Orthogonal Diffusion Anisotropy Measurements in the Dorsal Third Ventricle of Adult Rat Brain Reveal Cerebrospinal Fluid Flow

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Introduction: Diffusion Weighted Magnetic Resonance Imaging (DW-MRI) is a non-invasive technique able to evaluate the diffusion of water molecules and provide information on the microstructural environment of tissues and its changes in vivo. Water diffusion describes the random Brownian translational motion of water molecules from one spatial location to another within the region of interest over the interrogated time [1, 2]. The acquisition of DW images is based on the application of a set of paired diffusion gradient pulses in a spin-echo (SE) sequence able to encode the diffusion process along the gradient directions (Stejeskal-Tanner method). The properties of these gradient pulses are defined by the *b* parameter, the value of which depends on the strength of the diffusion gradient pulse, its duration (δ) and the time interval between the paired diffusion gradient pulses (Δ). Quantitative water diffusion measurements are achieved, on pixel by pixel basis, by fitting the Apparent Diffusion Coefficient (ADC) from intensity-*b* curves. However, it has been proposed that flow effects may interfere with the measurement of pure diffusion values since both diffusion and flow lead eventually to coherence loss [3]. The aim of the present study was to evaluate the effect of flow in the diffusion measurements. We acquired a set of DW images of the adult rat brain in three orthogonal directions, and calculated the corresponding ADC values in the Dorsal Third Ventricle (D3V). In the absence of significant flow effects, orthogonal measurements of regional ADC should provide very similar values in the three directions (isotropic diffusion) while the presence of appreciable flow effects would result in apparently larger ADC values in the direction of the flow. To test this hypothesis we implemented a live-sacrificed paradigm combining it with high-low *b* values sequences for orthogonal ADC determinations. Shortly after dead, the passive diffusion properties of CSF within the D3V are conserved while any directional flow effects on the ADC measurement are lost.

Material and Methods: All experimental protocols were approved by the bioethical committees of our institution and follow the recommendations of the appropriate governmental agency. Adult male Wistar rats (200-250g, n=6) were used. All animals were maintained anesthetized during the MRI procedure using a mixture of 2% isoflurane in 95% oxygen. All images were acquired on a 7T MR scanner (Bruker PharmaScan®) equipped with a 90 mm gradient insert (36 G/cm maximum intensity) and a rat head resonator (38 mm diameter). Acquisition was performed using a Diffusion Weighted protocol based on a SE sequence with 4-shot EPI-read gradient (SE-EPI); TR=3000 ms, TE=51.130 ms, FOV=38 mm, 128x128 matrix size, 0.297x0.297 mm^2 pixel resolution. The diffusion gradient pulses were defined for low *b* (10, 20, 30, 40, 50, 60, 80, 100 s/mm^2) and high *b* (100, 200, 300, 500, 800, 1200 s/mm^2) values along the three orthogonal directions of the magnet defined by the read, phase and slice encoding gradients (δ=4 ms, Δ=20 ms). We analyzed two rat-brain axial slices (1.5 mm thickness) from every animal. Reference images for the fitting were acquired twice, averaging both to increase signal to noise ratio. After these acquisitions, animals were sacrificed in the magnet by replacing oxygen by carbon dioxide to avoid any change in position. Then, the experiment was reproduced and low-high *b* values DW-images were acquired. The images obtained were analyzed using a home-made software application (Matlab R2007a). The intensity-*b* curves were fitted to an exponential curve ($S=S_0 \cdot e^{-b \cdot ADC}$) and ADC values were estimated for each of the orthogonal directions (Fig. 1) in the four experimental conditions: live-high *b* values, live-low *b* values, sacrificed-high *b* values and sacrificed-low *b* values.

Results: Our results demonstrate a preferential diffusion in the H-F direction in the D3V of live rats (Fig. 2), which becomes much larger for low *b* values (Fig. 3). This preferred diffusion direction corresponds to the dominant direction of the CSF flow in the D3V (head-feet in the slices we analyzed). However, a clear isotropic diffusion pattern is found when analyzing the orthogonal diffusion coefficients of the D3V in sacrificed rats (Fig. 2). It is important to remark that these flow effects are less prominent in the white matter region of the same animals under the same paradigms.

Conclusions: We have shown statistically significant CSF flow effects on the orthogonal ADC values measured by DW-MRI in the D3V of adult rat brain. The expected pure isotropic diffusion only occurs in the D3V of sacrificed animals, being replaced by an anisotropic diffusion pattern in the live specimens, with a dominant direction along the path of CSF flow. These results may allow us to obtain CSF flow values in the rat model, an important physiological variable previously difficult to obtain in rodent brain. Moreover, our results show that tissue perfusion has a distinct influence on the ADC values determined by SE-EPI, even when high *b* values are used. We conclude that current cerebral ADC measurements may contain region dependent contributions of CSF flow, a circumstance that merits further consideration when calculating diffusion anisotropy maps and axonal tracts.

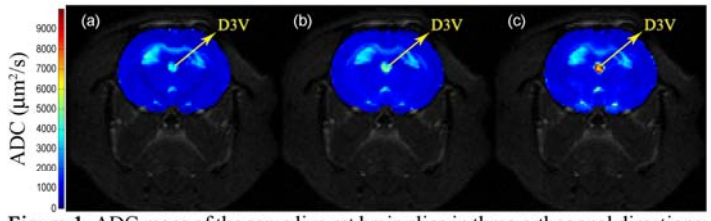

Figure 1. ADC maps of the same live rat brain slice in three orthogonal directions: (a) left-right (L-R→); (b) dorsal-ventral (D-V↓); (c) head-feet (H-F•). Note that ADC values are much larger in H-F direction than in L-R and D-V directions. Under these acquisition conditions free diffusion of water is not restricted by the dimensions of the D3V. In this slice, the CSF flow through the D3V occurs in the H-F direction, indicating that the ADC measurements are influenced by CSF flow.

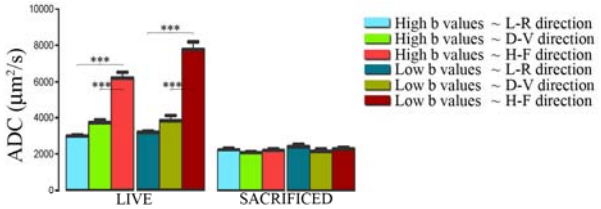

Figure 2. Orthogonal ADC values in the D3V of rat brain in the live-sacrificed paradigm. Live animals show significantly larger ADC values along the CSF flow direction (head-feet, H-F), while sacrificed rats depict an isotropic diffusion pattern with very similar ADC values in the three orthogonal directions. Note that the absence of CSF flow in the sacrificed animals results in a drastic reduction of the observed ADC in the H-F direction of live animals, and smaller changes in the dorsal-ventral (D-V) and left-right (L-R) directions, indicating that flow cessation results in a reduction of the measured ADC using SE-EPI sequences.

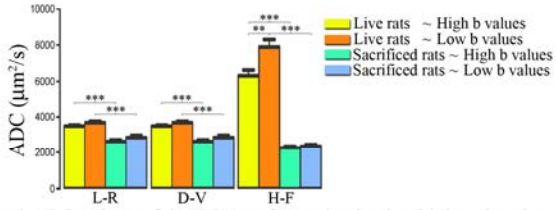

Figure 3. Orthogonal ADC values of the D3V as determined using high or low b values in live or sacrificed animals. Note that live animals show larger ADC values than the sacrificed ones, as expected from the absence of CSF flow in the dead animals. The difference is appreciably larger for the head-feet (H-F) direction with larger CSF flow influence at low b values.

References: [1] A. L. Alexander, J. E. Lee, M. Lazar, A. S. Field, *Diffusion Tensor Imaging of the Brain.* Neurotherapeutics, 2007. 4:316-329. **[2]** D. Le Bihan, *Looking into the functional architecture of the brain with diffusion MRI.* Nature Reviews - Neuroscience, 2003. 4(6):469-80. **[3]** J. S. Van Den Brink, *Method of Magnetic Resonance Perfusion Imaging.* Patent US 2006/0241375 A1, 2006.