morphometric analysis may also be more accurate. We sought to develop an active μ MRI optimised preparation and scanning protocol for imaging intact adult mouse brains in-skull, advancing our previous work on imaging mouse embryos[2]. We have used this protocol to perform an initial assessment of phenotypic differences in a transchromic (*Tc1*) mouse model of Down Syndrome[3].

Methods *Animal Preparation:* Mice were culled by an overdose of anaesthesia and then flushed with saline introduced via the left ventricle. They were then perfused-fixed with 4% buffered formol-saline (Pioneer Research Chemicals) with 8mM Gd-DTPA (Bayer-Schering). The head was decapitated and skin, muscle, lower jaw removed and post-fixed in 4% buffered formol-saline (with 8mM Gd-DTPA) at 4°C. *Imaging:* Performed on a Varian 9.4T VNMRs system with a 26mm quadrature birdcage coil (RAPID Biomedical). Brains were immersed in Fomblin (Solvay Solexis) and imaged in 20ml syringes. *Fixation Study:* 4 Wild-type *Tc1* (mixed 129s and C57Bl6 background) mice were fixed and imaged over a 5 week period. T_1 and T_2^* maps were obtained at each time point. Parameters: Single sagittal slice (0.5mm thick), matrix=128², FOV=19.5x13mm, NSA=4, TR was at least 5x T_1 . T_1 : IR-SE, 13 TIs, TE=11ms; T_2^* : GE, FA=90°, 8 TEs. Structural 3D GE images at unoptimised parameters were also obtained (FOV=20.48x13.04x13.04mm, matrix size=512x326x326, TE/TR/FA=6ms/20ms/60°, NSA=2 or 6). Measured tissue parameter values were input into a Matlab program based on the equation for spoiled gradient echo to determine the optimal scan parameters for a 3 hour scan-time. *Phenotyping study:* We imaged 28 (14 *Tc1*⁻, 14 *Tc1*⁺) male mouse brains (aged 18-21weeks), fixed for 9 weeks in 4% buffered formol-saline (with 8mM Gd-DTPA), using a spoiled-gradient echo sequence. Optimised 3D GE scan parameters: FOV=20.48x13.04x13.04mm, matrix size=512x326x326, TE/TR/FA/NSA=4ms/17ms/52°/6.

Results *Fixation Study:* While T_2^* values were found to stabilise relatively quickly, reaching a minimum at 3 weeks fixation (mean basal ganglia=2.9ms, cortex=3.8ms – data not shown), T_1 values took more time to plateau, approaching uniformity across the brain by the 5 week timepoint (Figure 1, top). Figure 1 (bottom) shows representative maps from a brain over 5 weeks. Maps of 4 mice from our 28-brain study population showed a uniform T_1 across the whole brain at 9 weeks fixation (example in Figure 1, bottom right). The reduction in T_1 resulted in a visible increase in SNR across the brain (Figure 2). The final images of our 28 study brains had an average SNR of 32 with 40 μ m isotropic resolution. They also demonstrated excellent contrast, which enabled the delineation of a number of structures (Figure 3).

Phenotyping Study: After visual inspection of the *Tc1* mice we noted that 10 individuals showed marked ventricular enlargement and defects in the choroid plexus as compared to wild-type brains (Figure 4). One individual showed particularly gross malformations in the ventricles and hippocampi.

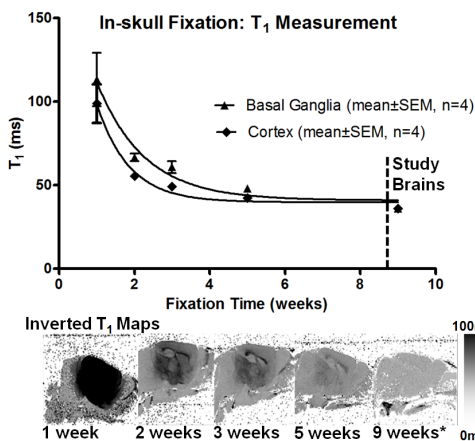


Figure 1: (Top) Graph showing timecourse of T_1 reduction in a central basal ganglia region of interest and the cortex. (Bottom) Example T_1 maps from an individual over 5 weeks; (*) map from an individual in our 28-brain study population.

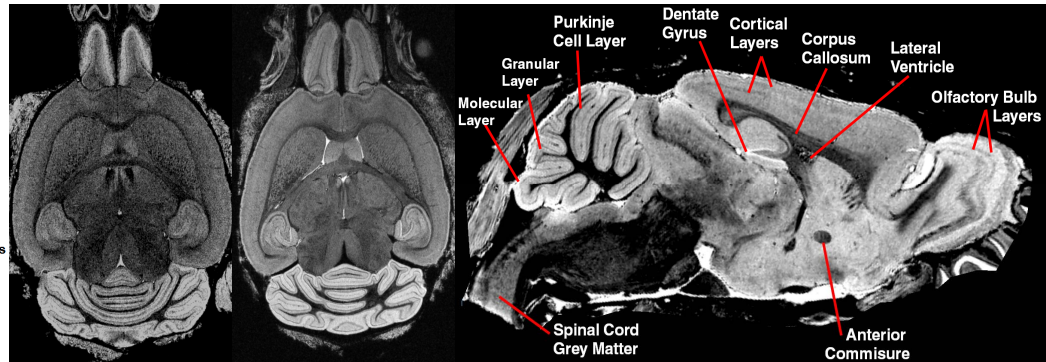


Figure 2: Similar axial slices through the same brain imaged after 1 week (left) and 5 weeks (right). SNR is visibly improved in the 5 week image, especially in the regions of the cortex and basal ganglia.

Figure 3: Sagittal slice through a wild-type *Tc1* brain fixed for 9 weeks and imaged at optimised parameters. A number of cortical and hippocampal structures are readily seen.

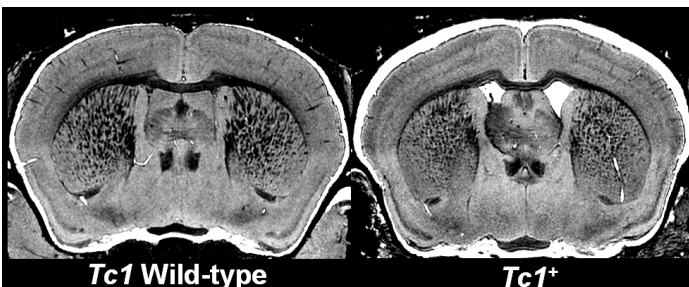


Figure 4: Coronal slices through *Tc1* wild-type (left) and transgenic brains (right). Ventricular enlargement and overall brain atrophy is seen in this typical *Tc1*⁺ individual.

Conclusion We have produced detailed MR images of mouse brains, prepared in-skull, with high SNR. We demonstrated that full penetration of Gd-DTPA, as assessed by T_1 values in the cortex and basal ganglia, required over 5 weeks fixation, and had equilibrated across the brain by 9 weeks. Optimisation of parameters facilitated imaging at a 40 μ m isotropic resolution in a scan time of 3 hours per brain. Individual layers in the hippocampus and cerebellum were easily delineated. Cortical layers could also be visualised. We achieved a SNR of ~ 32 , and this has been previously shown advantageous for visual assessments of brain data[4]. However, a lower SNR may be acceptable for automated, morphometric analysis[5] and this may enable higher resolution and/or a reduced scan time. In future studies we aim to use a coil with a longer z-axis profile, where multiple brains may be imaged in a single acquisition, increasing throughput. Finally, on application of this methodology, we have identified ventricular changes in the majority of *Tc1*⁺ mice, which is a new morphological phenotype in this mouse model of Down syndrome, indicating a structural consequence associated with the additional human chromosome in these mice. The development of active μ MRI histology provides anatomical detail that will enable greater sensitivity when performing morphometric phenotyping studies.

References [1]Kim S, et al. *NMR Biomed* 2008, 22(3): 303-309, [2] Cleary JO, Price AN et al. *NMR Biomed* 2009, 22(8): 857-866, [3] O'Doherty A, et al. *Science* 2005, 309(5743): 2033-2037, [4]Kale SC, et al. *NMR Biomed* 2009, 22(5), Pages 488-494, [5]Kale SC, et al. *Hum Brain Mapp* 2008, 29(10), 1111-1214

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