# TIME-DEPENDENT DIFFUSION MRI IN THE NEOCORTEX OF AQUAPORIN-4 DEFICIENT AND NORMAL MICE IN THE RESTING STATE AT 7T

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## Introduction

The goal of this work is to investigate whether and how non-invasive MRI methods can contribute to studying the role of Aquaporin-4 in water transport in the brain. Aquaporin 4 (AQP-4) is the predominant water channel protein in the neocortex and cerebellum. It is concentrated in the astrocyte end-feet membranes, adjacent to blood vessels to facilitate fast, selective and activity dependent water efflux from cortex to subarachnoidal space through the end-feet membranes [1]. We use time-dependent diffusion MRI to investigate how complete depletion of AQP-4 effects water transport in the mice brain. We focus on measurements during the resting state, which will serve as a reference for future work using fMRI.

## Materials and Methods

Animal: All experiments were approved by the Institutional Animal Care and Use Committee and conform to National Institutes of Health guidelines. Studies were conducted with 16 (male and female) C57B16 mice: 8 AQP-4 knockouts and 8 wild-type littermates of AQP-4 knockouts as controls. Animals were laid prone head first in the magnet, heated by a circulating water bed to keep at constant temperature. Anesthesia consisted of inhalation gas 67.5% N2/32.5% O2 + 1.5-3% isofluran. MRI: Experiments were performed on 7T Bruker Biospec 70/20 AS with BGA-12 400mT/m gradient unit, a 72mm volume resonator for transmit and an actively decoupled mouse brain quadrature surface coil for receive-only. The MRI protocol consisted of scout scans, localized shimming using Fastmap, single-shot EPI acquisition for optimization of EPI parameters, and DTI_EPI using pulsed gradient spin-echo (PGSE) and stimulated-echo (PGSTE). Diffusion parameters for PGSE: 3 diffusion directions, 10 *b* values from 50 to 500 s/mm$^2$ (not including imaging gradients), δ=4 ms, Δ=8, 9, 10, 11, 12 ms. Diffusion parameters for PGSTE: 3 diffusion directions, 16 *b* values from 50 to 800 s/mm$^2$, δ=4 ms, Δ=12.5, 13, 15, 20, 50, 100, 150 ms. Other imaging parameters: TE/TR=26.15 ms/2000 ms, NEX=1, aquisition time=1m 38s (max), BW=25 kHz, FOV=20x20 mm$^2$, Matrix=84x60, 2 mm slice thickness.

## Results

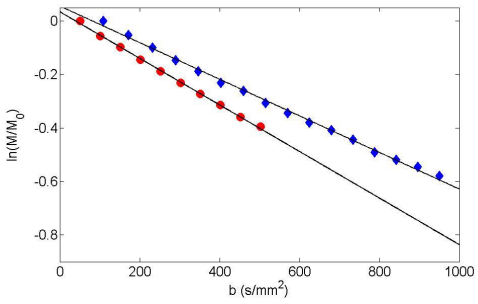


**Figure 1** Monoexponential signal decay as a function of *b*-value for two diffusion times, $t_D$, 6.67 ms (circles) and 148.67 ms (diamonds) in the AQP=4 knockout mouse brain. Figure clearly shows a single diffusion component which is time-dependent (see discussion).

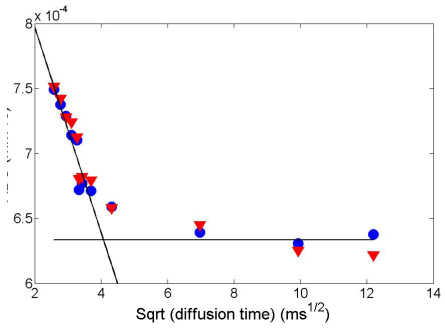


**Figure 2** Apparent diffusion coefficient (obtained from slopes such as in Figure 1) as a function of square root of diffusion time for normal (triangles) and AQP-4 deficient (circles) mouse brain. Fitting a straight line to short-time diffusion limit we can extract the 'bulk' diffusion coefficient $D_0$ [2]. Similarly, fitting an asymptote to the long-time diffusion data one can obtain the tortuosity ($T$) of the tissue since lim $t\rightarrow\infty$ $D(t)/D_0 = 1/T$ [2].

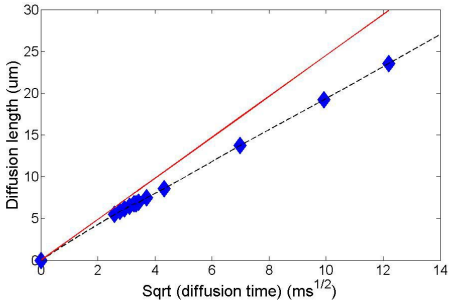


**Figure 3** Diffusion distance as a function of diffusion time using the Einstein diffusion equation, sqrt($6Dt_D$), and using apparent diffusion coefficients from Figure 2. We see from this plot that the diffusion component deviates from linearity (solid line) and is thus slightly restricted.

## Discussion and Conclusions

Short and long diffusion-time limits give respectively the bulk, unrestricted diffusion coefficient $D_0$=1x10$^{-5}$ cm$^2$/s and the tortuosity $T$=1.6. $D_0$ may appear to be low in our study, compared to bulk diffusion coefficient of water at 37°C (3.0 · 10$^{-5}$ cm$^2$/s). However, diffusion in cytoplasm is strongly influenced by proteins and macromolecules, while water in the extracellular space can be partly structured due to interactions with proteins [3], reducing the inherent value of $D_0$. The monoexponential signal decay for all diffusion times might also seem unusual at first in light of previous findings [4]. However, since we are in the low *q*-regime we are sensitive to displacements in the range between 20 µm and 80 µm, which is several orders more than the cell dimension and other smaller structures [2]. The tortuosity value depends partly on the permeability of the membranes, while diffusion behavior at short $t_D$ is sensitive to molecules being close to restricting surfaces. However, within the precision of our measurements we did not observe any differences in time-dependent diffusion between the AQP-4 knockouts and controls in the non-activated state. This is not unexpected, since the AQP-4 channels, and thus the permeability of the membranes, are only expected to be active when osmotic gradients are present across the membranes [5]. Future studies in the activated state need to be performed to see whether time-dependent diffusion could phenotype AQP-4 deficient mice.

## References

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