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# Microstructural effects of a neuro-modulating drug evaluated by diffusion tensor imaging

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ABSTRACT

In a longitudinal mouse study we evaluated whether diffusion tensor imaging (DTI) can monitor microstructural changes after administration of the neuromodulating drug EPO and whether erythropoietin (EPO) has an effect on cognitive performance. Twelve mice (2 groups with 6 mice each) were scanned in a 7T Bruker Biospin animal scanner with a highly resolved DTI sequence before and 16 days after intraperitoneal injections of EPO or saline. All mice underwent behavioral testing (Morris water maze) and histologic evaluation of hippocampal and corpus callosum cell proliferation and oligodendrogenesis. Whole brain DTI analysis showed significant Trace, RD and AD decrease within the dentate gyrus, subiculum, primary motor, somatosensory, and supplementary somatosensory areas and FA increase in the hippocampus, corpus callosum, and fimbria fornix in EPO treated mice only. ROI-based DTI analysis showed significant Trace and RD decrease and FA increase only in the corpus callosum of EPO treated mice, whereas in the dentate gyrus significant Trace, RD, and AD decrease occurred in both, EPO- and control-group. Behavioral tests showed that EPO treated mice performed better and learned faster than controls. Histologically, the number of BrdU-positive nuclei and optical density of DCX-labeled juvenile neurons significantly increased within the dentate gyrus, corpus callosum and fimbria fornix and the number of NG2-positive oligodendrocyte progenitors in corpus callosum and fimbria fornix, respectively. In conclusion we were able to monitor microstructural changes with DTI and showed EPO treatment-related alterations correlating with enhanced dentate gyrus and corpus callosum cell proliferation and better learning capabilities.





## Introduction

Diffusion tensor magnetic resonance imaging (DTI) is widely applied in the evaluation of neurological disorders and is considered a sensitive tool to detect neurodegenerative diseases at preclinical stages and to monitor disease progression and even drug-related effects (Amlien, Fjell, 2014; Cochrane, Ebmeier, 2013).

In a clinical study with Friedreich ataxia (FRDA) patients we showed significant correlations of DTI parameters (i.e. radial diffusivity — RD) with FRDA-specific clinical scores and genetic changes (von Hohenberg et al., 2013). Moreover, DTI parameters (i.e. axial diffusivity — AD, and fractional anisotropy — FA) changed in white matter structures after systemic treatment with recombinant human erythropoietin (EPO) (Egger et al., 2014). As these DTI changes occurred in a widespread whole

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brain distribution and spared primarily disease-specific brain areas, we hypothesized that they might reflect disease-unspecific EPO effects (Egger et al., 2014). This hypothesis was supported by a mouse study on the effects of EPO on adult hippocampal neurogenesis showing increased numbers of mitotically active neuronal progenitor cells and glial stem cells in the dentate gyrus of the hippocampus. Although the exact mechanism by which EPO acts on the brain is still not clear, these effects appeared to be mediated, at least in part, by mitogen-activated protein kinase signaling and are potentially regulated by a suppressor of cytokine signaling-3 (Ransome and Turnley, 2007).

In addition, EPO efficiently enhances the production of new oligodendrocytes (Iwai et al., 2010; Zhang et al., 2010; Kako et al., 2012) and promotes white matter reorganization (Li et al., 2009) after ischemic stroke. Beside the neuroprotective and neuroregenerative effects of EPO after neurological insult, observations derived from immunocytochemical staining clearly suggest an anatomical basis for direct transport of EPO within the systemic circulation into the central nervous system even in the absence of any neural insult (Brines et al., 2000).

The aim of this experimental mouse study was to evaluate EPO induced DTI changes under physiologic conditions using a combined whole brain as well as a region of interest (ROI) analysis. To prove microstructural changes we histologically evaluated the corpus callosum and the dentate gyrus. Finally, to elucidate EPO treatment effects we compared these changes with behavioral data.

## Material & methods

Twelve male C57BL/6 N mice approximately 8–9 weeks old, weighting 25–30 g (Charles River), were randomly separated in 2 groups. After 2 weeks of habituation in cages with 3 mice each and a baseline MRI, group 1 (6 mice) received intraperitoneal (i.p.) injections of EPO (5 U/g) every second day for 16 days. The EPO dosage regime was based on previous studies (Zhang et al., 2010; Brines et al., 2000). Accordingly, group 2 (6 mice) received i.p. injections of sterile 0.9% saline solution every second day for 16 days. Combined with the last 3 i.p. EPO-/saline-injections all mice received additionally 0.15 ml bromdesoxyuridin (BrdU) solution (10 mg/ml) to label proliferating cells. A follow-up MRI was performed after 16 days.

All experiments were performed in accordance with the local guidelines and ethics on animal experimentation.

### *MRI*

*In vivo* mouse brain MRI was performed twice (pre and post i.p. injections) for each mouse using a 7T Bruker Biospin animal scanner and a mouse brain adapted cryocoil (Bruker, Ettlingen, Germany). The usage of cryocoil technology provides a significant increase of signal to noise ratio and therefore a reduction in acquisition time.

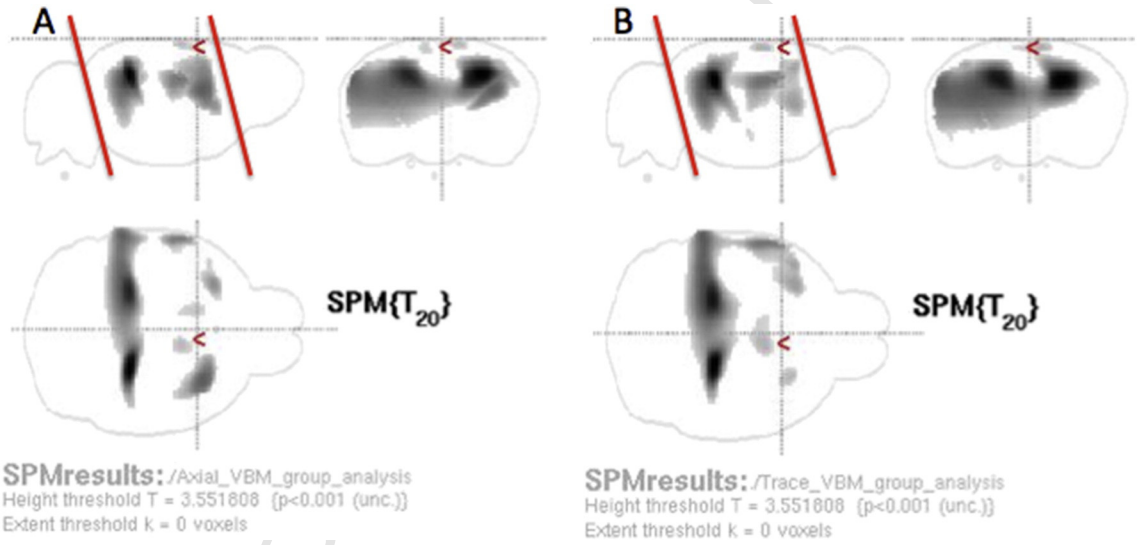


**Fig. 1.** Axial (A) and Trace (B) whole brain group analyses (post EPO versus pre EPO) results ($p < 0.001$ uncorr.) superimposed in gray values corresponding to significance (black showing areas with the highest significance) on a glass mouse brain image. The red lines show the rostral and caudal borders of the DTI field of view.

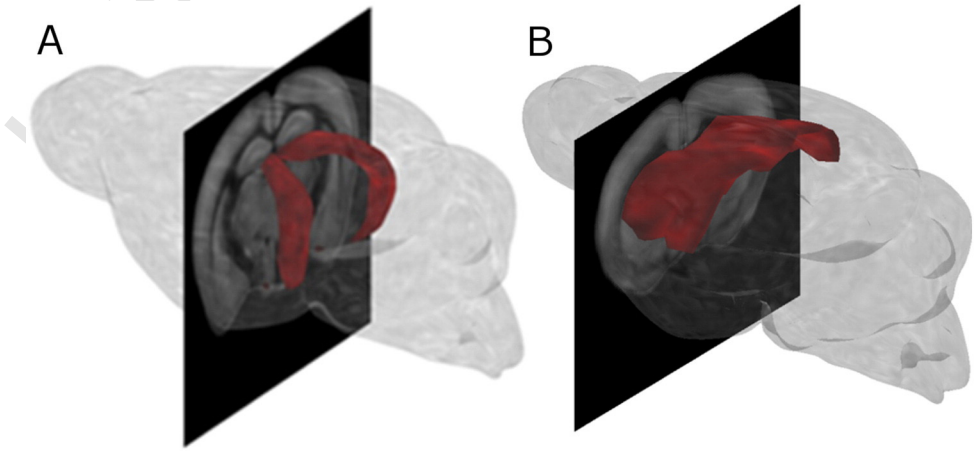


**Fig. 2.** "A priori" defined regions of interest. 3D model of the mouse brain showing dentate gyrus (A) and corpus callosum (B) in red.

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