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Studying the cellular sources of the DW-MRI signal with Organotypic cultures

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Introduction

Diffusion Weighted MRI (DW-MRI) of the brain is based on signal attenuation that originates from multiple morphological or functional sources [1]. Recently it was also suggested that some of these mechanisms can be used to detect neuronal activity directly by DW-MRI [2]. The quantification of the relative contribution of these physical factors to the DW-MRI signal *in-vivo* (in health and disease) is complicated due to various sources of physiological 'noise' such as bulk motion of the tissue, or flow of oxy/non-oxygenated blood. Organotypic neuronal cultures are one of the possible techniques applicable for the study of the sources of the DW-MRI signal. Organotypic cultures are obtained by slicing newborn rats' brains, and culturing them for several weeks in supportive conditions (temperature, oxygen, nutrients, etc.). During that period the cultures recover the initial trauma and dying cells are cleared by resident microglia. The surviving cells (neurons and glia) establish a functional neuronal network that can be used to study neuronal activity and networking [3]. Organotypic cultures are suitable analogs for the study of neuronal tissues: they mimic brain tissue and its response to perturbations and they survive for long times (weeks). On the other hand, they lack sources of MRI artifacts such as bulk motion and blood flow. Indeed, Shepherd *et al.* [4, 5] used multiple hippocampal organotypic cultures simultaneously and performed DWI in a 14.1T scanner at room temperature. However, they suffered from the low partial volume, from air bubbles and the need to perform multiple time consuming repetitions. Petridou *et al.* [6] used organotypic cultures to study an assumed modulation of NMR phase directly by neuronal activity. Their tissues were not perfused but were kept near physiological temperature. Our goal was to design a system for the use of vital, thermally controlled and perfused organotypic cultures in combination with DW-MRI. We aimed to use these cultures as a tool for the study of the biophysical origins of the diffusion-weighted MRI signal.

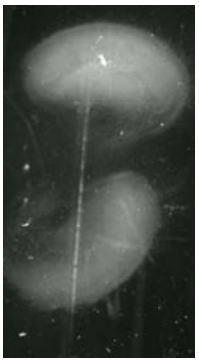

Fig. 1: Two spots of mature hippocampal organotypic cultures on a cover slip. The typical size of each spot is 2x2 mm.

Methods

Main experimental challenges – the unique challenges that we had to face include the following: **(a)** bubbles on the slides' surface, creating susceptibility artifacts; **(b)** low partial volume; **(c)** necessity to have the temperature set stably to 35°C; **(d)** low SNR, due to the effect of imaging gradients, while, **(e)** avoiding multiple time-consuming averages; **(f)** sensitivity of the tissue to manipulation, or to drying. These challenges were solves with the following setting.

Preparation of cultures – Brain slices were prepared as described in detail in [3]. Cortical and hippocampal coronal slices (400 µm) were cut from Sprague-Dawley rat brains at postnatal day 1-2 using a vibratome and attached to glass cover-slips [1]. Cultures were then submerged in Dulbecco's Modified Eagle's Medium and placed in a roller incubator for 2-3 weeks at 35°C. During the incubation period cultures flattened to the thickness of 100-200 µm [1].

MRI tissue chamber – Tissues were scanned in the MRI in a home-designed MRI chamber. The chamber was composed of an Ultem plastic that allows micro-imaging in high field, while preventing susceptibility artifacts close to the chamber's surface. The cover slips were attached to the chamber, facing one another, such that during the process of transfer of the cultures from the incubator to the MRI camber, no direct manipulation of the tissue (other than attachment of the slide) was required. The MRI chamber was than inserted into a standard 15mm NMR tube (Wilmad, NJ). Perfusion lines and optic fiber thermal probe were inserted through the tube's cap.

Tissue conditions inside the MRI – Throughout the MR scan the tissue was perfused with Artificial Cerebero Spinal Fluid (ACSF) saturated with oxygen (95% O_2; 5% CO_2). To avoid perfusion-driven flow, the perfusion was stopped before DW-MRI scans, and resumed immediately afterwards. Tissue temperature was kept at 35±0.2ºC throughout the entire experiment, (unless intentionally varied). Temperature was monitored by the optic fiber probe and regulated by the flow of hot gas and by warming up the perfusate.

MRI protocol – Experiments were performed in a 7T vertical scanner (Bruker, Ettlingen, Germany) equipped with a Micro2.5 gradient coil, and a 15 mm RF coil. Altogether, experiments included multiple DW-MRI scans under various conditions, and altogether took 2-3 hrs (after placement in the MRI), where during the first 40-60 minutes the temperature was stabilized and MRI parameters were prescribed. MRI scans used the following parameters: TR/TE = 2000/70 msec with no averages. Δ/δ=20/2.5 msec, FOV = 2.6x1.3cm^2 with 64x32 matrix and 150µm slice thickness, G_d=0-315 mT/m (5-8 values) in read and phase directions.

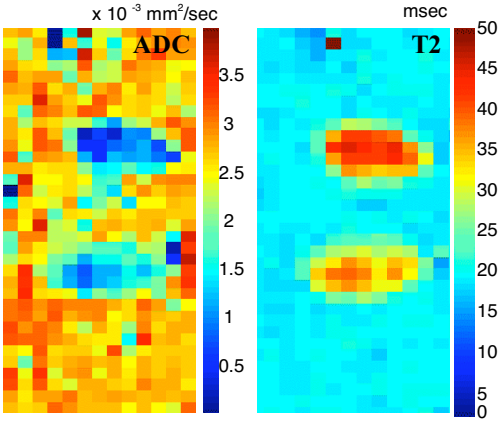

Fig. 2: ADC and T2 maps of the organotypic cultures. ADC map: TR/TE=3000/73 msec, Δ/δ=40/2.5 msec, Gd=0-220 mT/m (5-8 values). ADC values in 35ºC: ADC_{ACSF}: 3.0 ±0.2·10^{-3} ± mm^2/sec. ADC_{tissue}: 0.5-2·10^{-3} ± mm^2/sec. T2 map: TR/TE=3000/12.5 msec, FOV\= 2.6x1.4cm^2; 64x32 matrix and 150 µm slice thickness. T2 values in 35ºC: $T2_{ACSF}$: 20 ± 2 msec. $T2_{tissue}$: 36 ± 5 msec.

Results

Figures 2a & b show an ADC map and a T2 map of a typical organotypic culture at 35ºC. The clear discrimination between issue and medium is clear. The presented maps were obtained with 5 b-values and 2 repetitions. ADC values in the tissue areas varied in the range of 1-2.5·10^{-3}mm^2/sec depending on the medium-tissue volume ratio. We managed to avoid interfering bubble, and obtain measurements in stable temperatures. In following experiments we created perturbations in the chemical and thermal conditions of the cultures, to study the effect of these on the derved ADC and T2 (data not shown here). Figure 3 presents the attenuation curves for three regions of interest, in areas of the tissue and of the medium.

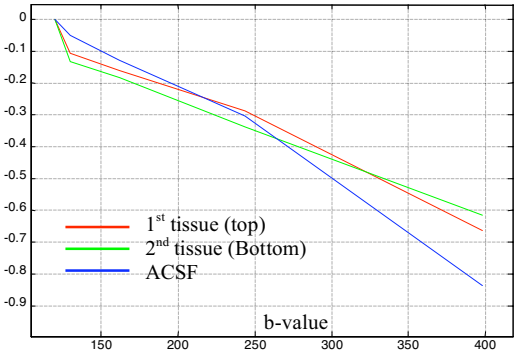

Figure 3: Attenuation curves for the DW-MRI data in Fig. 2. Each curve corresponds to an ROI in a tissue area or in the medium (ACSF).

Conclusions

This work presents a system for the study of micro-structural mechanisms influencing DW-MRI, by using perfused and thermally controlled organotypic cultures. The system is robust and can be used for the study of other types of tissues, and other factors influencing the MRI signal. Specifically, this system is based on spontaneously active neuronal cultures, while lacking sources of physiological noise (as motion and blood flow). It can thus serve to test newly suggested methods for MRI detection of nerve activation [2,6,7].

References

[1] Norris D.G., NMR Biomed, 2001. [2] Le Bihan D. *et al.*, PNAS 2006. [3] Plenz D. & Kitai S.T., Neurosci Lett. 1996. [4] Shepherd T.M. *et al.*, Mag. Res. Med. 2002. [5] Shepherd T.M. *et al.*, Neuroimage 2006. [6] Petridou N. *et al.*, PNAS 2006. [7] Truong T.K. & Song A.W., PNAS 2006.