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Diffusion Tensor Imaging Provides Evidence of Possible Axonal Overconnectivity in Frontal Lobes in Autism Spectrum Disorder Toddlers

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ABSTRACT

BACKGROUND: Theories of brain abnormality in autism spectrum disorder (ASD) have focused on underconnectivity as an explanation for social, language, and behavioral deficits but are based mainly on studies of older autistic children and adults.

METHODS: In 94 ASD and typical toddlers ages 1 to 4 years, we examined the microstructure (indexed by fractional anisotropy) and volume of axon pathways using in vivo diffusion tensor imaging of fronto-frontal, fronto-temporal, fronto-striatal, and fronto-amygdala axon pathways, as well as posterior contrast tracts. Differences between ASD and typical toddlers in the nature of the relationship of age to these measures were tested.

RESULTS: Frontal tracts in ASD toddlers displayed abnormal age-related changes with greater fractional anisotropy and volume than normal at younger ages but an overall slower than typical apparent rate of continued development across the span of years. Posterior cortical contrast tracts had few significant abnormalities.

CONCLUSIONS: Frontal fiber tracts displayed deviant early development and age-related changes that could underlie impaired brain functioning and impact social and communication behaviors in ASD.

Keywords: Autism, Development, DTI, Frontal tracts, Tract FA, Tract volume

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The first warning signs of autism spectrum disorder (ASD) involve abnormal social, communication, language, and emotional behavior (1–3). In typically developing infants and toddlers, these higher order abilities depend on the normal growth of long-distance connections and widely distributed neural networks in the brain, especially fiber tracts between frontal and temporal cortices, the amygdala, and the striatum (4). Across the early years, tracts normally display robust changes in microstructure, such as increases in axon size, myelination, and overall volume, and diffusion tensor imaging (DTI) measures of tract microstructure, such as fractional anisotropy (FA) and volume, and index these robust changes from fetal life through childhood (5), although DTI studies of normal development between ages 1 to 4 years remain rare. Whether these critical frontal, temporal, and limbic fiber tracts display pathologic development by the ages when autistic symptoms first begin has been studied little.

Almost 40 years ago, Damasio and Maurer (6) proposed that autism was due to frontal-temporal-limbic disconnection and dysfunction. The theory supposed ASD involved reduced numbers of cortical neurons and underdevelopment of axons and frontolimbic fiber tracts. This early speculation has been further refined and elaborated and is a prevalent theory today: namely, that disconnection or underconnectivity between different brain regions underlies ASD (7,8), despite structural evidence that cerebral white and gray matter in ASD at young ages may be increased (9–11). Reduced functional connectivity in functional magnetic resonance imaging (fMRI)-based studies (8,12) and reduced fiber tract FA in the great majority of DTI-based studies of older children (13–15), adolescents, and adults with ASD (16,17) do seem to support the original and prevalent view of structural/axonal, as well as functional, underconnectivity.

While abnormally reduced FA is one of the most consistent types of biological findings in ASD, few tracts have been consistently reported as abnormal, study sample sizes are typically small, volumes of specific tracts are seldom measured, and few studies have mapped and measured specific tracts (as opposed to measuring voxels within regions of interest) (17). Importantly, even though ASD is a disorder of very early development (18–20), nearly all DTI studies have been on older children, adolescents, and adults (17).

New fMRI and DTI data from the 1- to 4-year-old ASD brain do not fit neatly into the structural/functional disconnection model. Instead, new studies suggest a complex view of age-related changes in pathologic circuitry across early development in ASD. In toddlers with ASD, one fMRI study found reduced left-right synchronous functional activity (21), but another found hyperconnectivity of fronto-temporal-cerebellar activity (JH Manning, unpublished data, February 28, 2014).

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In contrast to numerous DTI studies of ASD adults and older ASD children that report decreased FA (e.g., decreased FA in fronto-frontal short-range fibers) (16,17,22), the three DTI studies of ASD infants and young children report the opposite: increased FA (23–25), including increased FA in frontal, but not posterior, regions of interest (23). In the only longitudinal DTI study of young ASD toddlers, Wolff *et al.* (24) found increased FA at 6 months of age but nonsignificant tendencies for decreased FA at 24 months in several fiber tracts including the left uncinate fasciculus, left inferior longitudinal fasciculus, and body of the corpus callosum; the comparison subjects were unaffected younger siblings of ASD children. In that study, increased tract FA at young ages in ASD was interpreted as indicating more compact, dense tracts. Whether this unexpected age-related change in FA in some tracts is general to ASD or specific to the ASD versus siblings at-risk for ASD comparison in Wolff *et al.* (24) remains untested and is an important question, especially in light of studies showing that behavior and neurobiology of unaffected siblings of children with ASD lies somewhere between that of ASD groups and typically developing infants and young children (26–28). Nonetheless, Ben Bashat *et al.* (23) and Wolff *et al.* (24) raise novel questions and possibilities about how fiber tracts develop in early life in ASD as compared with typical toddlers.

Postmortem data from ASD children, genetic findings (e.g., *CHD8, PTEN, EIF4A, WDFY3, KCTD13-CUL3-RHOA*) (29–37), and ASD animal models (31,28,39) point to disruption of cell cycle in fetal development, excess neuron proliferation, and brain overgrowth and call into question the anatomical underconnectivity hypothesis. For instance, in young ASD children with heavier than normal brain weight, prefrontal cortex has a 67% excess of neurons (40). This neuron excess predicts greater, not reduced, axon numbers in prefrontal tracts at young ages in ASD. Theoretical models predict doubling neuron numbers could quadruple axon numbers (41). Such postmortem data suggest that, at young ages, there should be an increase in prefrontal axon numbers, which could increase frontal tract volumes in ASD. If ASD does involve an excess of axons in prefrontal tracts at young ages, then ASD might be better modeled as a disorder of early overconnectivity, not underconnectivity, of prefrontal axons. Additionally, these frontal tracts might also display deviant growth trajectories across the first years of life, because, at young ages, genes and gene networks underlying cell differentiation and growth are downregulated (42), and by later childhood and adulthood in ASD, cell size in the cortex is reduced (40,43–44).

To test this general hypothesis of abnormal density, volume, and/or growth of frontal tracts, we DTI imaged 94 ASD and typically developing (TD) 1- to 4-year-olds and used probabilistic atlas-based mapping of multiple frontal fiber tracts; because ASD often displays an anterior to posterior gradient of neural pathology and dysfunction, posterior tracts served as a priori contrast tracts. Validation of this type of tractography methodology comes from a study of showing high correlations between anatomically dissected frontal tracts and DTI tractography-based measures of frontal tracts (45). We also examined the correlation between outcome ASD social and communication symptom severity and FA and volume.

The corpus callosum was measured because decreased FA in the callosum in older children, adolescents, and adults with ASD is the most consistently reported DTI abnormality in the literature (17); its measurement provides a strong test of the nature of early callosal development in ASD relative to a large literature on ASD at older ages.

METHODS AND MATERIALS

Subjects

Participants included 94 toddlers: 61 ASD and 33 TD toddlers ranging in age between 12 and 48 months (Table 1). A subsample of ASD (n = 14) and TD (n = 13) toddlers had a second DTI scan at a follow-up assessment that took place approximately 1 year after the initial scan. An additional 12 participants (7 ASD, 5 TD) were scanned but not included in analyses (Supplement). This project was reviewed and approved by the Human Subjects Protection Review Board at University of California San Diego. Informed consent was obtained from parents or guardians of toddlers.

Table 1. Diagnostic and Clinical Characteristics of ASD and TD Participants

Clinical Measurement	ASD (n = 61)	TD (n = 33)	p Value
Sex (M/F)	48/13	20/13	.103[a]
Age in Months	30.2 (8.4); 12–48	25.9 (11.1); 13–46	.056[b]
Mullen Subscale Scores			
Receptive language (earliest)	29.8 (10.8); 20–62	52.7 (8.8); 39–72	≤.001[c]
Receptive language (recent)	33.0 (11.6); 20–58	55.7 (8.3); 42–72	≤.001[c]
Expressive language (earliest)	32.3 (10.0); 20–62	55.3 (10.0); 38–75	≤.001[c]
Expressive language (recent)	33.8 (12.0); 20–60	58.1 (9.9); 41–80	≤.001[c]
ADOS Communication and Social Total Score (recent)	14.2 (3.9); 7-20	1.7 (1.5); 0–5	≤.001[b]
ADOS Restricted and Repetitive Behavior Score (recent)	3.6 (1.4); 1-6	.2 (.5); 0–2	≤.001[b]

Values for age and Mullen Early Scales of Learning scores are presented as mean (SD) and range.

ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; F, female; M, male; Mullen, Mullen Early Scales of Learning; TD, typically developing.

[a]Pearson's chi-squared test.

[b]Welch's t test.

[c]Accelerated failure time model.