# Structure Specific Analysis of white and gray matter in the rat brain after exposure to chronic stress

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**Introduction** Tract specific Analysis (TSA) [1] uses continuous medial modeling for structure-specific analysis of diffusion in sheet-like white matter tracts in the human brain. The medial geometry provides both a skeleton surface (the medial surface) and two "spoke" vectors from the skeleton to the boundary of the structure. Diffusion properties such as fractional anisotropy (FA) are sampled along the spokes and their mean is projected onto the skeleton for statistical testing on the medial surface. TSA enhances sensitivity and reduces multiple comparisons compared to voxelwise tests, by averaging and projecting voxel data onto a surface. The use of the medial model for projection preserves spatial specificity. TSA has been used in a variety of human studies, such as contrasting patterns of white matter degeneration in frontotemporal dementia and Alzheimer's disease [2]. In this work, we construct medial models of white matter tracts in the rat brain. We further apply the medial modeling approach to the isocortex. Compared to human cortex, the lissencephalic rat cortex is readily modeled with a medial surface and we can resolve higher FA with DTI. We apply the new medial models to the study of the neurobiology of chronic stress in rats. Tracts connecting to the hippocampus and prefrontal cortex are of particular interest, since these areas are widely reported to be affected by stress and are important in mediating stress response. We therefore built medial models of corpus callosum, fimbria / fornix, and the internal capsule.

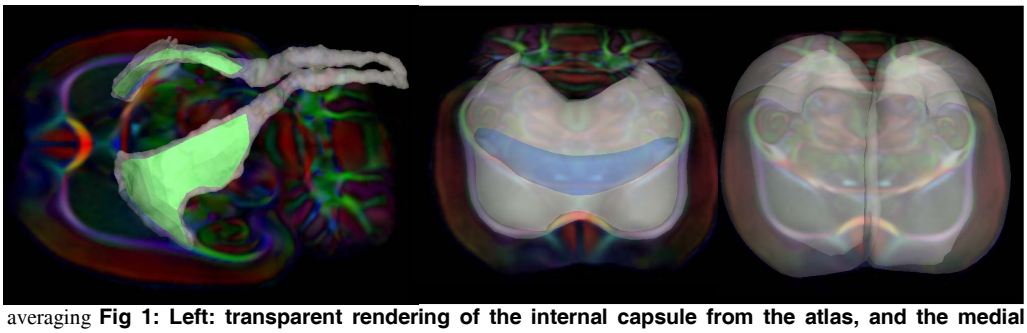

**Fig 1: Left: transparent rendering of the internal capsule from the atlas, and the medial skeleton (green) fitted to the anterior sheet-like part of the tract. Center: model boundaries for the corpus callosum (white) and fornix (blue). Right: model boundaries for isocortex.**

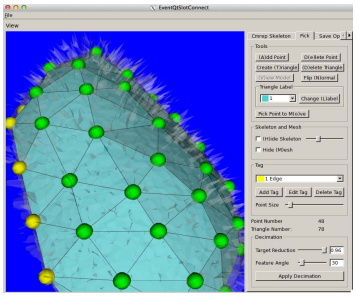

**Fig 2: A GUI tool is used to initialize the medial skeleton**

**Methods** A total of 15 male Sprague-Dawley rats were subject to chronic stress by "social defeat" [3]. The Sprague-Dawley rat is placed into the cage of a larger male rat for 30 minutes daily over 7 days. The large resident rat becomes aggressive and the intruder eventually exhibits a defeat posture, indicating surrender. The rats are then separated within the cage to continue the stress without injury to either animal. Of the intruder rats, 5 resisted domination by the resident for long latencies (LL) before being defeated. The remaining 10 rats had short latency (SL). SL rats submit to the resident faster and exhibit anxiety-like and depressive-like phenotypes in well-validated tests of behavior. A further 7 control rats were placed daily in new cages but without a resident rat to cause defeat. **Magnetic resonance imaging**: At the conclusion of the 7 days of defeat or handling, the rats were sacrificed and the brains were extracted and fixed in formalin for a minimum of 7 days, then immersed in phosphate-buffered saline with 0.2 mmol/kg M-GdDTPA contrast agent for 48 hours prior to image acquisition. Diffusion tensor imaging was acquired with 0.125×0.1875 mm in plane resolution and 0.17 mm coronal slices. A diffusion tensor (DT) template was constructed from the 22 images using DTI-TK [4]. The fractional anisotropy (FA) and radial diffusivity (RD) were computed on the template DT image and used for template segmentation. Initial template segmentation was performed by co-registering a high-resolution labeled magnetic resonance histology atlas [5] to the template space using multi-channel registration in ANTs [6]. The template FA and RD images were matched simultaneously to the corresponding images in the atlas. After normalization, the labels were manually edited for correctness and to smooth out aliasing, using ITK-SNAP [7]. Additionally, we removed parts of labeled structures that that could not be modeled as a sheet-like structure. As shown in fig 1, this means we retain only the anterior part of the internal capsule; we also removed the anterior pillars of the fornix. The corpus callosum label was cut axially below the level of the anterior commissure, and isocortex was divided into left and right hemispheres. **Medial modeling**: the edited labels were processed separately using the cmrep toolkit (available on Sourceforge.net). The medial skeleton was initialized manually (fig 2), the full medial model was then computed by the cm-rep tool. **Statistical analysis**: For each structure, DTI-TK was used to sample the local mean FA from each of the normalized images. At each point on the medial skeleton, a one-tailed t-test was performed for group contrasts: LL vs SL, control vs LL, control vs SL. Clusters where the t-value suggests significance at $p < 0.05$ were tested for significance at the cluster level with 10000 permutations.

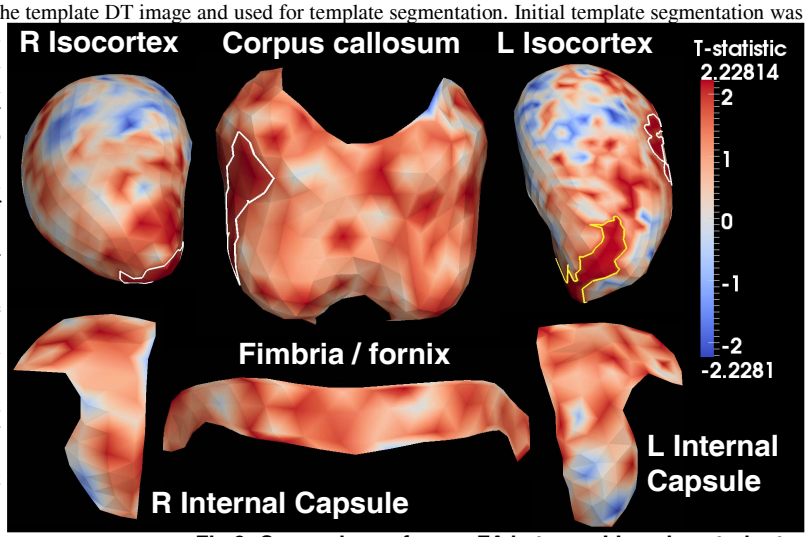


**Fig 3: Comparison of mean FA between LL and control rats.**

**Results** Fig 3 shows results for control vs LL, colored by the t-statistic. Red areas indicate a higher mean FA for control animals compared to LL stressed animals. All clusters significant at $p < 0.1$ under permutation (white outline) had lower FA in LL animals, in the corpus callosum ($p = 0.055$) and right isocortex ($p = 0.079$), and posterior left isocortex ($p = 0.099$). One cluster in the left prefrontal region of isocortex, was significant at $p = 0.0025$ (yellow outline). SL rats had a similar region of lower FA compared to controls in the corpus callosum ($p = 0.052$), but there were no corresponding clusters in isocortex. For the LL vs SL contrast, no clusters were significant under permutation with $p < 0.1$.

**Discussion** We have presented, to our knowledge, the first application of TSA to the rat brain. The results suggest that chronic stress reduce FA in the corpus callosum, since this was observed in both SL and LL animals compared to controls. In the prefrontal cortex, FA is significantly lower in LL rats compared to controls but not SL rats compared to controls. Further study is required to validate these results and explore whether the group differences pre-exist exposure to stress or reflect differential stress response between the SL and LL groups. Future work will also include refining the TSA models and making them available to other researchers.

**References** 1. Yushkevich et al, *NeuroImage* 2008 41(2) 448-461. 2. McMillan et al *Neurology* 2012 78(22):1761-1768. Wood et al, *Endocrinology* 2010 151(4):1795-1805. 4. Zhang et al *Med Image Anal* 2006 10(5):764-785. 5. Calabrese et al, *NeuroImage* in press doi:10.1016/j.neuroimage.2013.01.017. 6. Avants et al, *Med Image Anal* 2008 12:26-413. 7. Yushkevich et al *NeuroImage* 2006 31(3):1116-28.

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