# Abnormal diffusion properties in a rat model of dyslexia revealed by diffusion spectrum imaging (DSI) tractography

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## Introduction

Dyslexia is defined by severe and specific difficulty in reading acquisition. Abnormalities in brain development are increasingly reported in dyslexia (Galaburda et al., 2006), and recently, *Dyx1c1* was identified as one of the candidate dyslexia susceptibility genes and is involved in neuronal migration and other developmental processes (Meng et al., 2005; Paracchini et al., 2006; Wang et al., 2006). RNA interference (RNAi) of *Dyx1c1* disrupts neuronal migration in developing embryonic neocortex in the rat and resulted in pockets of unmigrated neurons similar to those seen in the brains of dyslexics (Rosen et al., 2007).

To determine if detectable cortical and white matter organizational changes were associated with the subtle heterotopia in the rat *Dyx1c1* knock-down, we used diffusion spectrum imaging (DSI). Unlike diffusion tensor imaging (DTI) where the local water diffusivity is modeled by an ellipsoid and often fails to accurately determine the direction of pathways at areas with low FA values, DSI is a technique that measures water diffusivity with numbers of direction-specific magnetic field gradients (Wedeen et al., 2005). Without fitting measured water diffusivity to an ellipsoid model, it can detect multiple directions of water diffusivity, which enables us to solve the tractography problem on crossing fibers.

## Methods

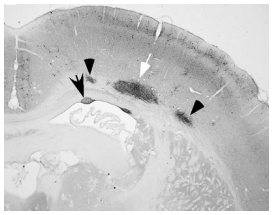
In utero electroporation was performed at embryonic day 14/15. In all *Dyx1c1* shRNA treatments, plasmids encoding shRNA and plasmids encoding enhanced green fluorescent protein (eGFP) were co-transfected into the ventricular zone. The remaining animals received transfection only with plasmids encoding monomeric red fluorescent protein (mRFP). The plasmids were microinjected by pressure through the uterine wall into one randomly chosen lateral ventricle of each embryo. Electroporation was achieved by discharge of a 500 uF 250V capacitor charged to 50-100 V.

At postnatal day 30, brains were perfused with 4 % paraformaldehyde after euthanasia, removed from the cranium and further soaked in 4 % paraformaldehyde containing 1 mM gadolinium (Gd-DTPA) MRI contrast agent for five days to reduce the $T_1$ relaxation time with minimal reduction of the $T_2$ relaxation time. At the fifth day, the solution was replaced with the phosphate buffer saline solution with 1 mM Gd-DTPA. The fixed brains were then scanned on a 9.4T Bruker scanner, with the brains immersed in Fomblin to minimize susceptibility artifacts.

The pulse sequence was a spin-echo (SE) 3D diffusion weighted echo-planar imaging (EPI) sequence, TR/TE 1000/50 ms, with an imaging matrix of 128 pixels with spatial resolution of 250 $\mu m^3$. We measured 515 points in Q-space with a maximum b-value = 40 x $10^3$ $cm^2$ $s^{-1}$. $\delta$ = 12 ms, $\Delta$ = 31 ms. Total acquisition time was 16 h.

Trajectories were propagated always pursuing the orientation vector of least curvature. Tracking terminated when the angle between two consecutive orientation vectors was greater than a given threshold (35 degrees), or when the fibers proceeded outside of the brain surface by using brain mask volumes. Trajectories were displayed on a workstation using a 3D tractography visualization tool (TrackVis).

## Results



Cortical heterotopia at the white/gray matter boundary and the ventricular zone (Rosen et al., 2007).

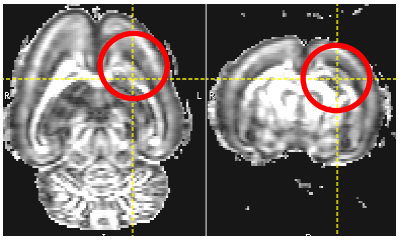


Fig. 1. Fractional anisotropy (FA) map of a *Dyx1c1* transfected brain.

The shRNA treated hemisphere (right side of image) showed lower FA values compared to the opposite hemisphere.

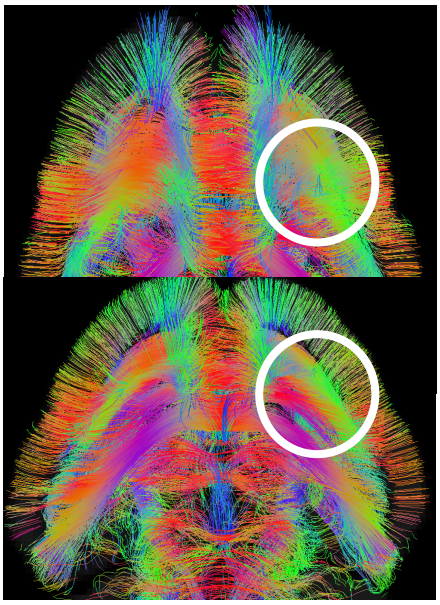


Fig. 2. DSI tractography of the transfected brain. Biased regional diffusion properties were observed in the shRNA treated hemisphere (right side of image) at the white/gray matter boundary.

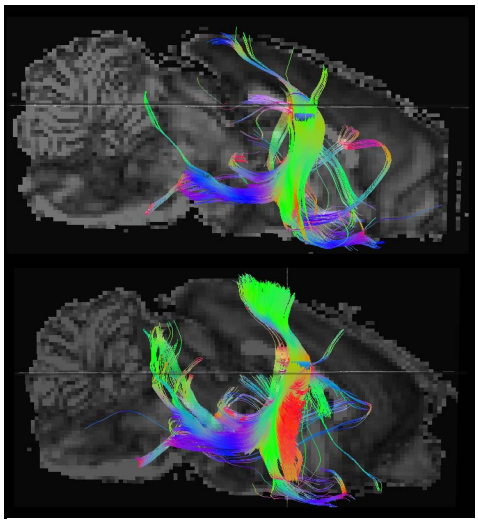


Fig. 3. DSI tractography from the region with abnormal diffusivity (upper) and from a corresponding region in the control rat brain (lower).

The transfected brain showed fewer cortical and white matter tracts from the abnormal region, although white matter tracts showed the same course compared to the control rat brain.

## Conclusion

DSI tractography detected abnormal tissue organization beyond the small focus of heterotopia at the white/gray matter boundary in *Dyx1c1* transfected rat brains with the transfected hemisphere having fewer cortical and white matter fibers compared to control brains. Studying the more widespread cortical organizational changes associated with these subtle cortical heterotopia could contribute to our understanding of one possible cause of dyslexia and help us understand what phenotypes to look for when imaging humans.