Deletion of the brain-specific link protein BRAL-1 facilitates extracellular diffusion in the mouse corpus callosum

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Introduction: Extracellular matrix molecules affect the diffusion of neuroactive substances in the central nervous tissue (Sykova et al., 2005). An altered extracellular matrix will inevitably affect neuron-glia interactions, synaptic efficacy as well as extrasynaptic „volume" transmission (Sykova, 2004). Link proteins are characterized as abundant extracellular matrix proteins, the function of which is to stabilizate the binding between lecticans and hyaluronic acid (HA) in cartilage. Recently, the brain-specific hyaluronan-binding link protein BRAL1 has been cloned, which co-localizes with the proteoglycan versican in the myelinated white matter of the mouse central nervous system at the nodes of Ranvier (Oohashi et al., 2002). It was suggested that a complex of HA, lecticans and tenascin-R stabilized by BRAL1 might play a role in neuronal conduction as this complex could represent a reservoir and/or diffusion barrier for sodium ions, the influx of which at the nodes is essential for saltatory conduction.

Subjects and methods: In the current study, we employed two methods to determine the diffusion properties in the primary somatosensory cortex (S1) and corpus callosum (CC) of BRAL1 positive (+/+) and negative (-/-) mice, in vivo as well as in vitro. The apparent diffusion coefficient of water (ADC_W) in the tissue was measured by diffusion-weighted MRI (mice under 1.5% isoflurane anaesthesia); the extracellular space (ECS) diffusion parameters volume fraction α (α = ECS volume/total tissue volume) and the geometrical factor tortuosity λ (λ^2 = free diffusion coefficient/ apparent diffusion coefficient) were determined in 400 µm thick coronal brain slices from concentration-time profiles of the extracellular marker tetramethylammonium (TMA^+) applied by iontophoresis (Nicholson and Phillips, 1981). Due to CC diffusion anisotropy, the measurements in the CC were performed along two prominent orthogonal axes: x-mediolateral and y-rostrocaudal (along and across the axons).

Results: Increased diffusivity, indicated by higher ADC_W values and lower tortuosity, was found along both axes in the corpus callosum of BRAL1 knockout mice in comparison with controls; however, the typical anisotropic diffusion in myelinated white matter persisted. BRAL1 deletion did not significantly influence ECS size (α). The values acquired in the CC are summarized in Table 1. In the cortex of BRAL1 +/+ mice, the ECS diffusion parameters were: α = 0.19±0.01, (mean ± S.E.M.), λ = 1.59±0.03, N = 12 (N = number of animals) and ADC_W = 597±8 $\mu m^2 s^{-1}$, N = 11; there were no significant differences in the cortex of BRAL1 -/- mice when compared to BRAL1 +/+ mice. Since diffusion in the cortex is isotropic, the values of the ECS diffusion parameters are the same along all axes (Vorisek and Sykova, 1997). The lack of significant changes in the primary somatosensory cortex correlates with the localization of BRAL1 protein exclusively at the nodes of Ranvier in the white matter.

	BRAL1 +/+	BRAL1 -/-
α	0.21±0.01	0.19±0.01
λ_x	1.41±0.03	1.32±0.02*
λ_y	1.70±0.02	1.56±0.01*
ADC_W - x axis ($\mu m^2 s^{-1}$)	1158±55	1340±25*
ADC_W - y axis ($\mu m^2 s^{-1}$)	442±19	521±24*

Table 1: ADC_W and ECS diffusion parameters measured along two distinct axes in the corpus callosum of BRAL1 +/+ and -/- mice. Significant differences ($p<0.05$) compared to BRAL1 +/+ animals are marked by asterisks. Values are expressed as mean ± S.E.M., N=10.

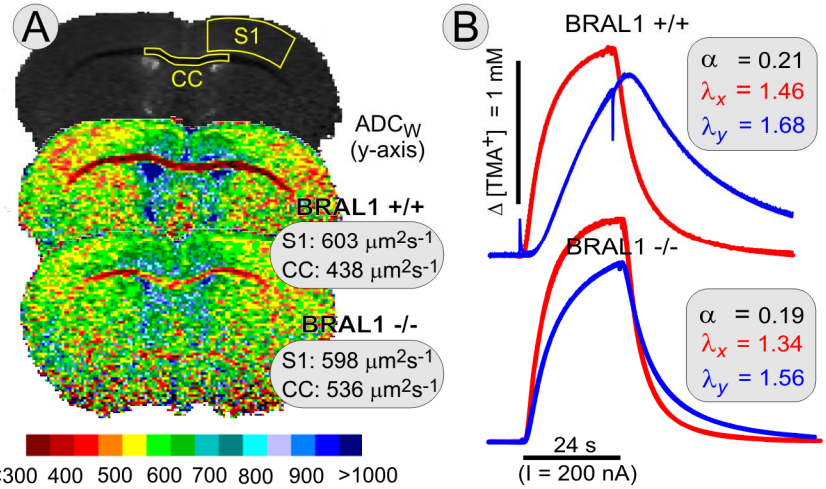

Figure 1: (A) Typical ADC_W maps of BRAL1 +/+ and -/- mice. Note the increased diffusivity in the corpus callosum of the BRAL1 -/- mouse. The mean values of ADC_W were calculated in regions of interest (ROI), which are outlined. (B) Examples of the TMA^+ concentration-time profiles recorded in the corpus callosum of BRAL1 positive and negative mice. The curves were used to calculate ECS volume and tortuosity.

Conclusion: We conclude that the deletion of the brain-specific hyaluronan-binding link protein, associated with a disruption of the HA-lecticans-tenascin-R complex, results in a reduction of the diffusion barriers formed by the extracellular matrix at the nodes of Ranvier, which in turn facilitates diffusion both across and also along the myelinated fibers. This might be important for neuronal conductivity as well as for extrasynaptic transmission based on the diffusion of neuroactive substances through the ECS.

References and acknowledgement:

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