Electrically Active In-Vitro Spinal Cords for the Study of Functional Diffusion Weighted Imaging

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Introduction

Diffusion weighted imaging (DWI) is explored in the last years as a tool for imaging brain activity. Darquie *et al.* reported that induced brain activity is reflected by a reduced diffusion coefficient, and attribute this to changes that occur in the activated neurons (Darquie et al. 2001). On the other hand, other biophysical mechanisms may induce the exact opposite effect upon brain activation. Intensive investigation is therefore needed in order to understand the actual interaction between brain function and water. A primary problem in this examination is that MRI measurements are significantly affected by multiple physiological factors that are not related to the neuronal mechanisms under question. *In vivo* measurements are affected by blood oxygenation level, flow and pulsation of blood in vessels and capillaries, motion artifacts and susceptibility differences in the microscopic and macroscopic level (Norris 2001). Moreover, the experimental set-up in itself interacts with the tissue: gradients create acoustic noise, affecting the tissue mechanically, or causing patient/animal movements. Thus, although whole brain imaging in live animals/subjects is obviously the subject of most studies, its biophysical investigation should be aided by more isolated experimental models.

We suggest the use of isolated and perfused spinal cords of newborn rats as an additional model for the study of the response of vital, mammalian neuronal tissues in the MRI. Perfusion of the spinal cords with oxygenated Artificial Cerebro Spinal Fluid (ACSF) allows maintaining the spinal cords in vital conditions such that the spontaneous and induced electric activity can be detected in these specimens, for up to 20 hours (Mor and Lev-Tov 2007). These cultures are advantageous for additional reasons: the spinal cord exhibits well defined morphology including known anisotropy and orientation of the nerve fibers and the perfusion is done with laminar flow, thus preventing motion of the spinal cord. **To the best of our knowledge, isolated and perfused (vital) spinal cords were never used in MRI applications.**

Methods

Spinal cord preparations were isolated from newborn (p=2) isoflurane-anesthetized rats. The preparations were then transferred to a recording chamber and perfused continuously with oxygenated ACSF. A closed loop of laminar flow (10cc per minute) was maintained using two peristaltic pumps. The chamber was located inside a 7T Bruker BioSpin MRI above a 10mm surface coil which served as the receiver coil, with an RF volume coil as the transmitter. T_2 weighted axial images and DW images were acquired following electrical activity recording to verify spinal cords viability. The electrical signals were recorded using suction electrodes placed on the preparations lumbar ventral roots and an electroencephalogram (EEG) system (Ives EEG solutions Inc., London, Ontario, Canada). It should be emphasized that the system is designed for recording inside the MRI during acquisitions. T_2 maps and ADC maps were computed using MATLAB.

Results & Conclusions

Despite of the small volume of the spinal cord, high resolution and high SNR images (Fig. 1) were acquired. Reproducible DW images were obtained despite the laminar flow (Fig. 2) and resulted in good ADC maps, with no physiological sources of noise and artifacts (as blood flow or breathing) (Fig. 3). The ADC values and AI agreed with the known values of the spinal cord and the differences between gray and white matter. In addition, the neuronal electrical activity could be seen well from the recorded signal (Fig. 4). Altogether we suggest that isolated and perfused newborn spinal cords may provide an optimal experimental system for the study of the biophysical mechanisms linked to neuronal activity. Specifically, these tissues are most fit for the study of the biophysical origins of the DWI signal and the variance in it, in health and disease.

References

Darquie A, Poline JB, Poupon C, Saint-Jalmes H, Le Bihan D. 2001. Proceedings of the National Academy of Sciences of the United States of America 98: 9391-9395.
Mor Y, Lev-Tov A. 2007. Journal of Neurophysiology 98: 2807-2817.
Norris DG. 2001. NMR in Biomedicine 14: 77-93.

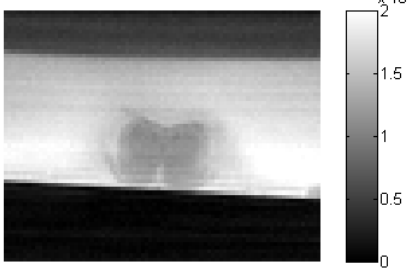

Fig. 1: T_2 weighted 3mm spinal cord axial image.

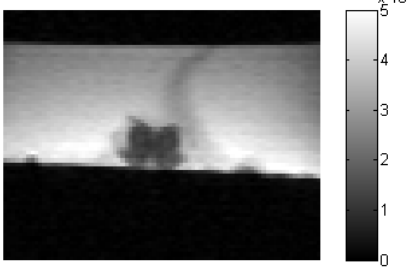

Fig. 2: DW 3mm spinal cord axial image.

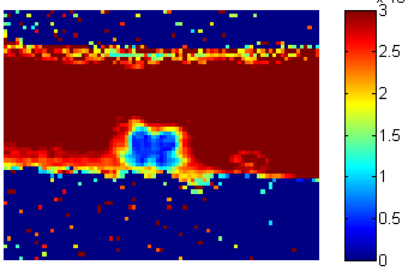

Fig. 3: Spinal cord axial ADC map [mm^2/s].

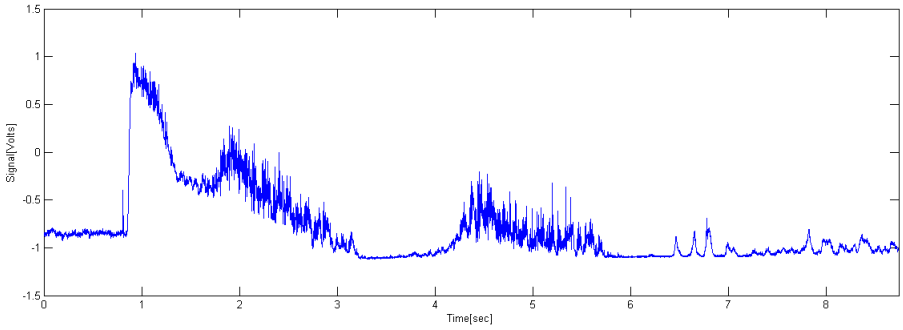

Fig. 4*:* A sample of neuronal activity signal which recorded from isolated viable spinal cord outside the MRI (amplification of x1000).