Significant reduction in ADC during enhanced neuronal activity in isolated spinal cord of newborn rat

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Introduction

Diffusion weighted imaging (DWI) is explored in the last years as a tool for imaging brain activity. Reduction in ADC was observed during brain stimulation [1, 2]. However it remained unclear whether the observed changes reflect biophysical mechanisms linked to neuronal activity (such as cell swelling) or that the reported variations are related to vascular effects [3, 4]. The purpose of this work was to design and build an isolated system for studying the origins of the diffusion signal changes during brain activation and to test the feasibility of using DWI as a direct (non-hemodynamic) probe of neuronal activity.

Methods

Isolated electrically active spinal cords of newborn Sprague Dawley rats (postnatal day=2, *n=7*) were used as a model for vital mammalian neuronal tissue. Using continuous flow of oxygenated artificial cerebrospinal fluid (aCSF) the preparations were maintained viable inside the MRI during the experiment time. T_2 weighted and DW axial images (slice thickness = 3mm) were acquired using a 7T/30 horizontal-bore MRI (Bruker Biospin, Germany) during three physiological phases: (a) Spontaneous baseline activity, (b) enhanced activity (induced by bath applied serotonin, NMDA and bicuculline (15, 5 and 15 μM, respectively)) and (c) reproducible baseline activity (induced by washing out the epileptic solution). DW images were measured with ten b-values, three diffusion times ($\Delta = 15, 60\ and\ 150ms$) and along three diffusion directions (left-right, anterior-posterior and along the spinal cord axis). In each physiological phase the same MRI protocols were repeated. During all scans the temperature of the solution was continuously sampled. Mono-exponential model was used to derive the ADC and M_0 parameters both pixel by pixel and regionally, and Student's t-test was used for statistical analysis, to find a relation between the neuronal activity and the water displacement as reflected by the ADC values. Histology was conducted on a sample of spinal cords to complement our knowledge about the tissue's anatomy.

Results

During elevated neuronal activity, the average ADC of the spinal cord region dropped by 18-27%. The observed changes were significant and repeated in all preparations (*n=7*) and in all protocols (*n=9*), while the most significant change was achieved for images acquired with 150 ms diffusion time. No changes in T_2 were observed. Upon return to baseline activity, ADC values were elevated back towards their baseline values but did not reach the high ADC values as in the initial resting state (Figures 1 and 2). Diffusion was found to be anisotropic in the spinal cord region. However, interestingly, the average ADC along the spinal cords axis was lower by 21% relative to both perpendicular directions. Moreover, the ADCs were reduced by 17% as the diffusion time was increased.

Discussion & Conclusions

The observed reduction in ADC during neuronal activity is in agreement with the literature; Flint *et al*. [5] saw a non-quantified, yet similar trend using acute hippocampal slices. Given the nature of the isolated experimental system, these results suggest that electrical activity affects water displacement in mammalian neurons and these changes are not an MR artifact due to a vascular effect. We propose that the observed changes are not related to diffusion alone, but also to changes in intracellular micro-streaming (fluctuations in the intracellular cytoplasm which are faster than diffusion). If correct, DWI may potentially serve as a new modality for functional imaging.

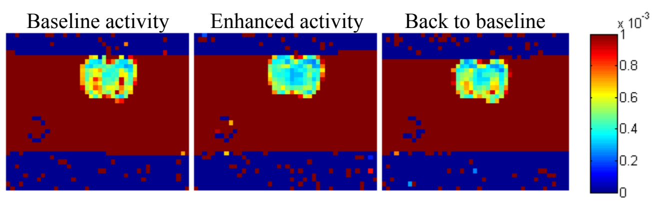

***Fig 1**: ADC maps [mm^2/s] of axial slice of isolated spinal cord of newborn rat in three different physiological phases. Left: baseline activity. Middle: enhanced activity. Right: return to baseline activity. The blue circle structure is the wall of a reference water tube. The maps clearly demonstrate reduction in the ADC in the enhanced neuronal activity phase relative to the baseline activity stage.*

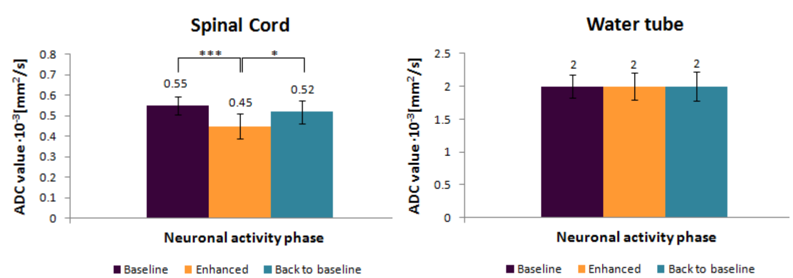

***Fig 2**: Left: comparison of average ADC values ± SD throughout three activity stages of the experiment: baseline activity, enhanced activity and back to baseline (n=7, excluding back to baseline n=4). Student's t-test results: *P<0.05; **P<0.005; ***P<0.00005. Right: comparison of average ADC values ± SD during the experiment, as calculated from two water tubes.*

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