Quantitative Tract-Based ROI Analysis: Altered Thalamo-Frontal Circuitry Conferred by Schizophrenia-Risk Gene NRXN1 Variant

David Rotenberg[1], James Kennedy[2,3], Benoit Mulsant[3,4], Aristotle N Voineskos[1,3], and Mallar Chakravarty[1,5]

[1]Research Imaging, Center for Addiction and Mental Health, Toronto, Ontario, Canada, [2]Neuroscience, Center for Addiction and Mental Health, Toronto, Ontario, Canada, [3]Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada, [4]Geriatric Mental Health, Center for Addiction and Mental Health, Toronto, Ontario, Canada, [5]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada

INTRODUCTION – Schizophrenia (SZ) symptoms, including delusions, hallucinations disorganized speech and behavior, have long been considered to be the result of disrupted brain circuitry, resulting from impaired neurological development. In particular, the time of maturation of fronto-thalamic pathways are coincident with SZ onset, and have been shown to be impaired in chronic SZ [1]. Furthermore, there is substantial evidence that schizophrenia is a highly heritable disorder. The SZ-risk gene neurexin-1 (NRXN1) has been indicated as conferring risk to both the frontal lobe and thalamus, and it has been shown that a NRNX1 variant leads to a 5.93% frontal white matter (WM) volume decrease in C/C homozygotes (C/C) versus heterozygous T-carriers (C/T) [2]. To assess possible impairment in specific pathways, a more refined approach is needed. Tract-based spatial statistics (TBSS) provides a means to perform quantitative analysis of voxel-wise group differences across whole brain white matter (WM) tracts, and improves upon the image-alignment sensitivity of voxel-based morphometry methods by projecting subject data onto an alignment-invariant skeleton on which statistics can be performed reliably. However, in cases with hypothesis-driven tracts of interest, a robust method of region of interest (ROI) based tract statistics is lacking. ROI-based analyses help control for Type I errors by limiting the number of statistical tests required, and thereby appropriately reduce the threshold for multiple comparison correction. We have developed a novel tract-based ROI approach that uses probabilistic tractography to segment tracts of interest across individuals, defining spatially restricted alignment-invariant skeletons for TBSS analysis. We apply this new technique to an investigation of the relationship between SZ-risk gene NRXN1 variants and thalamo-frontal circuitry.

MATERIALS AND METHODS - Diffusion weighted images (DWI) were collected from 52 healthy controls: 31 NRXN C/C participants (age 33±11 yrs, 32M, 20F), and 21 NRXN C/T participants, age and sex matched. All scans were carried out on a 1.5T GE Echospeed system (General Electric Medical Systems, Milwaukee, WI) at Toronto General Hospital. There were no differences in IQ, socio-economic status, and years of education observed based on genotype. Three diffusion-weighted scans were taken for each individual, (b=1000, 23 diffusion encoding directions), averaged to improve the signal-to-noise ratio. The data were corrected for motion and eddy currents, through affine registration to a non diffusion-weighted reference volume. Gradient directions were also adjusted to account for motion. Statistical analyses were conducted using a modified TBSS pipeline in the FSL package [3,4]. First, fractional anisotropy (FA) images were estimated for all subjects, and nonlinearly aligned to a common FA template in MNI space [5]. Standard, whole-brain, TBSS was first conducted, for a basis of comparison between ROI and whole-brain techniques. Probability distributions of fibre direction were estimated for each brain voxel using *bedpostx* software. Local probability distributions of fibre direction were used to perform probabilistic tractography using *probtrackx*, to track from a seed mask to a termination mask. The frontal [6] and thalamic seed [7] and terminations mask (see Fig. 1A) were used to select right and left, lateral orbitofrontal, inferior frontal, superior frontal, middle frontal and middle orbitofrontal to thalamus tracts. Each tract was then used to mask the original FA image. The masked FA images were then warped back to common space by non-linear registration. A center line skeleton was extracted, and the maximum FA value was projected onto the skeleton for statistical comparisons. All FA statistics were conducted with 500 permutations and corrected for multiple comparisons.

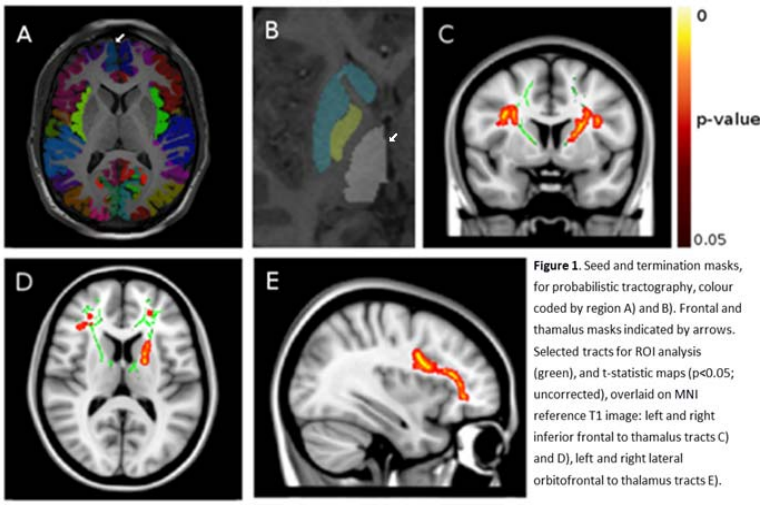

Figure 1. Seed and termination masks, for probabilistic tractography, colour coded by region A) and B). Frontal and thamalus masks indicated by arrows. Selected tracts for ROI analysis (green), and t-statistic maps (p<0.05; uncorrected), overlaid on MNI reference T1 image: left and right inferior frontal to thamalus tracts C) and D), left and right lateral orbitofrontal to thalamus tracts E).

RESULTS – The results of the whole-brain TBSS pipeline did not show significant differences between groups. Using the ROI approach, at p< 0.05 uncorrected, decreased FA was found in nine out of the ten tracts investigated (see Fig 1C-D) in C/C homozygotes compared to C/T heterozygotes (except for the right lateral orbitalfrontal tract). When corrected for multiple comparisons substantial decreases were observed in both the left and right inferior frontal (p<0.06), and right middle orbitofronal to thalamus tracts (p<0.06).

DISCUSSION – The results suggest that our refined, hypothesis driven TBSS approach is more sensitive to salient differences in WM tract-specific diffusion properties compared to standard whole-brain TBSS. These findings also suggest that our constrained tractography-based TBSS approach can be extended to other WM tracts throughout the brain. The significant FA decreases in WM tracts in C/C homozygotes contrasted with C/T heterozygotes supports recent findings that thalamo-frontal WM is altered in individuals at high-risk for SZ. The tracts connecting the inferior frontal cortex and thalamic nucleus, associated with working memory, and anterior cingulated cortex, associated with reward anticipation, both of which are impaired in SZ individuals, have significantly reduced FA in C/C homozygotes.

CONCLUSION - Identification of compromised WM pathways, along with better understanding of underlying genetic risk factors, will help to further our knowledge of SZ etiology and the development of new SZ biomarkers. The novel tract-based ROI analysis presented here has the potential to enable unique and focused statistical investigations of WM tracts that may not be available through standard TBSS analysis.

REFERENCES - [1]J. Oh *et. al.*, Hum Brain MappNov;30(11):3812-25. 2009 [2]A.Voineskos *et. al.*, PLoS One, 6(6), 2011 [3]S.Smith *et al.*, NeuroImage, 23(S1):208-219, 2004[4] S. Smith *et al.*, NeuroImage, 31:1487-1505, 2006 [5]D. Rueckert et al., IEEE 18(8):712-721, 1999. [6]D.Shattuck *et. al.,* Neuroimage 1;39(3):1064-80 2007 [7]M. Chakravarty, epub 2012.