Effects of hypotonic stress and ouabain on apparent diffusion coefficient at cellular and tissue levels.

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Target audience. The present work is within the range of interest of both preclinical and clinical MR neuroscientists.

Purpose. The Apparent Diffusion Coefficient (ADC) of water in the brain is known to decrease in various physiological (neuronal activation) or pathological conditions (acute ischemia)[1]. Although a link with cell swelling has been put forward, the exact mechanism of this ADC decrease has not been fully established yet. A popular view is that cell swelling causes the extracellular fraction to shrink in favor of intracellular space where diffusion is assumed slow. Another model suggests that cell swelling leads to an increase of membrane surfaces within a given volume, hence a larger pool of "bound" protons with a lower diffusion coefficient[1]. The aim of our study was to use a nervous system model where the resolution of MR microscopy is sufficient to image neurons both individually and at tissue level. This model is the *Aplysia californica*, which is widespread in neuroscience and has previously served for single cell MR diffusion measurements[2,3]. We evaluated the evolution of ADC in isolated cells and in ganglia tissue, following either hypotonic shock or exposure to ouabain.

Methods. *Aplysia* were anesthetized by injection of an $MgCl_2$ solution. The buccal, abdominal and pedal ganglia were resected and placed in artificial sea water (ASW). The first two were used for individual neuron extraction and imaging, while the latter was used whole for tissue imaging. MRI was performed in a 17.2T magnet (Bruker BioSpin) using home-built microcoils as RF transceivers. Single cell imaging: The ganglia were unsheathed and neurons were mechanically isolated. Cell integrity was verified under the microscope. Cells were inserted into a glass capillary filled with ASW and underwent two imaging sessions: an initial one and another one following a 30 minute stress: exposure to either 33% hypotonic ASW or 1 mM ouabain in ASW. ADC measurements were performed using a 3D DP-FISP[4] (b = 10 – 600 s/mm²; δ = 2.5 ms; Δ = 10 ms; centric encoding; FA = 20°; TE/TR = 2.6/5.2 ms; matrix 190x32x24; 25 µm isotropic resolution; TR between PE2 steps = 6 s; TA = 9min 36s / b-value).

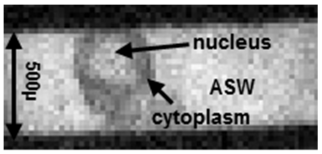

Fig. 1 DP-FISP image of a neuron. b=10 s/mm². 25 µm isotropic resolution.

Ganglia imaging: The bilateral buccal ganglia underwent the same three-step protocol: initial imaging, stress, final imaging. DP-FISP parameters differing from previously: TE/TR = 1.7/3.4 ms; matrix 128x44x40; 50 µm isotropic resolution; TA = 8min / b-value. Three neurons and one pair of buccal ganglia were used for each type of stress. One additional neuron underwent two subsequent imaging sessions with no exposure to hypotonic solution or ouabain, to serve as control. Data analysis: Single cells were manually segmented on the b=10 s/mm² images, including both cytoplasm and nucleus in the region of interest (ROI). Within the buccal ganglia, ROIs corresponding to clusters of 15 – 20 cells and comprising intra- and extra-cellular spaces were drawn. ADC was estimated before and after exposure to hypotonic solution or ouabain in the isolated cells and in cell clusters. In the case of single cells, the change in volume (in terms of voxel counts) was also evaluated.

Results. Fig.1 shows an example of a single cell image. Fig.2 illustrates the evolution in ADC with hypotonic shock and ouabain. In the baseline condition the ADC was significantly larger in the ganglia than in single cells. The exposure to a 33% hypotonic solution caused a substantial *increase* in ADC (53±26 %) and volume (24±6 %) in single cells, while producing a substantial *decrease* in ADC in the ganglia (-45%). The exposure to 1 mM ouabain also caused an *increase* in ADC (36±9 %) in single cells, while the ADC in the ganglia *decreased* (-17%). However, volume change with ouabain could not be reliably detected in our images. The control cell showed no significant change in ADC (6 %).

Discussion. The cell swelling and increase in ADC with 33% hypotonic shock is consistent with cell regulation of intra- and extra-cellular osmolarity and a dilution of the intracellular space. It is worth noting that Hsu et al. had found no change in ADC following 20% hypotonic perturbation[3], the shock the authors employed perhaps being too mild. Our single cell results are not compatible with the view that the overall ADC decrease observed at tissue level during cell swelling results from the inflation of the intracellular space, as the intracellular ADC itself increases substantially, even above tissue ADC value. The intracellular ADC increase and tissue ADC decrease pattern was also observed with ouabain stimulation. While cell volume increase is also expected with ouabain, the increase in cell size could not be reliably estimated at our image resolution. In previous studies on rat hippocampus, an 18% reduction in ADC following 1 mM ouabain perfusion was found[5]. Our ganglia measurements are consistent with this finding. In the light of these observations, the tissue ADC decrease could reflect variations in the amount of slow diffusing water bound to membranes (increase in the membrane surfaces) which outweighs the intracellular ADC increase. Experiments on a larger number of cells and ganglia need however to be carried out in order to confirm the observed trend.

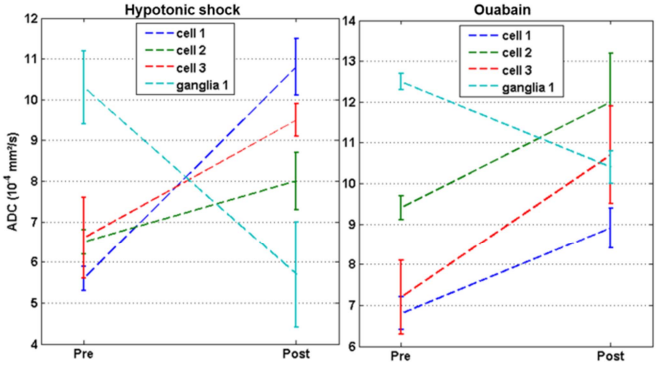

Fig. 2. Evolution of ADC in cells and ganglia with hypotonic shock and ouabain exposure. The errorbars represent the standard deviation of the ADC fit.

Conclusion. This study exploits for the first time the ability to look into diffusion changes induced by stress both at cellular and tissue level, within the same species. It could shed light on the mechanisms leading to ADC decrease in brain tissue following ischemia. Going to higher b-values would perhaps confirm the nature of the two "pools" of water identified in the biexponential behaviour of brain tissue. Work is in progress to render the DP-FISP sequence suitable for higher diffusion weighting and to increase spatial resolution, so as to depict ADC changes which could occur at membrane vicinity.

References. [1] Le Bihan D and Johansen-Berg H. Diffusion MRI at 25: exploring brain tissue structure and function. Neuroimage. 2012;61(2):324-41. **[2]** Schoeniger JS et al. Relaxation-time and diffusion NMR microscopy of single neurons. J of Magn Reson B 1994;103 (3):261-73. **[3]** Hsu EW, Aiken NR and Blackband SJ. Nuclear magnetic resonance microscopy of single neurons under hypotonic perturbation. Am J Physiol. 1996;271(6 Pt 1): C1895-900. **[4]** Lu L et al. Diffusion-prepared fast imaging with steady-state free precession (DP-FISP): a rapid diffusion MRI technique at 7T. Magn Reson Med. 2012;68(3):868-73. **[5]** Buckley DL et al. The effect of ouabain on water diffusion in the rat hippocampal slice measured by high resolution NMR imaging. Magn Reson Med. 1999; 41(1):137-42.