Altered fiber connectivity in adult brain of PAX6 knock-out mice revealed by DTI in vivo

S. Boretius[1], A. Stoykova[2], R. Tammer[1,3], J. Frahm[1], and T. Michaelis[1]

[1]Biomedizinische NMR Forschungs GmbH, Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany, [2]Molekulare Zellbiologie, Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany, [3]DFG Center of Molecular Physiology of the Brain (CMPB), Göttingen, Germany

Introduction

Despite the tremendous interest in genetically modified mice, in many instances only very specific questions are within the scientific focus, while more general consequences of the mutations on cerebral structure and function are incompletely studied. Here we applied diffusion tensor imaging (DTI) on mice in which PAX6 has been conditionally knocked out in the prefrontal cortex. As shown histologically, pertinent mice develop a general reduction of cortical thickness due to a lack of cortical layers 1 to 3 [1]. The purpose of this study was to investigate if this mutation results in associated changes of neuronal connectivity as now detectable by DTI in vivo.

Methods

MRI studies were performed at 2.35 T and 9.4 T (Bruker Biospin GmbH, Germany). Conditional PAX6 knock-out mice and their genetically unaltered littermates (n = 3/3) underwent T2-weighted MRI (2.35 T, 3D FSE, TR/TE = 3000/61 ms, 8 echoes, inter-echo spacing = 14.4 ms, 117 µm isotropic resolution) and DTI (9.4 T, DW single-shot STEAM, b=0/1000 s mm^{-2}, 125×125×500 μm^3 resolution) to obtain maps of fractional anisotropy (FA) and main diffusion direction (MDD). Fiber tracking was performed as described [2] using a FA threshold of 0.15 and a maximum directional change of 40° between consecutive steps. Fiber tracks were superimposed on manganese-enhanced T1-weighted images (9.4 T, 3D FLASH, TR/TE=17.0/4.0 ms, flip angle = 25°, 100 µm isotropic resolution) obtained 48 h after a subcutaneous injection of $MnCl_2$ (40 mg/kg). Directional color code: red = *left-right*, blue = *rostral-caudal*, green = *anterior-posterior.*

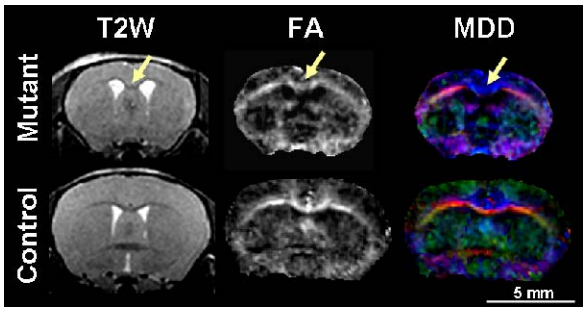

Fig.1: Axial T2-weighted images (T2W) as well as maps of fractional anisotropy (FA) and mean diffusion direction (MDD) of PAX6 knock-out mouse and control.

Results and Discussion

A comparison of T2-weighted images of mutant and control, see Fig. 1 (T2W), revealed a thinner cortex and a reduction of overall brain size. Whereas T2W and FA maps suggested the existence of a normal *corpus callosum* (cc, Fig. 1, arrow), the MDD map revealed a lack of interhemispheric connectivity in mutant (blue: *rostral-caudal* direction, arrow) compared to a clear right-left fiber orientation of the cc in controls (red). These findings were further supported by fiber tracking using a start ROI in the middle of the cc (Fig. 2, left, white ROI on the small MDD maps). Whereas controls (Fig. 2, bottom) presented with a clear connection of the two hemispheres and a projection into the cingulate and motor cortex, fibers of the cc in mutant showed a strong *rostral-caudal* orientation and a complete lack of interhemisperic projection. Furthermore, a vast fiber reorganization in mutants was seen for fibers crossing the septal region (Fig. 2, middle). Whereas controls revealed a clear delineation of the septum, mutants were characterized by a sprouting of fibers. Other evidence for altered fiber connectivity in PAX6 knock-out mice came from the enlargement of the fimbria and its *rostro-posterior* displacement (Fig. 2, right). These findings support an important role of PAX6 not only in the development of the cortex, but also in the formation of the fundamental neuroaxonal connectivity.

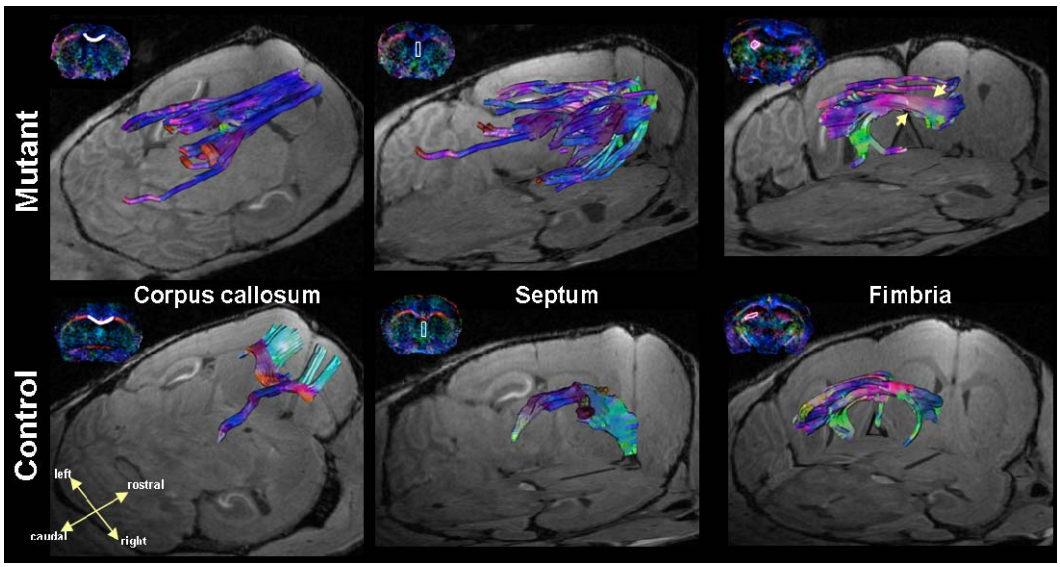

Fig. 2: Fiber tracks of PAX6 knock-out mouse and control. Start ROIs are marked in white on corresponding MDD maps in the left upper corner. Manganese-enhanced 3D MRI was used for anatomical reference.

Conclusion

DTI and fiber tracking in mice allowed for detection of major neuronal fiber connectivity in vivo. DTI offers new contributions to the study of neuronal development using genetically modified animals.

References

1. Stoykova et al., unpublished data;
2. Boretius et al., J Neurosci Methods, 2007, 161 :112