Journal of the International Neuropsychological Society (2008), **14**, 1–4.

SYMPOSIUM—SUPPLEMENTAL MATERIAL

Neuronal fiber pathway abnormalities in autism: An initial MRI diffusion tensor tracking study of hippocampo-fusiform and amygdalo-fusiform pathways

(Note: Sections are arranged in the order to which they are referred in the main article.)

THOMAS E. CONTURO,[1] DIANE L. WILLIAMS,[2] CHARLES D. SMITH,[3] EREN GULTEPE,[1] ERBIL AKBUDAK,[1] and NANCY J. MINSHEW[4]

[1]Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri
[2]Department of Speech-Language Pathology, Duquesne University, Pittsburgh, Pennsylvania
[3]Department of Neurology, University of Kentucky, Lexington, Kentucky
[4]Departments of Psychiatry and Neurology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Exclusion Criteria for Medication History, and Possibility of Medication Effects

The exclusion criteria relevant to the medication histories given in Table 1 were as follows: (1) no antipsychotics for 1 month prior to MRI; (2) no anticonvulsants 1 week prior to MRI; (3) no antidepressants the day of or night before MRI; (4) no central nervous system (CNS) stimulants/amphetamines 24 hours prior to MRI; and (5) no sedating cold/allergy medications the day of MRI. All medications had to have been taken at a constant dose for 2 weeks prior to MRI. In no cases were participants asked to refrain from taking any prescribed medications for the purpose of the study.

We expect medication effects on the diffusion-tensor tracking (DTT) metrics to be unlikely in this study for several reasons. First, only 7 of the 17 participants with autism were taking CNS medications during the 3 years prior to MRI (and none of these 17 participants were on chronic medications as children). Second, the medication history varied across participants (Table 1), causing any drug effects to average out in the group analysis. Third, the specific DTT findings in the different pathways would be unlikely to be related to the more global effects of medications (either acutely or chronically). Fourth, acute effects of current medications were reduced by using specific inclusion/exclusion criteria for recent medication history (see Methods). Fifth, any medication effects on brain water content would not alter the brain microstructure, and would not influence metrics such as anisotropy or D-min. Complete exclusion of individuals with any past/current medication history would not allow recruitment of a sufficient sample size, and withdrawal of medications for this study would not be ethical.

Scan-Rescan (Cross-Site) Variability Analysis

For assessment of cross-site variability, five individuals (two autism study participants; three outside controls) were scanned at both sites (the outside controls met the same inclusion criteria as the control study participants). Six individuals were re-scanned at the same site (balanced across groups/sites) to determine intra-site variation. For the right hippocampo-fusiform (HF) pathway, the cross-site/within-site coefficients of variation (CV) were, respectively, 3.48%/3.02% (D-min), 1.60%/3.72% (D-max), 5.72%/4.23% (A_σ), and 2.49%/3.16% (D-bar), where the scan-rescan CV was calculated as in (Bland & Altman, 1996) (A_σ and D-bar are secondary DTT metrics defined below). The similarity between the cross-site and within-site variability suggests that scan-site effects are negligible, obviating the need to individually match for scan site. These within-subject variabilities are comparable or low in relation to typical imaging studies, and are low relative to the cross-subject biological variability within subject groups (e.g., CV 8.1%/11.8% in autism/control groups for right HF D-min). Thus, the scan-rescan variability is significantly less than biological variability, allowing biological changes to be detected (with detectibility further enhanced by averaging across participants).

Computation of Whole-Brain Track Data from Diffusion-Weighted Images

The diffusion-weighted images were co-registered to the non–diffusion-weighted (b~0) images (T2-weighted I0 images) and averaged across the 10 frames to place all

data in the native diffusion imaging data space. From the averaged diffusion-weighted images, whole-brain track data were computed as in (Conturo et al., 1999; Lori et al., 2002) using in-house software. All computations and displays were performed using a SunFire V880 compute server (8 × 1.2 GHz, 64 GB RAM, XVR-600 graphics; Sun Microsystems, Santa Clara, CA). Tracks were generated from a whole-brain three-dimensional grid of seed points (1.0-mm spacings). This tracking algorithm was shown to be valid by the close agreement between ideal and calculated track lines in numerical simulations (Lori et al., 2002). Compared with attempts at seeding specific pathways, this whole-brain seeding is free of *a-priori* assumptions or bias about anatomic regions and their connections. From seeds having above-threshold anisotropy ($A_\sigma \geq 0.14$), tracks were reconstructed by iteratively stepping in the direction of fastest diffusion (major eigenvector) in 0.5-mm steps, terminating upon encountering a below-threshold anisotropy. Whole-brain track data were computed using all acquired data without editing.

DTT Pathway Selection

Pathway selection or "dissection" is a critical step in DTT studies (Catani et al., 2002; Conturo et al., 1999). The HF and amygdalo-fusiform (AF) pathways were selected from the whole-brain track data using a selection procedure designed to provide a consistent, objective selection with very low operator bias, without constraining the spatial extent of the pathways. Large ellipsoidal spatial selection volumes (SSVs) were placed at predefined coordinates in atlas space, and were combined in multi-step Boolean-logic operations using in-house software to select the groups of tracks representing the pathways. Tracks were selected that passed into both an SSV encompassing mid-fusiform area, and an SSV encompassing anteromedial temporal lobe, without passing into occipital lobe. The selectivity of this procedure is imparted mostly by the Boolean logic, rather than the anatomical borders of the SSVs, thus enabling large SSVs to be used to select the entire pathway (essential for quantitative comparisons), insensitive to the exact SSV locations. In contrast, small anatomically constraining SSVs can fail to select the entire pathway, and can introduce operator bias.

The mid-fusiform and anteromedial temporal SSVs were located in atlas space (Analyze 6.0, Mayo Foundation, Rochester, MN), and then the SSV locations were registered back to the subject's I0 volume (native diffusion imaging space). The mid-fusiform SSV was defined conservatively as having a rostro-caudal thickness of only 50% of the anterior-posterior limits of the mid-fusiform region (thus clearly within the rostro-caudal bounds of Brodmann area 37). The superior-inferior and medial-lateral dimensions of the mid-fusiform SSV were purposely set to be large enough to span the entire hemisphere in the coronal plane, to obtain the complete width and height of the pathway. The mid-fusiform and anteromedial-temporal SSVs were combined with Boolean "AND" logic to select tracks intersecting both SSVs. SSVs were inspected for track-free margins, ensuring that the pathway was selected without limiting its width. A final SSV was positioned midway along the pathway length to separate the AF and HF pathways at a naturally occurring separation between the pathways (Smith et al., 2008). The same operator performed all selection procedures, the selection was blinded to participant, and outcomes were not determined until all pathway selections were complete.

DTT Quality Control

Six quality control (QC) procedures were used for different aspects of the DTT process. For pathway selection, we (1) tested for track-free SSV margins and (2) evaluated inter-/intra-observer reliability. To assess the quality of track data and the degree to which the DT-MRI data support tracking of the pathways, we (3) used negative-control SSVs, (4) visualized tensor anisotropy and skewness in the pathway, and (5) viewed track lines with high-zoom. Finally, we tested the overall procedure by (6) scan-rescan analysis.

First, the SSVs had track-free margins (see above), indicating that the entire pathway of interest was selected. Second, nearly identical pathway selection results were obtained across observers (<1-mm inter-operator variation in SSV positions; <2% inter-operator variation in pathway track counts), indicative of the low bias resulting from using whole-brain track data, large atlas-placed SSVs, Boolean-logic operations, and measurement of tensor metrics in DTT-defined pathway space. Third, negative-control SSVs showed that tracks were not selected between regions not expected to be connected (e.g., mid-fusiform and primary motor cortex), indicating the specificity of the selection procedure. Fourth, visualization of tensor anisotropy and skewness (Conturo et al., 1996) along the pathway did not reveal regions of altered tensor characteristics that might cause termination or misdirection of tracks (e.g., due to noise effects or partial volume averaging between pathways). Fifth, high-zoom visualization of pathway track lines did not reveal artifacts such as sharp turns that might indicate tensor irregularities. Finally, scan-rescan variability was low (2–5%; see above), indicating that the overall DTT process had high reproducibility.

Secondary Microstructural DTT Metrics

For each pathway, various secondary microstructural DTT metrics were calculated for comparison. A parameter "radial diffusivity" (D-radial) or perpendicular diffusivity is sometimes calculated (Conturo et al., 1996; Lee et al., 2007; Song et al., 2002) as D-radial = (D-min+D-mid)/2, where D-mid is the middle principal diffusion coefficient. D-radial is often considered an index of the mean diffusivity across fibers. However, for pathways having features such as turns/

divergences (e.g., Conturo et al., 1999; Lori et al., 2002), D-min would represent the intrinsic across-fiber diffusion perpendicular to the plane of curvature/divergence, while D-radial would contain a mixture of along-fiber diffusion due to partial-volume averaging (Smith et al., 2008). Thus, we report D-min as a primary microstructural metric, with D-radial given for comparison.

The secondary microstructural DTT metric of anisotropy, A_σ (Conturo et al., 1996), represents the degree to which the microscopic water movements differ in different directions. Typically, water diffusion is stronger (molecules move over larger distances) along axonal fibers, while water diffusion is weaker (water moves over shorter distances) across axonal fibers. A_σ measures the degree to which along-fiber movements differ from across-fiber movements among a group of adjacent axons. A_σ is related to the standard deviation of the three principal diffusion coefficients (D-min/D-mid/D-max), whereas the directionally-averaged "mean diffusivity"D-bar is the average of these coefficients. A_σ is defined to have a linear response over the standard scale ranging from 0 (no anisotropy) to 1 (full anisotropy) (Conturo et al., 1996; Shimony et al., 1999), in contrast to fractional anisotropy (FA) (Basser, 1995). A_σ and FA are related by a nonlinear expression (Hasan & Narayana, 2003) enabling these parameters to be interconverted.

For anisotropy, a high A_σ indicates that water molecules have a strong preference to move along rather than across axons, and is often taken to indicate strong myelination. However, high A_σ could result from any of the above factors that cause slow D-min and/or fast D-max. Conversely, a low A_σ is often taken to indicate weak myelination, but could result from any of the above factors that cause fast D-min, provided that increases in D-max do not cancel the D-min effect on A_σ. Anisotropy is thus considered a secondary DTT metric because it is a combination of D-min and D-max, and in some cases can fail to change when both D-min and D-max change in the same direction.

Effect Size Calculation

Effect sizes for two-group comparisons are reported as %difference calculated as %Eff = 100%[mean(autism)-mean(control)]/mean(control) or, in the case of laterality, Eff=mean(autism)-mean(control). For behavioral subgroup analysis, effect sizes were calculated as %Eff = 100%[mean(lower-performance)-mean(higher-performance)]/mean(higher-performance).

Type I and Type II Error Rates and Paired Statistical Analysis

Statistical power was calculated (Power & Precision, Biostat, Englewood, NJ), and Type-I and Type-II error rates were estimated to assess the possibility of false-positive and false-negative errors. Type-I and Type-II error rates were estimated from the *p* values and power reported in the main text for the principal finding of reduced D-min (whole-brain normalized) in the right HF pathway. The Type-I error rate (i.e., the rate of false-positive errors) for the group differences in normalized D-min of the right HF pathway was estimated as 3.8% for unpaired analysis, reduced to 1.4% for paired analysis. The Type-I error rate for the D-min HF laterality effect was estimated as 0.40% unpaired, reduced to 0.17% paired (i.e., only 1.7 false positives in 1000 independent studies having demographics and methods identical to the study herein). While this Type-I error rate is very low, indicating that the findings are highly unlikely to be false positives, the Type-II error rate (i.e., the rate of false-negative errors) is also important, and should not be made excessively high to obtain a low Type-I error rate. When using the methods described herein to detect other unknown effects, a high Type-II error rate would indicate a high chance of not detecting a true biological effect. Based on the calculated power, the Type-II error rate for detecting the normalized D-min finding in right HF in a replication study would be 36% with an unpaired analysis, decreasing to 27% using a paired analysis. For the HF D-min laterality finding, the Type-II error rate would be 15% unpaired, decreasing to 9% paired. Thus, the sample size was sufficient to yield an acceptably low likelihood of false negatives. The power and Type-II error rate do not relate to the reliability of the D-min findings in the HF pathway system, because these were positive (not negative) results. The power and Type-II error rate relate more to the ability to replicate the findings in a future study. For example, in replication studies with identical demographics/methods, only 0.9 out of 10 studies would fail to replicate the D-min laterality findings using a paired analysis. Importantly, these results indicate that both Type-I and Type-II error rates are reduced by using a paired as opposed to unpaired statistical analysis (in combination with close pair-wise matching), such that statistical power does not have to be sacrificed to obtain a low Type-I error rate.

Results for Secondary Microstructural DTT Metrics: D-radial, A_σ, and D-bar

The observed D-min results (see main text) are reflected in the D-radial metric, although with a weaker effect. The D-radial laterality shift was statistically significant with $(+0.47 \pm 0.92)\%$ for the autism group and $(-2.23 \pm 0.81)\%$ for controls (Eff = +2.70%; $p = .031$). Trends in normalized D-radial nearly reached statistical significance in right HF (autism 1.199 ± 0.038, controls 1.266 ± 0.025; %Eff = −5.23%; $p = .131$ unpaired; $p = .064$ paired).

For A_σ, there was a statistically-significant shift in HF laterality, with $(-0.40 \pm 1.15)\%$ in the autism group and $(+3.06 \pm 0.79)\%$ in controls (Eff = −3.46%; $p = .016$). Consistent with the D-min HF results, there was a loss of normal A_σ lateralization and a shift toward higher A_σ on the right. Considering the D-min and D-max results above

(Figs. 2, 3), this A_σ laterality shift was driven predominantly by changes in D-min in the HF pathways. The A_σ showed trends toward increased anisotropy in the right HF pathway (autism 0.3167 ± 0.0070, controls 0.3079 ± 0.0074; %Eff = +2.86%; $p = .37$), and decreased anisotropy in the left AF (%Eff = −2.49%; $p = .37$) and left HF (%Eff = −3.88%; $p = .26$) pathways. These trends were not augmented by whole-brain normalization. The anisotropy was between the ranges measured for typical projection/association white matter (Shimony et al., 1999).

Similar to the D-max effects, the directionally-averaged mean diffusivity D-bar was elevated in the right AF pathway in the autism group compared with controls (autism 0.8150 ± 0.0073 μm^2/ms, controls 0.7921 ± 0.0086 μm^2/ms; %Eff = +2.89%; $p = .044$ unpaired; $p = .028$ paired).

REFERENCES

Basser, P.J. (1995). Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. *NMR in Biomedicine*, *8*, 333–344.

Bland, J. & Altman, D. (1996). Measurement error. *British Medical Journal*, *313*, 744.

Catani, M., Howard, R.J., Pajevic, S., & Jones, D.K. (2002). Virtual in vivo interactive dissection of white matter fasciculi in the human brain. *Neuroimage*, *17*, 77–94.

Conturo, T.E., Lori, N.F., Cull, T.S., Akbudak, E., Snyder, A.Z., Shimony, J.S., McKinstry, R.C., Burton, H., & Raichle, M.E. (1999). Tracking neuronal fiber pathways in the living human brain. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 10422–10427.

Conturo, T.E., McKinstry, R.C., Akbudak, E., & Robinson, B.H. (1996). Encoding of anisotropic diffusion with tetrahedral gradients: A general mathematical diffusion formalism and experimental results. *Magnetic Resonance in Medicine*, *35*, 399–412.

Hasan, K.M. & Narayana, P.A. (2003). Computation of the fractional anisotropy and mean diffusivity maps without tensor decoding and diagonalization: Theoretical analysis and validation. *Magnetic Resonance in Medicine*, *50*, 589–598.

Lee, J.E., Bigler, E.D., Alexander, A.L., Lazar, M., DuBray, M.B., Chung, M.K., Johnson, M., Morgan, J., Miller, J.N., McMahon, W.M., Lu, J., Jeong, E.K., & Lainhart, J.E. (2007). Diffusion tensor imaging of white matter in the superior temporal gyrus and temporal stem in autism. *Neuroscience Letters*, *424*, 127–132.

Lori, N.F., Akbudak, E., Shimony, J.S., Cull, T.S., Snyder, A.Z., Guillory, R.K., & Conturo, T.E. (2002). Diffusion tensor fiber tracking of human brain connectivity: Aquisition methods, reliability analysis and biological results. *NMR in Biomedicine*, *15*, 494–515.

Shimony, J.S., McKinstry, R.C., Akbudak, E., Aronovitz, J.A., Snyder, A.Z., Lori, N.F., Cull, T.S., & Conturo, T.E. (1999). Quantitative diffusion-tensor anisotropy brain MR imaging: Normative human data and anatomic analysis. *Radiology*, *212*, 770–784.

Smith, C.D., Lori, N., Akbudak, E., Sorar, E., Gultepe, E., Shimony, J., McKinstry, R.C., & Conturo, T.E. (2008). MRI diffusion tensor tracking of a new amygdalo-fusiform and hippocampo-fusiform pathway system in humans. *Journal of Magnetic Resonance Imaging* (submitted).

Song, S.K., Sun, S.W., Ramsbottom, M.J., Chang, C., Russell, J., & Cross, A.H. (2002). Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage*, *17*, 1429–1436.