# Intracellular Water Preexchange Lifetime in Cultured Mixed Neurons and Astrocytes

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**INTRODUCTION:** Knowledge of intracellular water preexchange lifetime ($\tau_{IN}$) in central nervous system cells is essential for studying intracellular water diffusion properties over different time scales[1]. Herein, by using a thin-slice spin-echo inversion recovery (IR) sequence, we separate the intracellular water MR signal from that of flowing extracellular water (perfusate) based on the distinctly different apparent longitudinal relaxation times ($T_1$'s) of these water populations in a "Brains on Beads" system. The true intracellular water $T_1$ is ~50 fold greater than the apparent $T_1$ of the flowing perfusate. Thus the system is in the MR slow exchange limit, allowing the intracellular water $\tau_{IN}$ to be determined as 0.26 ± 0.03 s. **METHODS:** Mixed neurons and astrocytes were obtained from newborn rat cerebral cortex as previously described[2] and cultured on polystyrene microbeads (125-212 µm diameter, SoloHill), Fig. 1. Microbeads with cells attached were transferred into an 8-mm ID glass tube and perfused with 27 °C oxygenated Tyrode's solution. Data were acquired on an 11.74-tesla Agilent/Varian DirectDrive™ MRI system using a slice-selective IR spin-echo sequence. Crafted 90° and 180° pulses[3] were employed to select a group of 75-µm thick slices with slice-planes oriented orthogonal to flow direction. Other scan parameters include: TR 2 s, TE 25 ms, 8 averages, and scan time less than 9 min. Bayesian analysis (http://bayesiananalysis.wustl.edu/) was used to estimate the water $^1$H MRS signal amplitude from each slice and to model the IR data. In some experiments (Studies #5-6, Table 1), Gd-BOPTA (Bracco) was added to Tyrode's solution to shorten the extracellular water $T_1$.

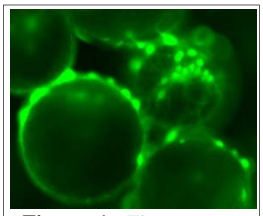

**Figure 1.** Fluorescence micrograph of "Brains on Beads". Green color denotes alive cells stained by Calcein AM (Invitrogen).

**RESULTS AND DISCUSSION:** At a flow rate of 40 mL/min, > 99% of the spin-echo signal from media flowing through cell-free packed microbeads was suppressed due to both a time-of-flight effect and gradient/turbulent-flow induced transverse magnetization phase dispersion (data not shown). The residual signal was characterized by a short apparent $T_1$ of 12 ± 1 ms, Fig. 2. In the presence of microbead-adherent cells, however, the IR curve was well modeled as a bi-exponential, Fig. 2, thus defining two water populations by their different $T_1$'s. Table 1 summarizes the $T_1$ estimates and amplitude ratios of the two components from four independent studies. In another experiment (Study #5), the media was replaced by Gd-BOPTA-doped (4 mM) Tyrode's solution where the intrinsic $T_1$ of the media was decreased from 2.7 s to 47 ms. $^1$H IR MRS was performed in this case without flow. Bi-exponential relaxation was clearly evident. Interestingly, the $T_1$ of the slowly relaxing (long-$T_{1,OBS}$) component (A) was essentially equivalent to that observed with flow in the absence of Gd-BOPTA, while the rapidly relaxing component's time constant [$T_{1,OBS}$(B)] was in accord with the $T_1$ of the Gd-BOPTA-doped media, i.e., extracellular water (47 ms). A slowly relaxing component (A) was not found in a control experiment employing Gd-BOPTA-doped media with cell-free packed microbeads (Study #6). Here the IR data were well modeled as mono-exponential with $T_1$ = 46.4 ± 0.1 ms. Based on this clear evidence, bi-exponential IR component A is assigned to the intracellular water $^1$H MRS signal and component B to that from extracellular water.

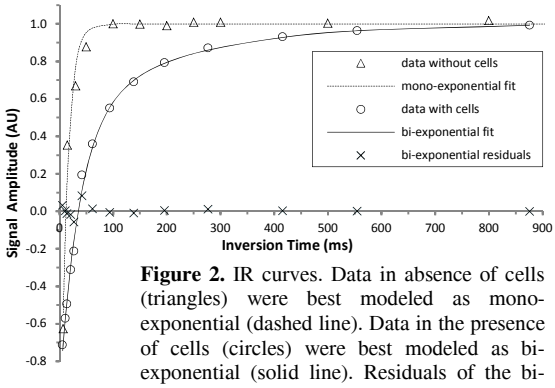


**Figure 2.** IR curves. Data in absence of cells (triangles) were best modeled as mono-exponential (dashed line). Data in the presence of cells (circles) were best modeled as bi-exponential (solid line). Residuals of the bi-exponential fit were marked by crosses.

**Table 1.** IR MRS modeling results. Flow studies #1-4 used normal Tyrode's solution; non-flow studies #5-6 used Tyrode's solution with 4 mM Gd-BOPTA.

| Study | $T_{1,OBS}$(A) (s) (Mean ± SD) | $T_{1,OBS}$(B) (s) (Mean ± SD) | Amplitude Ratio (A : B) | $\tau_{IN}$ (s) |
|---|---|---|---|---|
| 1 (n=1) | 0.19 ± 0.03 | 0.030 ± 0.003 | 1 : 2 | 0.21 |
| 2 (n=3) | 0.21 ± 0.02 | 0.036 ± 0.002 | 1 : 3 | 0.24 |
| 3 (n=3) | 0.21 ± 0.02 | 0.038 ± 0.002 | 1 : 3 | 0.24 |
| 4 (n=4) | 0.25 ± 0.03 | 0.037 ± 0.002 | 1 : 3 | 0.29 |
| 5 (n=1)[a] | 0.24 ± 0.03 | 0.049 ± 0.000 | 1 : 41 | 0.28 |
| 6 (n=1)[a,b] | N/A | 0.0464 ± 0.0001 | N/A | N/A |

a) Single slice experiment with slice thickness 4 mm; no flow. b) no cells.

A previous preliminary report from this lab employing the "Brains on Beads" system postulated magnetization transfer between microbeads and extracellular water as the source of the rapidly relaxing IR component.[4] More detailed follow-up studies described herein support assignment of this component instead to residual signal from flowing extracellular perfusate water whose signal is not adequately suppressed in a system of packed "brain-cell"-adherent microbeads. Indeed, careful examination of this system with its highly dendritic neurons and astrocytes reveals that the "brain cells" engage in entanglements that cause the microbeads to "clump", thus packing inefficiently and resulting in "fast" and "less fast" flowing media populations. Signal from the fast moving media is highly suppressed (apparent $T_2 = T_1 \approx 10$ ms) and essentially not detected. However, residual signal from the less fast moving media is detected as a rapidly relaxing IR component ($T_2 = T_1 \approx$ 30-40 ms). Importantly, the more slowly relaxing component ($T_1 \approx$ 0.19-0.25 s) is also present in the absence of flow-enabled extracellular-media signal suppression when $T_1$ and $T_2$ of the media are greatly reduced ($T_1$ = 46.4 ms) *via* Gd-BOPTA. Thus, the more slowly relaxing IR component cannot be ascribed to extracellular media and is assigned to the intracellular water population. The Block-McConnell equations describing exchange between two compartments (in this case, intracellular and extracellular) result in a relatively simple expression for the observed intracellular IR relaxation rate constant, $R_{1,IN}^{obs}$, Eq. [1]. Using the intrinsic $T_1$ of "brain cell" intracellular water at 11.74 tesla, $T_{1,IN}$, = 1.8 s, reported previously[4], the intracellular preexchange lifetime is readily obtained (Table 1) and falls in the range 0.2-0.3 s.

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

**CONCLUSIONS:** Experiments have been carried out to determine the intracellular water preexchange lifetime, $\tau_{IN}$, of cultured mixed neurons and astrocytes using a microbead ("Brains on Beads") system. Bi-exponential inversion recovery was observed in this system, the origins of which were clarified by control (non-flowing, Gd-BOPTA) experiments. The more rapidly relaxing (short-$T_{1,OBS}$) component reflects the residual from poorly suppressed more slowly flowing media (extracellular water) and the more slowly relaxing (long-$T_{1,OBS}$) component arises from intracellular water. Intracellular water $\tau_{IN}$ was estimated as 0.26 ± 0.03 s. Cell viability was maintained by minimizing perfusion rate (40 mL/min) and shortening experiment time (< 1 hour).

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