DW-MRI of perfused and thermally controlled neuronal organotypic cultures

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Objectives

Signal attenuation in Diffusion Weighted MRI (DW-MRI) of the brain originates from multiple sources [1] that are being studied extensively, in order to quantify their relative contributions to the DW-MRI signal in health and disease. Recently it was also suggested that some of these mechanisms can be used to detect neuronal activity directly by DW-MRI [2]. However, sources of physiological 'noise' such as bulk motion of the tissue, or flow of oxy/non-oxygenated blood, complicate the study of the physical origins of DW-MRI signal *in-vivo*, and the interpretations of signal changes. To address the question of biophysical origins of DW-MRI signal, while avoiding the use of fixed tissues or of *in-vivo* experiments, we use the experimental model of neuronal organotypic cultures. We mimic the physiological conditions by continuously perfusing the tissue, and maintaining it near physiological temperature.

Introduction

Organotypic cultures are used extensively in the study of neuronal networks and activity [3]. Brain slices are taken from newborn rats and cultured for several weeks, prior to use, in supportive conditions (temperature, oxygen, nutrients, etc.). During that period the cultures recover after the initial trauma, dying cells are cleared by resident microglia, and the surviving cells (neurons and glia cells) establish a functional tissue culture with an organotypic neuronal network. 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. Shepherd *et al.* [4, 5] devised ways to use multiple hippocampal organotypic cultures simultaneously and performed DWI in a 14.1T scanner at room temperature. Petridou *et al.* [6] used organotypic cultures to study an assumed modulation of NMR phase directly by neuronal activity. Their tissue was not perfused but was kept near physiological temperature. We designed a system for the use of organotypic cultures that allows us to study their response to various types of well defined perturbations by performing DW-MRI while perfusing the tissues and closely monitoring their temperature.

Methods

Preparation of organotypic cultures – Brain slices were prepared as described in detail in [3]. Cortical and hippocampal coronal slices (500µm) were cut from Sprague-Dawley rat brains at postnatal day 1-2 using a vibratome, glued to rectangular pieces of a Millicell-CM membrane (Millipore), and attached to glass cover-slips. 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 100-200µm.

MRI tissue chamber – Tissues were scanned in the MRI in a modified 5mm NMR tube that served as a culture chamber (Fig. 1). The culture, with its carrying membrane, was glued to a 5mm Ultem bottom plug with a thin layer of plasma-thrombin. Perfusion lines and an optic-fiber temperature probe were inserted through the tube's cap. Moving the tissue to the chamber, while moist, took 3-4 minutes.

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 37±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.

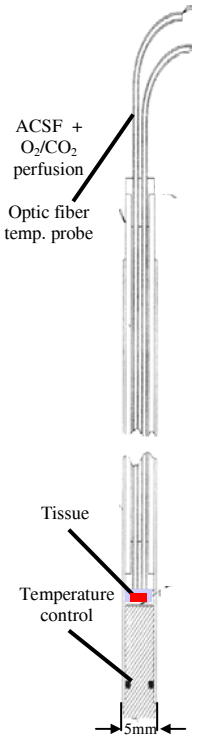

Figure 1: The MRI tissue chamber

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 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 2 averages. Δ/δ=50/5 msec, FOV = 0.6x0.6cm^2 with 32x32 matrix and 500µm slice thickness (2 slices), G_d=0-90 mT/m (5-8 values) in read and phase directions. To study the response to temperature changes, temperature was varied significantly (lowered to room temp. for a few hours, or raised to 45ºC for several minutes) or alternatively, varied in the 'safe' regime of 30-38ºC. All DW-MRI scans were performed once the temperature was returned to baseline (37ºC).

Experimental difficulties At this point, an unresolved issue is the trapping of bubbles below/on-top of the tissue, which create susceptibility artifacts that mask part of the tissue.

Results and Discussion

Figures 2a&b show a diffusion map obtained from a sample at 37ºC. ADC values varied in the range of 1-2.5·10^{-3}mm^2/sec depending on the medium-tissue volume ratio. 'Black' spots in the ADC map are attributed to noisy pixels, in which ADC could not be derived reliably (in most cases caused by bubbles). Applying a heat shock of 45ºC for ~2 minutes (Total time of perturbation: 37º→45º→37ºC ~8min) resulted in a drop in the ADCs of the organotypic culture, as show the maps of ADC values (Fig. 2b) and ADC change (Fig. 2c). Figure 2d shows the mean response of the ADC to temperature change across the entire tissue-slice (tissue in red, medium in blue). In a second experiment temperature was lowered for 6 hours to room temperature. Organotypic cultures are assumed to survive in low temperature for long times. Nevertheless, upon return to 37ºC, a drop in the ADC was observed in this experiment as well (Fig.3). The microstructural mechanisms that were triggered by the heat or cold and that resulted in ADC-drop may be cellular dis-function that resulted in swelling, or alternatively, protein denaturation or conformation changes, in the cytoplasm or on the cells' surfaces.

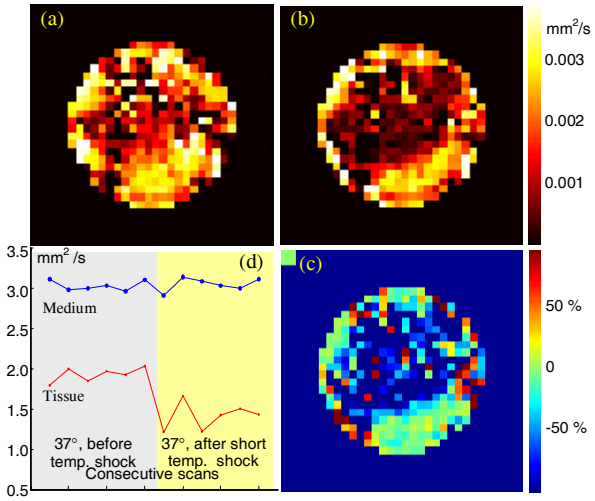

Figure 2: Response of ADC to high temperature shock. ADC maps before (a) and after (b) drop in temp., ADC difference map (c) and mean ADC dynamics (d).

Conclusions

This work presents a system for the study of microstructural mechanisms influencing DW-MRI, by using perfused and thermally controlled organotypic cultures. We demonstrated one application in which variations in temperature caused ADC changes. The system is robust and can be used to further study other tissues, and other factors influencing the cellular response and activity. Specifically, it can serve to test newly suggested methods for MRI detection of nerve activation [2,6,7].

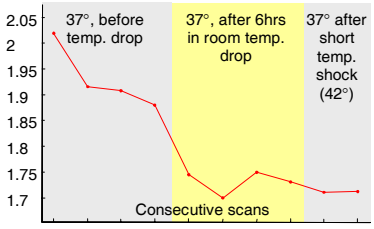

Figure 3: Response of cellular ADC to low temperatures. All measurements were performed in 37°.

References

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