Cingulate and sensorimotor cortical changes in the R6/2 Huntington's disease mouse: a study of 116 brains

S. J. Sawiak[1,2], N. I. Wood[3], G. B. Williams[1], A. J. Morton[3], and T. A. Carpenter[1]
[1]Wolfson Brain Imaging Centre, University of Cambridge, Cambridge, Cambs, United Kingdom, [2]Behavioural and Clinical Neurosciences Institute, University of Cambridge, Cambridge, United Kingdom, [3]Department of Pharmacology, University of Cambridge, United Kingdom

Introduction Huntington's disease (HD) is a fatal, inherited, neurodegenerative condition with no known cure. The R6/2 mouse is the most widely used mouse model used to study this condition and prospective preclinical treatments. We have previously used MRI to create an atlas of this mouse brain and applied voxel-based and deformation-based morphometry (VBM and DBM respectively) to assess its structural phenotype [1]. Historically, most attention is paid to the subcortical structures in this model, particularly the basal ganglia. Recent evidence suggests there are significant cortical changes so here we have used a voxel-based cortical thickness (VBCT) method [2] to find focal differences in cortical structure between the R6/2 mouse and wildtype (WT) controls.

Methods The brains of 116 mice (58 WT, 58 R6/2, aged 9-18 weeks) were scanned *ex vivo* at 70µm isotropic resolution using a 1T Bruker console (TR/TE 2000/50ms ETL 4 NEX 4, matrix 256×192×128 FOV 18.9×13.4×9.0mm^3). Images were brought into the same stereotactic space in the production of a minimum-deformation atlas [3], the cortex of which was then segmented and transformed to each individual image using the high-resolution deformation fields produced with the atlas. Cortical thickness was found by solving Laplace's equation with outer and inner cortical surfaces as isopotentials, using resistive boundaries around the corpus callosum following Lerch et al [4] (see figure 1A). The gradient of the solution (Figure 1B) gives a unique path (figure 1C) which can be followed at each point and integrated to define a thickness value at each voxel. A median filter was applied to the gradient fields prior to integration with a simple Euler method with stepsize 0.2 voxels (14 µm). Each voxel was thus given a value for cortical thickness to produce a thickness map, the entire algorithm taking approximately 2 minutes per brain. A map was produced for each brain and these volumes were then transformed back to atlas space before smoothing with an isotropic 200µm Gaussian kernel. SPM5 [5] with the SPMMouse toolbox [6] was used to perform a two-tailed *t*-test between WT and R6/2 brains including overall brain volume and age as covariates in the analysis. The false-discovery rate (FDR) was controlled at $p < 0.05$ to correct for multiple comparisons.

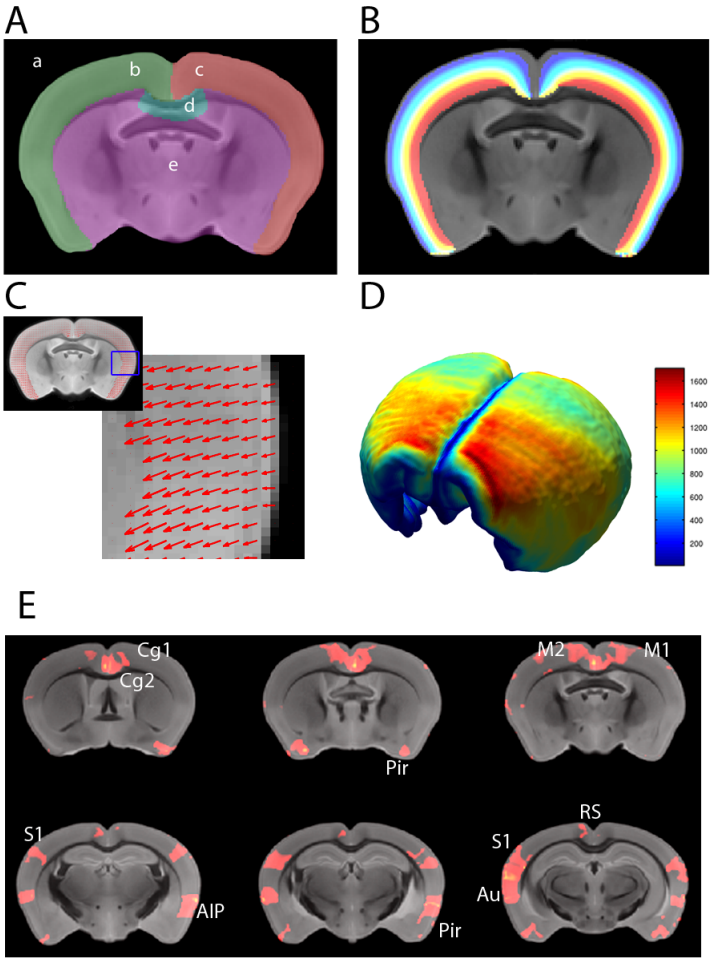

Figure 1. (**A**): atlas segmented to produce boundary conditions for Laplace's equation (a: external, b-c: right and left cortices, d: resistive boundary, e: internal). (**B**) the solved potential; (**C**) illustration of gradient vectors integrated to produce thickness values; (**D**) a map of thickness in the atlas of 116 brains (µm); (**E**) significant differences between WT and R6/2 brains ($p_{FDR} < 0.05$)

Results
Figure 1D shows the cortical thickness map found for the atlas [1]. The statistical parametric map (SPM) showing differences between WT and R6/2 brains is shown in Figure 1E. Differences are found in motor cortices (predominantly M1), cingulate cortices (Cg1, Cg2) and sensory cortices (particularly S1).

Conclusion
The significant thinning of the motor, sensory and cingulate cortex found in R6/2 mice in the current study reflects the motor, sensorimotor and learning and memory deficits found previously in these mice [7, 8], and also mirrors the extensive degeneration of the cingulate found by conventional histology in R6/2 mice [9]. Thinning of the motor and sensory cortex has been found previously using MRI in HD patients [10], which reinforces the value of the R6/2 mouse as a model of HD. The use of a voxel-based method with volume data instead of the alternative surface-based approaches has several advantages including reduced importance of registration accuracy and increased signal to noise ratio. The algorithms for VBCT are also simpler and do not require specialist segmentation software.

In the YAC128 model of HD it has been suggested early cortical changes may be associated with a potential compensatory mechanism before the onset of later atrophy [4]. We intend to extend our study with longitudinal *in vivo* imaging using an aging cohort of R6/2 mice to shed light on this hypothesis. We will also apply it to the hippocampus, which has been implicated in cognitive deficits in this model.

References
[1] Sawiak, S.J. et al (2009) Neurobiol. Dis. 33(1) pp12-20; [2] Hutton, C et al (2008) NeuroImage 40(4) pp1701-1710; [3] Sawiak, S.J. (2007) et al Proc ISMRM Berlin; [4] Lerch, J (2008) NeuroImage 41(2) pp243-251; [5] Ashburner et al NeuroImage (2005) 26(3) pp939-51; [6] Sawiak, S.J. (2009) et al Proc ISMRM Hawaii; [7] Carter, J. et al (1999) J. Neurosci. 19(8) pp3248-3257; [8] Lione, L. et al (1999) J. Neurosci. 19(23) pp10428-10437; [9] Turmaine, M. et al (2000) PNAS 97(14) pp8093-8097; [10] Rosas H.D. et al (2008) Brain 131 pp1057-1068.