

# ASTROCYTIC AQUAPORIN-4 CONTRIBUTES SIGNIFICANTLY TO WATER MOBILITY IN THE RAT BRAIN

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**Background:** Diffusion weighted imaging (DWI) is routinely used for clinical prognosis and in experimental studies. Its quantitative parameter, the apparent diffusion coefficient (ADC) is thought to reflect water mobility in brain tissues. After injury, reduced ADC values have been considered secondary to decreases in the extracellular space caused by cell swelling. However, the physiological mechanisms associated with such changes remain uncertain. Water channels (aquaporins, AQPs) facilitate water diffusion through the plasma membrane and provide a unique opportunity to examine the molecular mechanisms underlying water mobility in brain tissues. AQP4 is highly expressed in the brain and is distributed within astrocytic cell membranes. We hypothesize that AQP4 contributes to the regulation of water diffusion and variations in expression levels of affect ADC values in normal brain.

**Methods:** We used RNA interference (siRNA), to acutely knock down the expression of AQP4 in rat brain. MRI was performed at 3 days after siRNA and siGLO injection on a Bruker Avance 11.7 T MRI (Bruker Biospin, Billerica MA). Two imaging data sets were acquired: 1) a 10 echo T2, and 2) a diffusion weighted sequence where each sequence collected twenty coronal slices (1 mm thickness and interleaved by a 1 mm). Western blot analysis and immunohistochemistry were performed on siGLO and siAQP4 treated rats after the MRI.

**Results:** Injection of siGLO-tagged with CY3, a fluorescent dye, showed diffusion of siRNA along the corpus callosum, the contralateral cortex and bilaterally in the striatum (Fig. 1A). The presence of the siGLO-CY3 was observed in positive GFAP cells (green, Fig. 1B). Western blot analysis Fig. 1C) on brain showed that AQP4 expression was decreased by 27% in siAQP4 treated rats compared to controls ( $1.23 \pm 0.11$  in

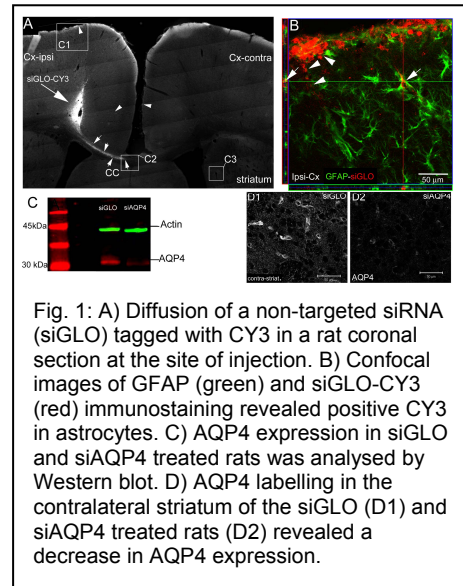


Fig. 1: A) Diffusion of a non-targeted siRNA (siGLO) tagged with CY3 in a rat coronal section at the site of injection. B) Confocal images of GFAP (green) and siGLO-CY3 (red) immunostaining revealed positive CY3 in astrocytes. C) AQP4 expression in siGLO and siAQP4 treated rats was analysed by Western blot. D) AQP4 labelling in the contralateral striatum of the siGLO (D1) and siAQP4 treated rats (D2) revealed a decrease in AQP4 expression.

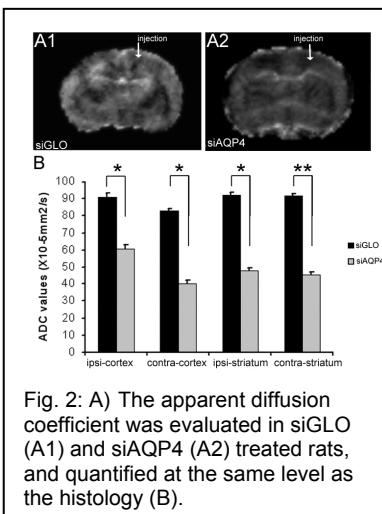


Fig. 2: A) The apparent diffusion coefficient was evaluated in siGLO (A1) and siAQP4 (A2) treated rats, and quantified at the same level as the histology (B).

control vs  $0.90 \pm 0.09$  in siAQP4,  $p < 0.05$ ). AQP4 immunolabelling showed that perivascular AQP4 expression was decreased in the ipsilateral cortex adjacent to the site of injection and in the contralateral striatum (Fig.1D). Acute AQP4 silencing resulted in a significant decrease in ADC values bilaterally in both the cortex and striatum (Figure 2A, B). In the ipsilateral cortex, ADC values were decreased 33% after siAQP4 ( $60.8 \pm 2.5 \times 10^{-5} \text{ mm}^2/\text{s}$ ) compared to siGLO treated controls ( $91.1 \pm 2.5 \times 10^{-5} \text{ mm}^2/\text{s}$ ;  $p < 0.05$ ). Decreased ADC values were also observed at distance in the contralateral striatum, ADC values were decreased 51% (siAQP4,  $45.4 \pm 1.7 \times 10^{-5} \text{ mm}^2/\text{s}$ ; siGLO,  $92.1 \pm 1.0 \times 10^{-5} \text{ mm}^2/\text{s}$ ,  $p < 0.01$ ). Water content, based on T2-weighted images, showed no changes.

**Discussion:** Our results demonstrate that ADC values in normal brain can be modulated by astrocytic AQP4 activity.

These findings have major clinical relevance as they suggest that the imaging changes seen in acute neurological disorders such as stroke and trauma are in part due to changes in tissue AQP4 levels. These results open the question of the contribution of the astrocyte and AQP4 in other images generated on the basis of the water diffusion properties such as diffusion tensor images.