Correlation between diffusion tensor imaging indices and sociability, a behavioral endophenotype relevant to autism: A longitudinal study in the BALB/cJ mouse strain

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Introduction: Autism spectrum disorders (ASD) are a genetically complex set of neurodevelopmental disorders that present with core symptoms of impairments in social interaction and communication, as well as restricted and repetitive interests and behaviors[1]. ASD is characterized by disrupted connectivity across neural circuits, in which multiple networks throughout the brain are affected[2]. Histopathological studies on postmortem brain sections and MRI studies have reported involvement of cerebellum, limbic system and cerebral cortex in autism[3]. Availability of animal models of reduced sociability can aid in further elucidation of the biology of social behaviors, and may ultimately shed light on the biology of ASD. Low sociability is one of the most prominent and disabling symptoms of ASD and the BALB/cJ inbred mouse strain may be a useful model as it exhibits several social behavioral traits that resemble those seen in ASD[4]. The advantage of animal models is that they allow the study of developmental and behavioral abnormalities over the entire life-span of the animal. Diffusion tensor imaging (DTI) has been proposed as a noninvasive method to probe microscopic structure of the tissue in vivo[5], currently there are no reports on longitudinal studies in ASD, in human patients or in animal models. The aim of this study was thus to investigate the correlation between mouse behavior and DTI indices and to observe longitudinal changes in DTI indices in BALB/cJ mice. These studies may provide non-invasive biomarkers to assess neurobehavioral abnormalities and may eventually aid in the diagnosis and treatment of ASD.

Materials and Methods: ***Behavioral Testing:*** Eleven male BALB/cJ mice were included in this study. Behavioral testing was performed at 28 (prepubescence), 48 (post pubescence) and 68 (early adulthood) days of age and a test for anxiety-related behavior was performed on the following day. Sociability was measured by a social choice test using a 3-chambered apparatus as previously reported[6, 7], while anxiety was measured using the elevated zero-maze test[8].

In vivo DTI imaging: In vivo MRI was performed on a 9.4 T horizontal bore scanner (Varian, Palo Alto, CA) equipped with 25 G/cm gradients. A 20mm i.d. quadrature birdcage coil (M2M, Cleveland, OH) was used for signal transmit and receive. A diffusion weighted spin echo sequence was used to acquire 2D multi-slice images with diffusion weighting along six directions optimally selected for anisotropy measurement[9] using a b-value=757.68 s/mm^2; TR=2s; TE=33ms; matrix size=128x128, FOV of 20mm, averages= 2; 15 axial slices; thickness = 0.8mm; acquisition time = 2 hours.

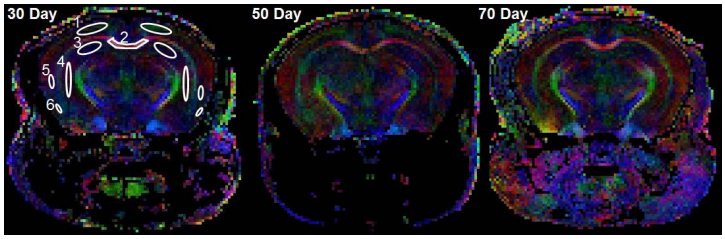
Data quantification: Acquired data were processed to images and saved in DTI studio format using custom software developed in the IDL programming environment. DTI studio was used for region of interest (ROI) analysis to measure different DTI indices [fractional anisotropy (FA), relative anisotropy (RA), linear anisotropy (CL), planar anisotropy (CP), spherical anisotropy (CS) and mean diffusivity (MD×10^{-3}mm^2/s)]. ROI's were placed in mid axial slice of the color coded map on the corpus callosum (CC) and bilaterally on the cerebral cortex, hippocampus (Hipp), caudate putamen (CPu), entorhinal (ento) and piriform cortex (Fig.1), based on the mouse brain atlas[10]. While several regions were analyzed, data is presented from selected regions which are most relevant in ASD[11].

Statistical analysis and Results: Multiple comparisons using Bonferroni, Post Hoc tests were performed to determine the changes in DTI indices at different time points i.e. 30, 50 and 70 days (Table, Fig. 2). We also observed significant differences from the CC FA and right entorhinal cortex MD between day 30 and 50 (data not shown). Pearson correlation was also performed between DTI indices and behavioral [chamber preference score (CPS)] and anxiety score at different time points. When the anxiety score was compared at 3 time points, no significant difference between different time points was observed; suggesting that the social behaviors were not fully attributable to levels of anxiety. Social scores were significantly lower on 30 day compared to 50 and 70 day and these scores showed significant correlation with DTI indices in different regions of the brain (Fig. 3).

Table: Bonferroni post hoc analysis of DTI indices at 2 time points in BALB/cJ mice.

Regions	Variable	30 day (mean±SD)	70 day (mean±SD)	p
LCPu	FA	0.23±0.05	0.28±0.04	0.02
	RA	0.19±0.04	0.23±0.03	0.03
	CL	0.19±0.04	0.26±0.03	0.01
CC	RA	0.56±0.08	0.66±0.01	0.05
	CS	0.30±0.05	0.22±0.05	0.02
R Piriform cortex	MD	0.77±0.04	0.74±0.07	0.04
R Cerebral cortex	MD	0.74±0.03	0.69±0.02	0.02

Figure 1: Color maps showing placement of ROIs.



1=Cerebral cortex, 2=CC, 3=Hippo, 4=CPu, 5=Entorhinal cortex, 6=Piriform cortex

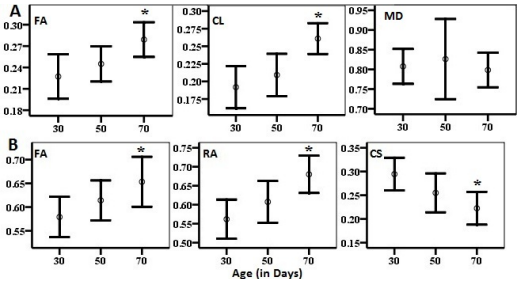


Figure 2: DTI indices at 3 time points. (A) DTI indices from LCPu, (B) DTI indices from CC. *sig. in 30vs70 days

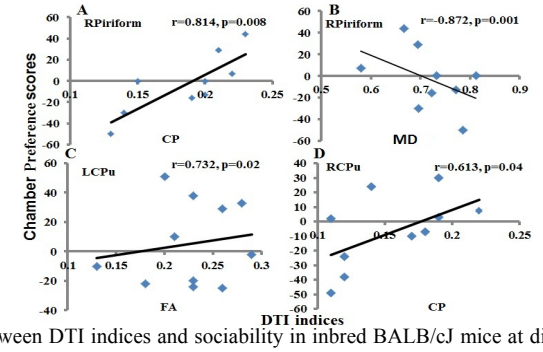


Figure 3: shows correlation between DTI indices and behavioral scores. CP (a) and MD (b) showed correlation at 30 day in R Piriform cortex, while FA (c) exhibited correlation from L CPu at 30 day. CP (d) showed correlation at 70 day in left and right caudate putamen, respectively.

Discussion: In this study, we observed a significant correlation between DTI indices and sociability in inbred BALB/cJ mice at different time points corresponding to the developmental age of these animals. A positive correlation between FA and chamber preference score at 30 day is suggestive of a deficit in early behavioral development as the BALB/cJ mice exhibit low sociability at this age. Lewis et al have reported altered cortical-basal ganglia circuitry in the development and expression of restricted and repetitive behavior in an animal model of ASD[12]. Abnormality in the CC has also been reported as a structural brain underconnectivity and was associated with impaired social and cognitive functioning in humans[13, 14]. In the present study, reduced FA and low sociability score may be suggestive of abnormal development of the CC. This preliminary data demonstrates that abnormalities in social behavior may be assessed by structural changes in DTI and that DTI indices may be used as surrogate markers for studying other neurodevelopmental disorders like schizophrenia.

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