Diffusion weighted magnetic resonance imaging of neuronal activity in the hippocampal slice model

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Introduction: Functional magnetic resonance imaging (fMRI) has become the leading modality for studying the working brain. Blood oxygenation level dependent (BOLD) fMRI, like all haemodynamic-based modalities, measures a surrogate signal (changes in blood oxygenation level rather than actual neuronal activity) whose spatial specificity and temporal response are subject to both physical and biological constraints [1]. Alternative MR-based methods for detection of brain activity have been proposed and investigated and studies have reported functional imaging based on diffusion weighted (DW) MRI [2, 3]. The basis for such DW fMRI is believed to be the sensitivity of diffusion weighted MRI to changes in tissue micro-structure, which have been shown to follow neuronal activity closely [4-6]: however, it remains unclear whether the observed DW MRI signal changes reflect cell swelling related to neural activation [3], residual vascular effects [7], or a combination of both. Here we present evidence of a detectable activity related change in the diffusion weighted MR-signal from the cellular level in live hippocampal slices in the complete absence of vasculature. Slices are exposed to activity-inducing substances and the effects evaluated and compared.

Materials and methods: Brains from male Sprague Dawley rats (150g) were removed by craniotomy, grossly dissected, and cut into 500µm-thick sections using a Lancer vibratome (Ted Pella, series 1000). Live hippocampal slices were then transferred to a 10mm NMR tube containing a multi-slice perfusion apparatus as described previously [8]. Briefly, hippocampal slices were placed in pairs between adjacent inserts arranged in a stack (2-3 layers). Circular inserts consisted of nylon mesh interiors to support tissue while allowing for the flow of perfusate affixed to the bottom of Delrin rings (0.6-0.7 mm thick) to accommodate the depth of each tissue slice. Perfusion with ACSF was carried out at a rate of 2ml/min. Outflow lines were positioned in the NMR tube as to allow for a close proximity between tissue slices and the air interface (approx. 4-6 mm). Diffusion-weighted images (δ = 6.0ms, Δ = 13.4ms, TR = 2000ms, TE = 28ms, in-plane resolution 156^2 µm^2, b-values = 37, 600, 1200, 1800, 2400 s/mm^2) were acquired using a DW spin-echo sequence on a vertical-bore, 600MHz (14.1T) Oxford magnet with a Bruker console. In each experiment, images were replicated three times as well as staggered in their overall order to test for system and tissue stability respectively. Once placed inside the magnet, tissue slices were perfused with oxygenated ACSF (30min) prior to imaging (30min) during which the perfusion was paused to eliminate flow artifacts. The measurements from this first imaging period established a baseline for each experiment that images from the second imaging period were compared to. After the first imaging period, slices were again perfused with oxygenated ACSF solution (15 min) followed by an additional perfusion (30min) of oxygenated ACSF alone (to test model stability, 4 slices) or ACSF containing a compound intended to evoke neural activity (27mM KCl (4 slices) or 10µM kainate (6 slices)). For these ACSF solutions the osmolality was maintained at physiological levels by removal of equimolar amounts of NaCl. The ACSF containing these compounds was introduced into the perfusate stream by means of a bubble trap device that served the dual purpose of preventing air bubbles from entering the NMR tube and ensuring minimal dilution of the treated ACSF containing KCl or kainate. Lastly, perfusion was interrupted before running the imaging protocol (30 min) a second time. A series of experiments that employed chemical inhibition was also carried out. For these experiments the inhibitor (10µM MK-801 (6 slices) or 10µM CNQX (6 slices)) was present in the ACSF as well as the tissue cutting medium as previous studies have shown increased efficacy with pretreatment [9]. All experiments were carried out with a bore temperature matching the ambient temperature of 23° C.

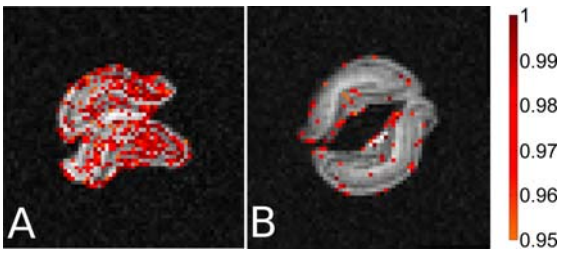

Figure 2: A) Pixels showing significantly different ($p<0.05$) signal at b=2400 s/mm^2 between resting state and active state (exposure to kainate). B) Significantly different pixels ($p<0.05$) upon treatment with kainate in slices pretreated with kainate inhibitor CNQX. Colorbar shows 1-p.

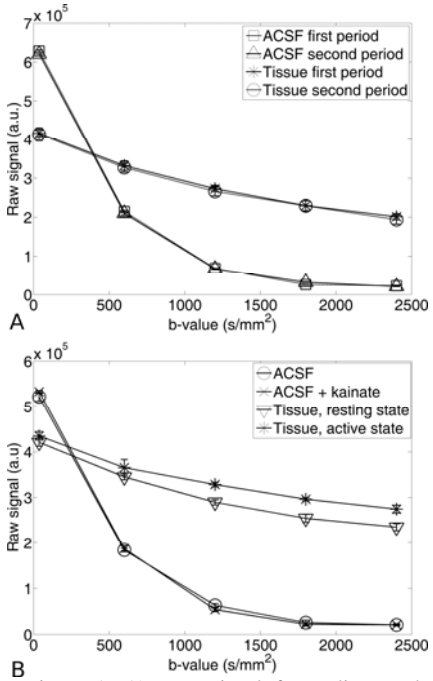

Figure 1: A) Raw signal from slices and perfusate in the two imaging periods. The tissue signal is seen to be stable between imaging periods. B) Raw signal from tissue (rest, active) and perfusates (ACSF, ACSF with kainate) in the two imaging periods. The signals from the perfusates are identical but the tissue signal changes upon exposure to kainate.

Results: The test of slice stability (untreated ACSF throughout) showed no sign of tissue change between the two imaging periods. This is seen in Figure 1A which shows the diffusion weighted signal as function of b-value for the same tissue region in both imaging periods. The signal curves from the perfusate in both periods are also included. Treatment with kainate lead to a widespread diffusivity decrease in the hippocampus as can be seen in Fig. 2B. Figure 2A which shows tissue areas with significantly different signal intensity between resting state (exposed to ACSF) and the active state (exposed to ACSF containing kainate) at b=2400 s/mm^2. Figure 2B shows the reduced effect of kainate in slices pretreated with kainate inhibitor CNQX. Exposure to kainate in slices pretreated with neuroprotective MK-801 produced patterns of signal change comparable to Fig. 2A but showed a more distinct regional response to kainate. In the case of KCl a more general effect was seen across the slices.

Discussion and conclusion: The methods of neural activation described in the current study are well established. By documenting slice stability and using chemical inhibition we have shown the observed signal changes to be caused by the agents introduced to the perfusate prior to the second imaging period. Different regional signal changes are seen with kainate and KCl reflecting the different tissue response to each agent. For the case of KCl we see a general signal change across the slice which we attribute to glial swelling as K^+ is taken up by the glial network. The same effect has been reported in previous studies [10]. This swelling has been shown to be reversible when using equivalent concentrations of potassium [11]. In the present study, we have described the ability to monitor periods of increased neural activation using diffusion MRI methods with the signal change attributed to micro-structural changes occurring at the cellular level.

References: [1] Logothetis N-K: Nature 2008;453(7197):869-878. **[2]** Darquie et al.: *PNAS* 2001; 98(16):9391-9395. **[3]** Le Bihan et al.: PNAS 2006;103(21):8263–8268. **[4]** Iwasa et al: *Science* 1980;210:338–339. **[5]** Cohen et al.: *J Physiology* 1996;203:489–509 **[6]** Kim et al.: *Biophys J* 2007;92(9):3122–3129. **[7]** Miller *et al.* PNAS 2007;104(52):20967-20972. **[8]** Shepherd et al: MRM 2002;48(3):565-569. **[9]** Wang *et al. PNAS* 2006;103(27):10461-10466. **[10]** Andrew et al. *J Neurophys* 1996;76(4):2707-2717. **[11]** Stroman et al. MRM 2008;59:700-706.