Functional magnetic resonance imaging of cortical tissue slices by means of signal enhancement by extravascular water protons (SEEP) contrast

P. W. Stroman[1], and D. Andrew[2]

[1]Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada, [2]Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada

Introduction: A novel contrast mechanism for functional magnetic resonance imaging, that does not rely on the blood oxygenation-level dependent (BOLD) effect has been proposed[(1,2)], and it has been theorized to arise from changes in tissue proton-density at sites of neuronal activity. The underlying mechanism is theorized to be exudation of water from capillary beds with increased perfusion pressure, and swelling of neurons and/or glial cells with water as a consequence of ion transport as cells depolarize[(3)]. The consequence of this mechanism is that fMRI can be carried out using proton-density weighted imaging methods in areas of poor magnetic field homogeneity, or at low fields. Here we confirm the existence of this contrast mechanism, which we have termed "Signal Enhancement by Extravascular water Protons" (SEEP), by carrying out fMRI studies of superfused rat cortical tissue slices, in the absence of blood or any contribution from the BOLD effect.

Materials and Methods: Brain tissue slices were obtained from male Sprague-Dawley rats, aged 21-28 days, following methods established for light transmittance microscopy studies of neuronal activity[(4)]. Eight to ten coronal slices, 600 µm thick, were taken from the fronto-parietal cerebral region using a vibrating blade mictrotome, and were transferred to a submerged net in a beaker containing oxygenated (95% O_2-5% CO_2) artificial cerebrospinal fluid (aCSF) at 22°C. Superfusion was continued at all times during time-course experiments, with oxygenated aCSF during baseline and recovery periods, and oxygenated high-K^+ aCSF (containing 26mM KCl in place of the equimolar amount of NaCl), during stimulation periods. Control experiments were also carried out with hyper- and hypo-osmotic (±80 mosm) solutions.

Neuronal activity elicited by depolarization with high K^+ for 1, 2, or 3 minutes, followed by 25 minutes of recovery, was confirmed by imaging intrinsic optical signals (IOSs) which are generated by changes in the amount of light being scattered or absorbed within living tissue.[(5)] Each slice was placed in an imaging chamber and transilluminated using a broadband light source and viewed on an inverted light microscope. Video frames were acquired using a charged couple device (CCD), and frames were acquired at 30 Hz and averaged to produce one frame every 6 seconds. Light transmittance (LT) changes over time in the 690-1000 nm wavelength range were determined in reference to the first image in each series.[(5)]

Functional MRI studies were carried out separately in a 3 tesla Siemens Magnetom Trio MRI system, using a posterior head-coil as a volume imaging coil. A custom-made chamber was used for holding two cerebral slices at a time in the MRI system, and for superfusion with a gravity-feed system at a rate of 2 mL/min at room temperature (22 °C) to replicate the light microscopy setup. Time-course MRI data were acquired from a single imaging slice with a thickness of 6 mm centered on, and co-planar with, the 2 mm deep imaging chamber, with 0.7 mm x 0.7 mm in-plane resolution. A half-fourier single-shot fast spin-echo (HASTE) imaging sequence was used, with TE/TR = 39/2000 msec, consistent with the method used for fMRI of the spinal cord, except that images were averaged 3 times.[(6)] Images were acquired across a 38 minute rest/stimulation paradigm.

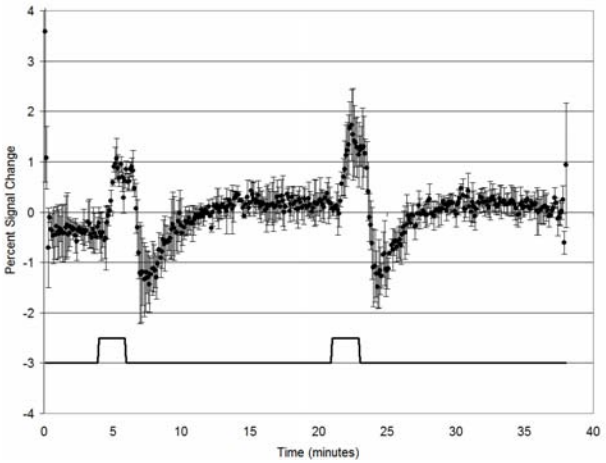

Figure 1: Average signal intensity time courses (mean ± s.d.) from gray matter regions in 6 cortical tissue slices in response to 2 two-minute periods of depolarization with high K^+ followed by recovery (indicated with solid line).

Data analysis was carried out in MatLab by first selecting two background reference regions inside the chamber, and an analysis region spanning the slices. Signal intensity time courses in the reference regions were used to subtract global effects (due to movement of the superfusion fluid), and the corrected time courses were then assessed within gray and white matter regions.

Results and Discussion: Functional MRI of superfused cortical tissue slices demonstrated consistent signal intensity changes upon stimulation with high K+, with time courses that were distinct between gray matter and white matter regions. The gray matter alone demonstrated an initial signal increase that lagged the onset of the change in superfusate, and with the return to superfusion with aCSF was followed by a temporary decrease and then recovery to baseline (Fig 1). This temporal pattern was corroborated by light transmittance changes. Control experiments confirmed that signal intensity changes observed with fMRI were the result of stimulation with high K^+. Superfusion with hyper- and hypo-osmotic aCSF solutions demonstrated signal decreases, and increases, respectively, confirming that MR signal intensity changes in tissues arise as a result of cell swelling or shrinking. This study confirms the existence of the SEEP contrast mechanism for fMRI that, in the intact CNS, originates from neuronal and glial swelling as neurons discharge during brain or spinal cord function.

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