Validation of Diffusion-Weighted FMRI signal in vitro using brain slices

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Introduction: Blood-oxygenation-level dependent (BOLD) functional MRI (fMRI) is widely used to map brain activation. It is an indirect measure of neuronal activity, with signal change depending on a coupled increase in blood flow and oxyhemoglobin concentration. The maximum hemodynamic response occurs at a delay of up to 4s after stimulus onset and may be displaced by up to 10 mm from the site of neuronal activation. In contrast, more recently described fMRI techniques using diffusion-weighted imaging (DFMRI) [1,2] are less well understood than BOLD fMRI but may provide a more direct measurement of neuronal activation. DFMRI methods are thought to reflect cell swelling and a reduction in extracellular space accompanying the influx of water and ions in active neurons [3]. DFMRI and BOLD may provide complementary information because of the differences in source of the signal. There is some evidence, however, that DFMRI signals may be contaminated by haemodynamic/BOLD effects [4,5]. ***This study aimed to verify the origin of DFMRI signal in vitro using perfused brain slices free from haemodynamic effects.***

Methods: Imaging data were acquired using (1) a fluorescence microscope connected to a Princeton Instruments Acton CCD camera and (2) a 16.4T Bruker microimaging MRI scanner. Brain slices from 2-week-old Wistar rats were prepared using a vibratome and kept viable in carboxygenated artificial cerebrospinal fluid (aCSF).
(1) *Fluorescence imaging*: 500 µm brain and hippocampal slices were bulk-loaded with a fluorescent calcium-sensitive reporter, Oregon-Green-BAPTA-1-AM (Invitrogen), washed, and continuously perfused with aCSF. Neuronal activation was initiated by: (i) a topical pulse of 1 µl KCl (1 M), which was immediately washed away by aCSF perfusion (n=3), and (ii) via switching the perfusate to a high K^+ aCSF (28mM) for 5 min to simulate the activation setup used for brain slice fMRI. Fluorescence was continuously recorded using the CCD camera.
(2) *DFMRI experiment*: A time series of DWI scans (1 min each) of 500 µm hippocampal slices (n=3) was acquired using the spin-echo Stejskal-Tanner sequence at b=2000 s/mm^2 with 78 µm in-plane resolution. Slices were maintained inside the MRI chamber using carboxygenated aCSF (5 mL/min). A 5 mL of bolus injection of aCSF containing 26 mM KCl was used to stimulate activation. Serial diffusion images were affine registered and each image was subtracted from an average baseline image.

Results:
In the topical pulse experiment, extracellular calcium influx was detected instantaneously after potassium administration and the fluorescence signal reached its maximum in ~2s and slowly decreased over a period of 15s (Figure 1C). In the global potassium stimulation experiment, this response profile is slower and more dependent on the exchange speed of K+ aCSF (Figure 1G). The difference in fluorescence intensity response between these two experiments may be due to a different level of tissue auto-fluorescence and KCl concentrations used in the two experiments.
A similar pattern of signal enhancement was observed in the DFMRI experiment. The largest diffusion signal change was greatest in the dentate gyrus approximately 40% above baseline (Figure 2D). 2,3,5-Triphenyltetrazolium chloride staining of the slices post MRI showed that the area with signal increase remained viable.

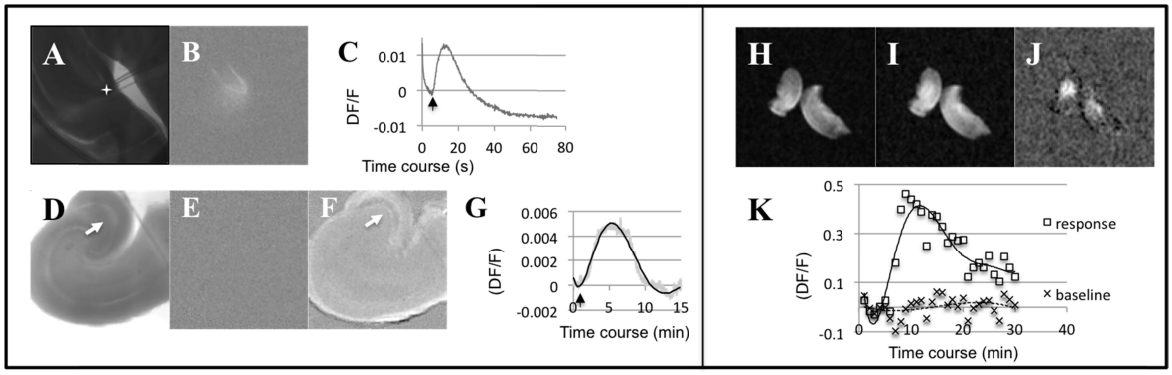

Figure 1. Detection of potassium-induced neuronal activation.
Using fluorescence microscopy (A-G) *Activation was achieved by topical application of 1M KCl (row A-C), or by switching the perfusate to a high K^+ aCSF (28mM) for 5 min (row D-G). (A) bright-field image showing the position of a micro pipette tip on the surface of the dentate gyrus (5x magnification). (B) Calcium-activation map showing fluorescence signal in the dentate gyrus. (C) Calcium-sensitive fluorescence response signals showing the activation profile from a short pulse of K^+.*
(D) A bright-field image of a whole hippocampal slice (5x magnification). (E) A calcium-sensitive fluorescence response before activation and (F) during a global 5-mins K^+ stimulation. Dentate gyrus is shown by white arrows. (G) Time course calcium-sensitive fluorescence response signals of a hippocampal slice from the 5-mins K^+ stimulation. The start of K^+ application is indicated by vertical arrows.
Using DFMRI (H-K) *(H) MRI of the hippocampal slices before and (I) during the peak of K^+ stimulation. (J) Area with positive DFMRI signal highlighting activity in the dentate gyrus. (K) A recording of DFMRI response signal during the course of global potassium stimulation.*

Conclusion: DFMRI detects activation signal in the absence of hemodynamic effects. In hippocampal slices, K^+ stimulation induced changes in DFMRI signal with a similar spatial distribution and time course to that observed with fluorescence microscopy.

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