# Using Continuous Time Random Walk Diffusion to Quantify the Progression of Huntington's Disease

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**Target Audience** – Investigators in the MR community interested in developing new tools for evaluating axonal degeneration in Huntington's Disease (HD).

**Purpose** – HD results from accumulation in the brain of a malformed huntingtin protein that expresses an abnormal repetition of the amino acid glutamine. Prior work[1] indicated that degeneration of axons projecting from specific neuronal populations in the cerebral cortex might play an important role in HD pathogenesis. This makes imperative the development of non-invasive imaging tools aimed to monitor changes in axonal integrity prior to the development of clinical symptoms. Previous studies of HD-affected patients documented quantitative changes in diffusion MRI parameters, such as fractional anisotropy in the corpus callosum[2] and subcortical gray regions[3], albeit using the standard Gaussian random walk (Brownian motion) assumptions for the diffusion of water in brain tissues. By extending the Gaussian model by using the continuous time random walk (CTRW) description of diffusion, a greater sensitivity to micro-architectural "heterogeneity and tortuosity" in the brain has been attained[4]. This technique encodes additional information in the fractional order parameters, $\alpha$ and $\beta$, which correspond to the fractional order of the time and space derivatives, respectively, in the governing CTRW anomalous diffusion equation[4]. *Here, we examine the utility of CTRW-based diffusion MRI techniques to distinguish brain tissue alterations in R6/2 mice, a well-characterized HD animal model.*

**Methods** – R6/2 transgenic mice and wild type littermate controls were sacrificed and perfused with a 4% praformaldehide solution at post-natal days 21 (P21, n=3 control and n=1 R6/2) and 60 (P60, n=3 control and n=3 R6/2, currently being acquired) using an approved animal protocol. Brains were imaged at a vertical bore 750 MHz Bruker Avance III HD magnet using Micro-2.5 gradients (1500 mT/m) at the AMRIS MR imaging center, in Gainesville, FL. Images for localization were first acquired using a rapid acquisition with refocused echoes (RARE) sequence. Subsequent diffusion images were acquired using a stimulated echo diffusion sequence with standard pulsed diffusion gradients of duration $\delta$=3.5 ms and with G=400 mT/m amplitude. A b0 image was acquired along with trace images at 8 b-values, measured by evenly sampling $\Delta$ from 15.6 to 115 ms, giving us b-values from 0 to 22,700 s/mm$^2$. Additional imaging parameters were 100x100x500 $\mu$m$^3$ voxel resolution, a TR of 3000 ms, and a TE of 22.86 ms. Data was fit pixel by pixel to the CTRW model using a nonlinear least squares fitting algorithm in Matlab. In this analysis, the $\beta$ (space-varying) parameter was fixed to 2 in the CTRW model for two reasons: first, we did not expect *ex vivo* tissue to show any super-diffusive properties (**Figure 1**) and second, due to varying $\Delta$, we expected greater sensitivity in the $\alpha$ parameter. For comparison purposes, corresponding regions in the striatum and the corpus callosum (CC) – two brain regions displaying increased vulnerability to neurodegeneration in HD[2,3] – were evaluated.

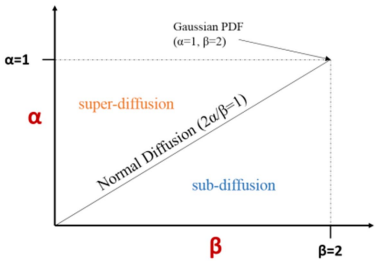


**Figure 1:** A phase diagram for $\alpha$ and $\beta$ and how it relates to different types of CTRW diffusion.

**Results** – Typical T2-weighted images of control and HD brains imaged of P21-aged mice are shown in **Figure 2A** and the results of the $\alpha$ fitting can be seen in **Figure 2B**. The alpha map is able to differentiate between gray and white matter, with the lower $\alpha$ values representing tissue having more diffusion-restricting boundaries and heterogeneity. A small difference was observed between the wild-type and the R6/2 CC at P21 when $\alpha$ values taken from ROIs compared (**Figure 3).** Consistent with these observations, electron microscopic analysis (**Figure 4**) showed differences in CC myelin cohesion and inclusions between the control (left) and R6/2 (right).

**Discussion and Conclusion** – These results suggest that CTRW parameters may be diagnostically useful in Huntington's disease, specifically in white matter regions. Reductions in striatal volume typically occur after the onset of motor symptoms in R6/2 mice (P60); in this study, the $\alpha$ value was relatively insensitive to changes in the striatum at P21 and results at P60 remain to be seen. However, if we examine the changes in the corpus callosum, we can see that there is a net decrease in $\alpha$ for the diseased mouse at P21, suggesting an increase in tissue heterogeneity. Whether this increase is due to a loss of tissue boundaries/membranes, an increase in intracellular inclusions, or a change in populations of neural cells remains to be determined. We plan further experiments to better stratify these groups of mice and these regions of the brain. Future work also includes increased sample size, validation through histology (both electron and fluorescent microscopy), as well as biochemical assays to determine cell integrity using biomarkers such as myelin content.

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**References** 1. Han, I., *et al. J. Neurochem.* (2010). 2. Rosas, H. D. *et al. NeuroImage* **49,** 2995–3004 (2010). 3. Douaud, G. *et al. NeuroImage* **46,** 958–966 (2009). 4. Ingo, C., *et al. Magn. Reson. Med.* **71,** 617–627 (2014).

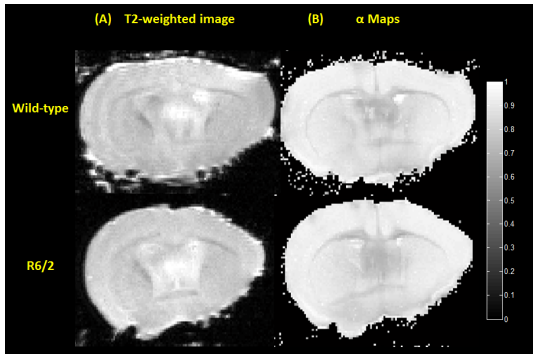


**Figure 2:** T2 weighted images and $\alpha$ maps of the coronal sections of wild-type and R6/2 transgenic mice. T2-w image intensity is windowed and normalized to match the range of $\alpha$.

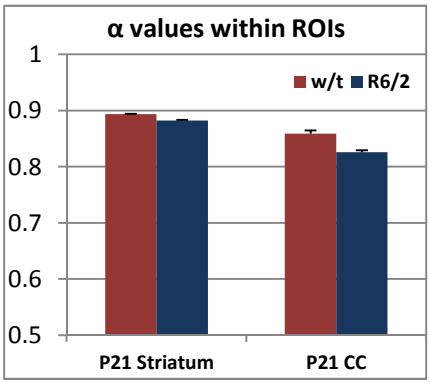


**Figure 3:** Comparisons of $\alpha$ values from ROIs the striatum and corpus callosum. Error bars indicate variance within each region.

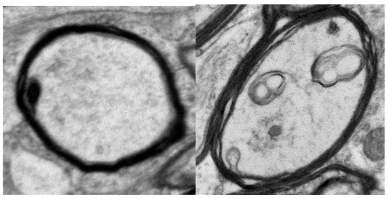

**Figure 4:** Transmission electron micrographs showing a transverse cut of the prefrontal corpus callosum in control (left) versus R6/2 (right) mouse brains at P60. The inclusions seen in the diseased brain could lead to changes in $\alpha$.