EXTREMELY SLOW WATER DIFFUSION IN RODENT BRAIN AS A INTRACELLULAR BIOMARKER FOR AGING

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INTRODUCTION: Extremely slow water diffusion at ultrahigh b value in brain has been long well observed (1, 2, 3), however, its source is still not clear. Evidence showed that such slow-decaying component mainly originates from restricted water diffusion in the nerve fibers (3), is related to transmembrane water flux (5) and can be utilized to investigate changes in axonal morphology (4). Brain aging is of paramount research interest. It is known that degenerative changes of nerve fibers underlie the cognitive function decline in the aged population (6). In this study, we investigated whether extremely slow water diffusion measured at ultra-high b values can potentially serve as a biomarker for objective assessment of brain aging *in vivo*.

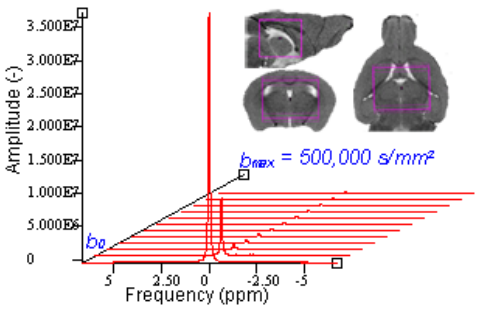

Fig. 1 Typical diffusion-weighted spectra acquired with 12 b values within a cubic voxel of 6×4×5mm^3, shown by the purple boxes in sagittal, coronal and axial T_2-weighted images.

METHODS: **Animal Preparation:** Fifteen normal mice (C57BL/6N) were studied at the age of 3 (n=6), 7 (n=6), 24 (n=6) and 28 (n=6) month. Some mice were studied twice. Seven SD rat pubs (n=7) were scanned repeatedly during postnatal day 8 to 30. During experiment, animals were anesthetized with isoflurane (3% for induction and 2.0% for maintenance) and kept warm at 37°C. **MRI Protocols:** In vivo T_2-weighted images and diffusion-weighted (DW) ^{1}H spectra were acquired on a 7T MRI scanner with a 25-mm-diameter volume coil using a previously validated stimulated echo acquisition mode (STEAM)-based single-voxel MRS sequence (7, 8). All DW spectra were acquired during the plateau of the respiration waveform within a cubic voxel (120 to 180 mm^3). For water diffusion measurement, diffusion duration time (δ) = 40ms, diffusion time (Δ) = 80 or 160ms, TR/TE=1500/80ms, NEX = 32 to 64 for 12 b values (0 to 5.0×10^{5}s/mm^2). For NAA diffusion measurement, DW spectra were acquired with water suppression, δ/Δ=40/80ms, TR/TE=1500/80 ms and NEX = 160 for 12 b values (0 to 12,667s/mm^2). **Data Analysis:** The twelve FIDs corresponding to 12 b values in each scan were stored individually, then frequency-realigned and phase-corrected using home-developed software to mitigate motion contamination. Spectral analysis was performed using the JMRUI package (AMARES for water peak and QUEST for NAA peak). Signal amplitudes were plotted on a logarithmic scale. Water signals within the b value range from 1.0 to 5.0 ×10^{5}s/mm^2 (nine b vales) and NAA signals at all the b vales used were fitted to a mono-exponential model. Apparent diffusion coefficient (ADC) was derived from the decay rate, while the relative slow-diffusing water fraction was computed from the intersection by an exponential function. Paired or unpaired t-tests were performed for statistical analysis.

RESULTS: Fig.1 shows one typical DW spectra without water diffusion and the voxel position in a mouse brain. Consistently the voxel was placed in the brain center in each animal, maximally covering the majority of thalamus and striatum, part of hippocampus and a small portion of cortex while avoiding dura or air. Fig.2 demonstrates the water diffusion difference between young and aged mice. Significant water ADC decrease with a significant increase of its relative fraction was observed in aged mice. Both young and aged mice showed increased slow-diffusing water fraction at 4 month following the first measurement, with the aged mice also show ADC decrease. No significant difference was observed in NAA ADC. Fig.3 shows the water signal decays on a logarithmic scale from seven rat pups at different age. At P8 to P15, the water signals were hard to differentiate from noise in most P8 to P15 rat pups when b value was larger than 2.0×10^{5}s/mm^2 (Fig.3a). At P30, the water signal decay was much slower and largely monoexponential at b values from 1.0 to 5.0 ×10^{5}s/mm^2 (Fig.3b). When measured with longer diffusion time, the decay rate was even smaller (note the intersection between the green line and the decay curve in Fig. 3b and c). Individually fitted water ADC decreased while the estimated slow-diffusing water fraction increased at longer diffusion time (Fig.3d).

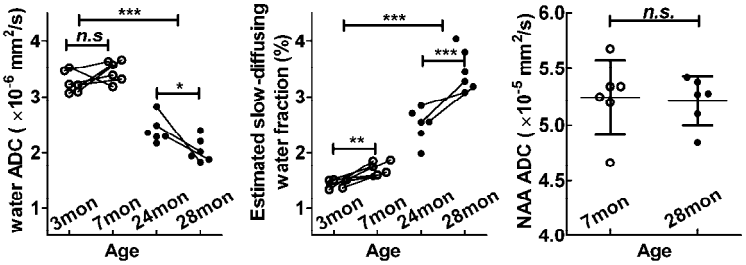

Fig. 2 Diffusion measurement results in young and aged mice. Connected points refer to the mice that were scanned repeatedly. Paired t-tests were performed for comparing results obtained at 3 and 7 month. Unpaired t-tests were performed for other comparisons. *p< 0.05, **p< 0.01, ***p< 0.001, *n.s.*, not significant.

DISCUSSIONS AND CONCLUSION: The results of this study demonstrated a steady decrease of slow water ADC at very high b value range and increase of its fraction with age. Furthermore, such change progressed faster in aged mice than in young mice within a 4-month normal living period. The NAA ADC, which was measured at lower b value range and mainly serve to probe the properties of cellular soma instead of axonal space (9), showed no significant difference between young and old. This not only indirectly suggested that the water ADC decrease in aged mice was unlikely caused by increased magnetic field inhomogeneity due to iron deposition, but also indicated that the neuronal soma structure may not change significantly during normal aging. The gradual development of the slow-diffusion water pool in rat brain during the first 4 postnatal weeks, when fast increase of axonal membrane structures and myelin sheath took place, further confirmed the role of white matter changes in the slow water diffusion properties. The smaller water ADC at longer diffusion time in P30 rat brain was consistent with previous experiments [10], implying the water signal decay was dependent on the restricted dimension. All together, the water diffusion ADC decrease and its fraction increase with age was potentially related to the degenerative changes in nerve fiber such as the formation of myelin balloons and axonal membrane splits during the late stage of life. Thus it holds the potential to serve as an intracellular biomarker for objectively and dynamically assessing aging-related structural brain changes *in vivo*.

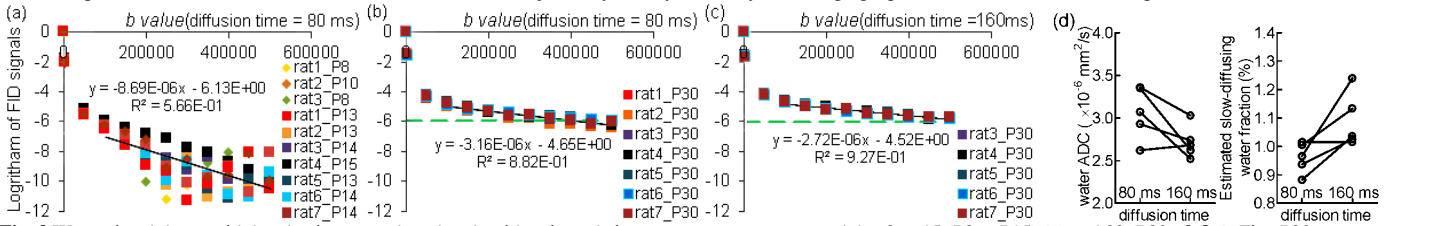

Fig. 3 Water signal decay with b value increase plotted on logrithamic scale in seven rat pups at postnatal day 8 to 15 (P8 to P15) (**a**) and 30 (P30) (**b&c**). Five P30 rat pups were measured with two diffusion times. With diffusion time increased from 80ms to 160ms while other parameters were kept constant, water ADC decrease while the slow-diffusion water fraction increase in general (**d**).

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