The Intracellular Water Preexchange Lifetime of Neurons and Astrocytes Are Different and Decrease Rapidly under Oxygen-Glucose-Deprivation Conditions

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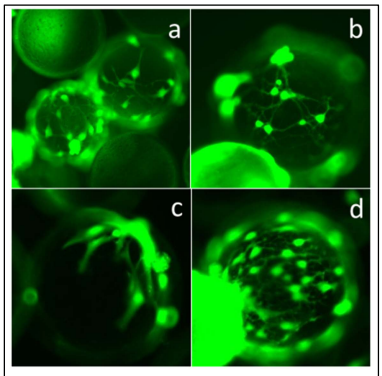

Figure 1. Fluorescence micrograph of microbead-adherent neurons (a and b) and astrocytes (c and d) stained by Calcein AM.

INTRODUCTION Knowledge of intracellular water preexchange lifetime (τ_{IN}) in central nervous system (CNS) cells is of fundamental importance for interpreting diffusion MR results over different time scales. Herein, by employing a previously described "Brains on Beads" methodology[1], we measure the τ_{IN} in cultured rat neurons and astrocytes under normal conditions (oxygen-glucose-perfusion) and upon oxygen-glucose-deprivation (OGD).

METHODS Neurons or astrocytes from newborn rat cerebral cortex were cultured on polystyrene microbeads (125 – 212 μm in diameter), Fig. 1. Microbead-adherent cultures were perfused at 36.5° C with oxygenated, glucose-containing, Tyrode's solution inside an 8-mm ID glass tube. In some experiments, OGD was achieved by replacing oxygen with nitrogen and glucose with 2-deoxy-D-glucose. MR data were acquired on an 11.74-tesla Agilent/Varian DirectDrive™ scanner using a slice-selective inversion-recovery (IR) spin-echo sequence. The slice-selective spin-echo sequence greatly suppresses signal from flowing medium. Fifteen inversion times were used, logarithmically spaced from 5.5 ms to 5 s. Bayesian analysis was employed to estimate the water ^{1}H signal amplitude and to model the IR data (http://bayesiananalysis.wustl.edu).

RESULTS AND DISCUSSION IR data from medium flowing through cell-free packed microbeads were well modeled as a mono-exponential. The flow-dominated apparent T_1 was estimated to be very short (10-30 ms) and was sensitive to flow rate (data not shown). In the presence of microbead-adherent cells, however, IR data were well modeled as a bi-exponential, Fig. 2, thus defining two relaxing components (A and B) by their different T_1's. Component A, with an observed T_1 varying between ~0.3 – 0.7 s depending on the experimental condition, was not detected in the cell-free experiment and contributed to an average of 25% of the total signal. The average T_1 of component B ($T_{1,OBS}$(B)) was 48 ± 7 ms, similar to that of the flowing medium in cell-free experiment. Therefore, the long-T_1 component (A) was assigned to the intracellular water population. Using the Bloch-McConnell equations describing longitudinal relaxation in the two-compartment slow-exchange regime, Eq. [1], and an intrinsic intracellular water T_1, $T_{1,IN}$, of 1.8 s as previously reported[2], τ_{IN} was determined as 0.88 ± 0.24 s for neurons (n = 9) and 0.66 ± 0.17 s for astrocytes (n = 13). OGD experiments were employed to mimic cerebral hypoxia-ischemia. The τ_{IN} in both types of cells decreased within 30 min after OGD onset, Fig. 3. At 75 min after OGD onset, neuron and astrocyte τ_{IN} decreased to 0.48 ± 0.07 s (n = 3) and 0.18 ± 0.04 s (n = 5), respectively. The decrease in τ_{IN}, interpreted as an increase in apparent cell-membrane water permeability, may reflect disruption of energy-dependent ion pumps but could also indicate disruption of aquaporin function and/or change in membrane structure upon OGD. Importantly, $T_{1,OBS}$(B) remained unchanged in each OGD trial, wholly consistent with the interpretation that component B represents the flowing medium. Non-mono-exponential decay of the diffusion-weighted MR signal in CNS is often ascribed to contributions from water populations with distinct apparent diffusion coefficients. In a simplified two-compartment-exchange model, τ_{IN} determines in what exchange regime the diffusion-weighted data should be assessed[3]. The diffusion times typically used in clinical studies place them in the slow-exchange regime. However, as τ_{IN} decreases significantly shortly following injury, the transmembrane exchange moves towards a more intermediate-exchange regime. This should be taken into account when interpreting diffusion data. Moreover, the intrinsic, non-negligible difference between neuron and astrocyte τ_{IN}, especially in injured cells, should also be taken into account in the analysis of time-scale-sensitive data.

$$\frac{1}{T_{1,IN}^{OBS}} = \frac{1}{T_{1,IN}} + \frac{1}{\tau_{IN}} \quad [1]$$

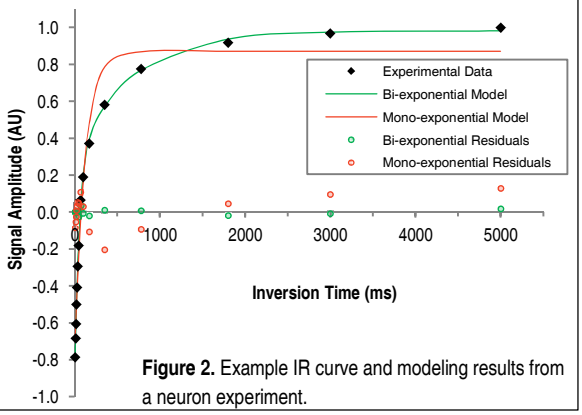

Figure 2. Example IR curve and modeling results from a neuron experiment.

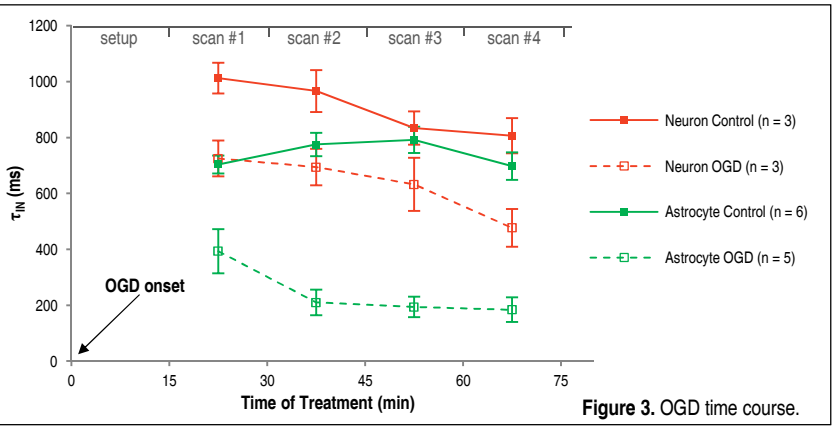

Figure 3. OGD time course.

CONCLUSION To our knowledge these are the first MR measurements of intracellular water preexchange lifetime in cultured neurons (0.88 ± 0.24 s) and astrocytes (0.66 ± 0.17 s). These lifetimes decreased quickly upon oxygen-glucose-deprivation to 0.48 ± 0.07 s and 0.18 ± 0.04 s, respectively.

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REFERENCES 1. Yang D, *et al.*, abstract #0535, *2013 ISMRM Annual Meeting*; 2. Ye Q, *et al.*, abstract #2744, *2010 ISMRM Annual Meeting*; 3. Lee J, *et al.*, *Magn Reson Med.* **2003**; 49: 450.