NeuroImage 155 (2017) 113–119

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

Combined fMRI-MRS acquires simultaneous glutamate and BOLD-fMRI signals in the human brain

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ARTICLE INFO

Keywords:
Functional Spectroscopy
BOLD fMRI
Glutamate
Neurochemistry
Visual cortex

ABSTRACT

Combined fMRI-MRS is a novel method to non-invasively investigate functional activation in the human brain using simultaneous acquisition of hemodynamic and neurochemical measures. The aim of the current study was to quantify neural activity using combined fMRI-MRS at 7 T. BOLD-fMRI and semi-LASER localization MRS data were acquired from the visual cortex of 13 participants during short blocks (64 s) of flickering checkerboards. We demonstrate a correlation between glutamate and BOLD-fMRI time courses (R=0.381, p=0.031). In addition, we show increases in BOLD-fMRI (1.43 ± 0.17%) and glutamate concentrations (0.15 ± 0.05 I.U., ~2%) during visual stimulation. In contrast, we observed no change in glutamate concentrations in resting state MRS data during sham stimulation periods. Spectral line width changes generated by the BOLD-response were corrected using line broadening. In summary, our results establish the feasibility of concurrent measurements of BOLD-fMRI and neurochemicals using a novel combined fMRI-MRS sequence. Our findings strengthen the link between glutamate and functional activity in the human brain by demonstrating a significant correlation of BOLD-fMRI and glutamate over time, and by showing ~2% glutamate increases during 64 s of visual stimulation. Our tool may become useful for studies characterizing functional dynamics between neurochemicals and hemodynamics in health and disease.

Introduction

The blood-oxygenation level dependent (BOLD)-fMRI response is one of the most widely used measures of neural activity (Ogawa et al., 1990) yet is not a direct measure of action potentials, or synaptic activity. BOLD-fMRI reflects a spectrum of energy and blood-flow dependent processes (Logothetis et al., 2001) which are not fully understood (Logothetis, 2008; Hall et al., 2016). ^{1}H-MRS is a non-invasive measure of absolute concentrations of neurochemicals and, particularly in the absence of any sensory stimulation, has been exploited to identify biomarkers of normal and pathological brain states (Oz et al., 2014). While several recent studies have measured functional ^{1}H-MRS during specific tasks (Mangia et al., 2006, 2007; Lin et al., 2012; Schaller et al., 2013, 2014; Apsvalka et al., 2015; Bednarik et al., 2015), no study to date has quantified simultaneous changes in neurochemicals and brain activity using BOLD-fMRI. Here, we provide the first demonstration of combined fMRI-MRS measurements, and reveal a specific relationship between changes in BOLD-fMRI and glutamate at time scales relevant to conventional fMRI block design experiments (64 s). These results cannot be explained either by line narrowing during BOLD-changes (Zhu and Chen, 2001) or resting state variations in glutamate.

Materials and methods

Participants

Eighteen volunteers (9 females, mean age 28.71 ± 5.62 years), including two of the authors, were recruited for the main study. All had normal, or corrected-to-normal, vision and normal stereo-acuity (< 120 arc sec, TNO Stereo test, Lameris, Utrecht). Five participants were excluded from analysis due to one or several of the following reasons: early termination of experiment; difficult MRS voxel placement as evidenced by negative BOLD-fMRI signal and/or poor signal-to-noise in the metabolite spectra. The final data set was composed of 13 subjects (7 females). Each volunteer took part in one behavioral

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http://dx.doi.org/10.1016/j.neuroimage.2017.04.030
Received 19 December 2016; Accepted 13 April 2017
Available online 19 April 2017

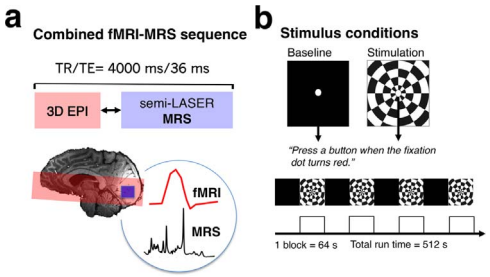

Fig. 1. (a) Diagram shows the MR sequence consisting of a 3D BOLD echo planar imaging (3D-EPI) and semi-LASER MR-spectroscopy sequence in the same TR, with diagram below illustrating the 2×2×2 cm MRS voxel in the occipital lobe (blue square) and the EPI slice coverage (red outline), overlaid on a high resolution anatomical image. (b) Experimental design showing stimulus conditions, consisting of a baseline (black screen, 64 s) and a flashing checkerboard (64 s). Each participant took part in a single functional MRS visual stimulation experiment, consisting of four cycles of baseline and stimulation blocks. Subjects performed a fixation task throughout the 8 min 24 s experiment.

session to assess their vision, and one MRI session. Volunteers received a reimbursement of £10 for the behavioral testing session and £25 for the MRI session. All gave informed written consent, approved by the University of Oxford Research Ethics Committee (MSD-IDREC-C1-2014-146).

MR protocol

MR data were collected using a 7 T whole body MR-scanner (Siemens, Erlangen) with a Nova Medical head coil (single transmit, 32 receive channels). Anatomical images were collected with a 1-mm isotropic resolution (MPRAGE, repetition time TR=2.2 s, inversion time T_I=1.05 s, echo time TE=2.82 ms, FOV=192×192×176 mm, flip angle=7°, total acquisition time=171 s) for the placement of the visual cortex voxel-of-interest (VOI). A 2×2×2 cm MRS VOI was positioned in the occipital lobe, centered along the midline and the calcarine sulcus.

Fig. 1a shows a diagram of the combined fMRI-MRS sequence, based on a sequence developed by Hess et al. (2011). In the same TR of 4 s, BOLD-fMRI (3D EPI, resolution=4.3×4.3×4.3 mm; flip angle=5°, repetition time TR_{epi}=40 ms, TE=25 ms, FOV=240 mm, 16 slices) and MRS data were acquired. MRS data were acquired using short-echo semi-localisation by adiabatic selective refocusing (semi-LASER) pulse sequence (TE=36 ms, TR_{mrs}=4 s) with VAPOR water suppression and outer volume suppression (Oz and Tkac, 2011; van de Bank et al., 2015). Semi-LASER sequences have a high test-retest reliability at 7 T (Terpstra et al., 2015) and minimal chemical shift displacement at ultra-high field MR imaging. A delay between fMRI and MRS acquisition (250 ms) was inserted to minimize potential eddy current effects from the EPI read-out (Hess et al., 2011).

Experimental design

Stimuli were generated on a MacBook Pro laptop using Psychtoolbox-3 (Brainard, 1997) and custom written Matlab scripts. An Eiki LC-XL 100 projector (1028×768 pixels, 60 Hz) displayed the images onto a back-projection screen (DA-LITE Milestone AV Technologies, Minnesota). For each subject, a resting state experiment ('eyes shut') was collected during which no visual stimulation was delivered, and subjects were instructed to keep their eyes shut for the entire scan. During visual stimulation, subjects viewed the screen using an angled mirror, mounted on the head-coil (viewing distance=60 cm). Fig. 1b shows a diagram of the visual stimuli and experimental protocol. Visual stimulation consisted of a baseline-stimulation block design (stimulus size=19.82°×14.25°, block length=64 s, number of cycles=4). During the stimulation period a full-field contrast-reversing checkerboard was presented (8 Hz flicker, mean luminance=385 cd/m^2; 50% contrast, 64 s duration). The baseline period was a uniform black screen (2.33 cd/m^2; 64 s duration). A white central fixation dot (0.5°) was visible at all times, and subjects pressed a button on a button-box when it randomly turned red (500 ms) about once every three seconds. The purpose of the task was to encourage central fixation and steady levels of attention. The type of visual stimulation used is known to target the visual cortex and is unlikely to generalize across cortical regions. In support, a previous study using similar checkerboard stimuli in a prolonged stimulation design has found no changes in metabolites outside of the visual cortex (Mangia et al., 2006). Each participant took part in two combined fMRI-MRS experiments: The resting state scan was collected first (128 averages, 'eyes closed no stimulation'), followed by the functional scan (128 averages, 'four cycles of flashing checkerboards and baseline'). Supporting scans for anatomical registration were collected last.

fMRI analysis

fMRI data analysis was performed using FEAT (FMRI Expert Analysis Tool) v. 6.00, part of the FSL software distribution (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Data were pre-processed using motion correction MCFLIRT (Jenkinson et al., 2002); non-brain tissue extraction (Smith, 2002); spatial smoothing using Gaussian kernel of FWHM=5 mm, grand-mean intensity normalization and high pass temporal filtering (Gaussian-weighted least squares straight line fitting, main experiment=132 s; resting state data=250 s). Functional images were registered to an initial 2-mm structural image (6 DOF), and then to the 1-mm isotropic T1-weighted structural image using boundary-based registration (BBR) in FLIRT (Jenkinson and Smith, 2001; Jenkinson et al., 2002). Percentage BOLD-change in the MRS-voxel was calculated using Featquery. The group activation map was calculated using FLAME (FMRIB's Local Analysis of Mixed Effects), with z-statistic threshold > 2.3 and cluster-correction threshold of $p < 0.05$. Participants maintained very steady head position throughout the scan, as indicated by motion estimates from MCFLIRT (Jenkinson et al., 2002). Absolute motion displacement referenced to the center of slice was 0.228 ± 0.056 mm (mean N=13, ± std). Relative motion displacement referenced to the preceding time point was 0.173 ± 0.056 mm.

Dielectric pad

A dielectric pad measuring 110×110×5 mm^3 containing a suspension of Barium Titanate ($BaTiO_3$) and deuterated water (mass-mass ratio of 3:1) was placed behind the occiput of each subject to increase the extent of the effective transmit field (Luo et al., 2013; Lemke et al., 2015). The pad was positioned so that the center of the bias field was symmetric with the midline. Dielectric pads can increase transmit field efficiency (> 100%) in regions close to the pad without affecting specific absorption rate and B_0 field homogeneity (Teeuwisse et al., 2012).

Data reduction

Previous functional MRS studies have excluded up to 50% of data to obtain stable metabolite measurements, by using the second half of prolonged visual stimulation blocks (Mangia et al., 2006, 2007; Schaller et al., 2013; Bednarik et al., 2015); or focused on specific experimental cycles (Just et al., 2013). We excluded the first two time averages (2 TR=8 s) of each block, under the assumption that metabolite spectra are unstable during the period where the BOLD-amplitude is known to peak (Buxton et al., 2004). Excluding the first two TRs from every block is equivalent to excluding 12.5% of the data.

Metabolite quantification

MRS data were eddy-current corrected using the unsuppressed