Fractional anisotropy correlates with social behavior symptoms in a mouse model relevant to autism

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Introduction: Patients with autism spectrum disorders (ASD) exhibit impairments in social behaviors, impairments in social communication, and restricted, repetitive, or stereotypical behaviors. A leading hypothesis is that ASD behavioral phenotypes are mediated by a combination of underconnectivity of white matter fiber tracts connecting distal brain regions, as well as over-connectivity in more proximal brain regions (1). The BALB/cJ inbred mouse strain has been proposed as a model relevant to autism behavioral endophenotypes, because, on average, juvenile BALB/cJ mice show low sociability (low tendency to seek social interaction) in comparison to the more normal and highly social C57BL/6J mice (2). Interestingly, genetically homogeneous BALB/cJ mice also show within-strain variability in both sociability and brain white matter development (2). Since diffusion tensor imaging (DTI) has been widely used in studying the brain connectivity of both human and animal models, we hypothesized that DTI studies of the BALB/cJ mouse brain may be able to detect abnormal brain connectivity that is associated with the reduced sociability in these mice. Development of DTI as surrogate markers for detection of social behavioral abnormalities may help in identifying specific anatomical pathway disruptions and microstructural changes that underlie autism-relevant behavioral phenotypes.

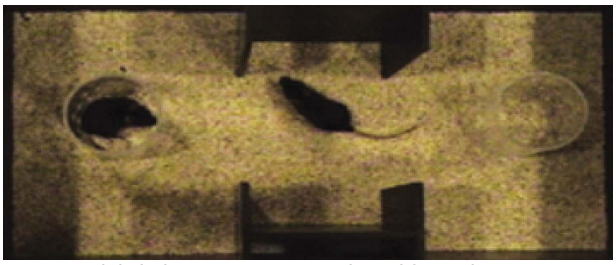

Figure 1. Social choice test apparatus viewed from above.

Materials and Methods: BALB/cJ mice were bred in house. A social choice test was performed in these animals at age 31 days-of-age. This test was performed using a three chambered apparatus shown in Fig.1. The apparatus has a clear Plexiglas cylinder in each of the two end chambers. A "test" mouse is shown in the center chamber and a "stimulus" mouse is shown in the cylinder on the "social" side. The cylinder on the other side contains an inanimate object. The test mouse is initially allowed to acclimatize with the apparatus without the presence of the stimulus mouse or inanimate object for 10 min (Phase 1, habituation phase). The stimulus mouse and the inanimate object are then simultaneously placed in the two cylinders. The time spent by the test mouse sniffing each cylinder is then recorded for 5 minutes to generate a cylinder sniffing time (Phase 2, social choice phase). A "social" mouse would typically spend more time in olfactory investigation of the cylinder containing the stimulus mouse, while the "less social" mouse would spend equal time sniffing the cylinder containing the object vs. the cylinder containing the stimulus mouse. Following the social choice test, the mice were sacrificed (n=15) and brains were fixed using trans-cardiac perfusion and extracted for ex vivo DTI studies.

High-resolution DTI scans were performed using a 9.4 T, 89 mm vertical bore magnet and a specially designed loop gap resonator probe. A 3D bipolar multi echo PGSE sequence was used with TR = 250 ms, TE = 52 ms, FOV = 18.0 x 7.5 x 11.0 mm, matrix size = 256 x 104 x 156, and b-values =0 and 882 s^2/mm. Four age-matched C57BL/6J brains were also scanned using the same protocol and were used to generate an averaged brain template of the fractional anisotropy (FA) map. The FA maps of all BALB/cJ mice were then co-registered to this template. A regression analysis was performed between the FA values in each voxel and the cylinder sniffing time of the animals using the social choice test mentioned above. SPM (UCL, UK) was used for data analysis. T-test was used to select the regions with regression coefficients significantly different from zero ($p<0.001$), and to separate them with positive and negative relationships.

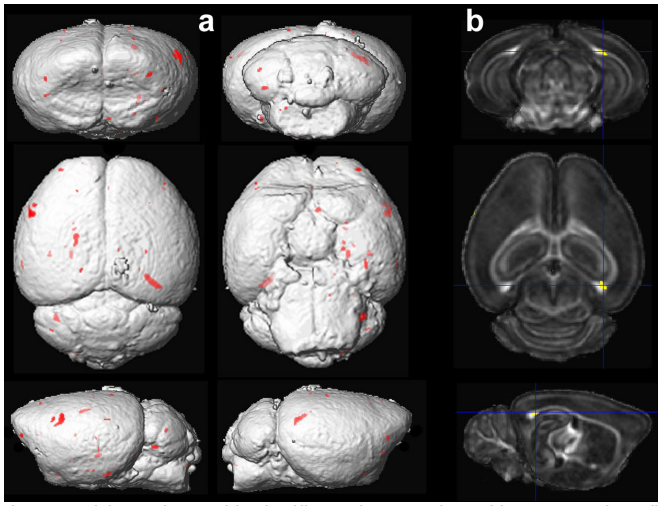

Figure 2. (a) Regions with significant ($p<0.001$) positive regression. (b) Example of a cluster in posterior commissure overlaid in the averaged FA template image.

Results and Discussion: Fig.2 shows regions where significant ($p<0.001$) positive regression was found between FA and the cylinder sniffing time. Most regions with the positive relationship were observed in the white matter as shown in Fig.2b. In contrast, significant ($p<0.001$) negative regression was found mostly in the gray matter as shown in Fig.3. Although preliminary, these results demonstrate the relationship of DTI to social behaviors in this mouse model. These early results are suggestive of the potential of DTI in confirming the hypothesized long range neural underconnectivity and short range neural overconnectivity disorder in ASD. Further studies along with histological validation may be necessary to establish this hypothesis. In conclusion, DTI may play an important role in better understanding the underlying causes of the social behavioral abnormalities observed in ASD.

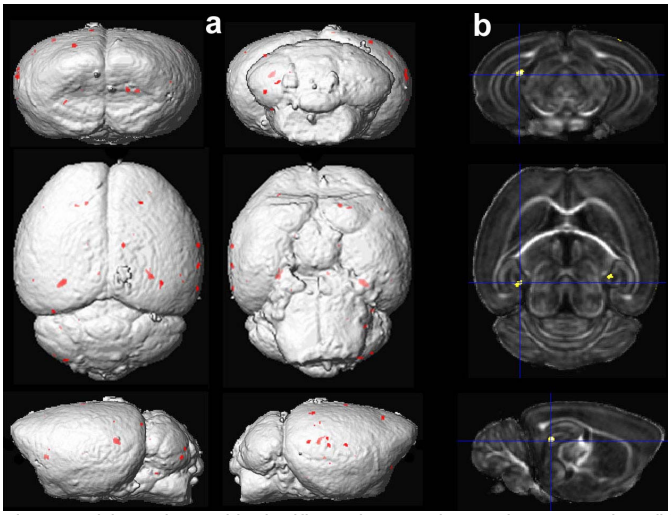

Figure 3. (a) Regions with significant ($p<0.001$) negative regression. (b) Example of a cluster in hippocampus overlaid in the averaged FA template image.

Reference: 1. Belmont, M.K. et al. (2004) J Neurosci 24, 9228-9231. 2. Brodkin, E.S. et al. (2007) Behav Brain Res 176, 53-65

Acknowledgement: This study was funded by the NIH grant R01MH080718.