Recording BOLD, ASL and CBV fMRI responses to epileptic spikes in rats

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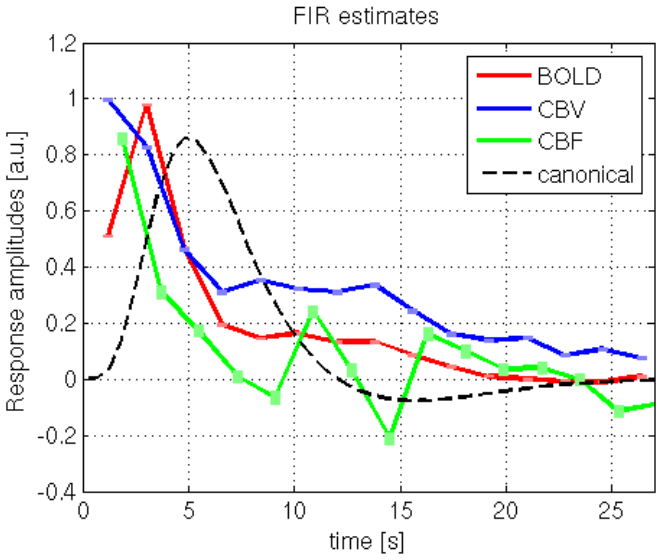
PURPOSE The interpretation of the BOLD response to epileptic discharges (EDs) requires understanding the mechanisms underlying neurovascular coupling. These latter are poorly known, and several puzzling and sometimes contradictory findings have been presented in the literature. In this study we tried to characterize the hemodynamic response and to investigate the neurovascular coupling to EDs via multimodal EEG fMRI by recording LFPs together with BOLD, CBF (ASL) and CBV (Mion) fMRI signals.

METHODS Eight male Wistar rats (310+-60 g) underwent surgery under isoflurane anesthesia to stereotaxically implant a guide for a cortical injection catheter targeting the somatosensory cortex, two extradural carbon EEG electrodes ~1 mm caudally and cranially of the injection site, and one occipital reference electrode. One week after initial surgery, a catheter for contrast agent injection was placed in the tail vein and a fine silica capillary (o.d. 0.19 mm) was inserted into the cortex via the cannula guide to a depth of 1–1.5 mm (layers III to VI). Throughout the MRI session, rectal temperature was recorded and maintained at 37 °C. Comprehensive physiological recordings were performed via a pulse oximeter (Starr Life Sciences, Pittsburgh, PA, USA). Animals were placed in a stereotaxic holder and maintained under isoflurane anesthesia (1.5–2%). Oxygen was supplemented as necessary to maintain aSO_2 at ~98%. EDs were elicited locally by intra-cortical infusion of bicuculline methochloride (2.5 mM, abcam, UK) at 200 nl/min for 5 min during MR imaging, using silica fiber connected to a 5-µl microsyringe (Hamilton) placed in a micropump via polyethylene tubing filled with bicuculline. Additional infusions were performed as needed (4–6 per animal).

MRI was performed in a horizontal-bore 4.7-T magnet (Biospec 47/40, Bruker, Germany) at the Grenoble MRI facility IRMaGe. A linear volume coil was used for RF transmission, and an open linear surface coil (Rapid Biomedial, Rimpar, Germany) permitting routing of EEG cables and injection catheter was used for detection. The imaging protocol consisted of a RARE T_2 reference scan followed by GE EPI BOLD-fMRI time-series (5 slices, TR 600 ms, TE 20 ms, FOV 32x32x5 mm, resolution 0.35x0.35x1 mm, 300 volumes, Tacq 3 min) and SE EPI pCASL fMRI time series (5 slices, label duration 1050 ms, post-label delay 200 ms, TR 1500 ms, TE 20 ms, FOV 32x32x5 mm, resolution 0.35x0.35x1 mm, 120 ctl + 120 tag volumes, Tacq 6 min). Ten BOLD and ASL scans were acquired in an interleaved fashion. Subsequently a MION contrast agent (P904, Guerbet, France) was injected intravenously (200 µMol Fe/kg) and 2–3 CBV-weighted fMRI time series were acquired (parameters as for BOLD, except TE 15 ms, 1000 volumes, Tacq 10 min). EDs were recorded using Micromed systemPLUS (SD MRI, Micromed, Italy), along with triggers from the MR system for synchronization.

fMRI and EEG data were processed in the Matlab environment (Mathworks, Natick, MA, USA). Briefly, EEG data were filtered to remove MRI artifacts and epileptic spikes were automatically labeled and used as events in the fMRI analysis. fMRI data were automatically examined for outliers (spikes), repaired if necessary, realigned to correct for (apparent) motion due to scanner drifts, spatially normalized to a homemade rat brain template and smoothed using SPM (FIL, UCL, London, UK) and custom routines. fMRI analysis was performed using SPM using a GLM based on a FIR basis set (duration 27 s, 15th order). Regressors accounting for correlations with any of the physiological parameters were included as parameters of non-interest. Estimates of response amplitude and its standard error for each FIR basis were obtained from each scan and averaged over a cortical ROI surrounding the injection site (~5 mm diameter) as well as across scans and animals to calculate the optimally weighted mean response kinetics.



RESULTS Animals were physiologically stable for 4–5 h, permitting acquisition of the entire protocol. The high SAR of the pCASL sequence (body coil Tx) required cooling of the animals by circulating ice water during pCASL/BOLD acquisitions. Bicuculline elicited robust subcritical EDs for 30–50 min after each intra-cortical injection, with frequent interictal-like discharges [1] occurring at a variable frequency of 0.2–2 Hz. Implanting an injection guide and carbon electrodes one week prior to fMRI acquisition minimized artifacts related to bleeding at the injection site. fMRI data show significant signal increases in the target ROI ($p<0.001$, uncorrected) in 63 out of 84 BOLD scans, 58 out of 78 pCASL scans and signal decreases in 24 out of 24 CBV scans. Estimation of the fMRI response kinetics was considerably hampered by the short inter-spike interval. The figure shows preliminary data on the average unfiltered HRFs obtained for BOLD, pCASL and CBV responses, compared to the canonical HRF.

DISCUSSION Multimodal EEG-fMRI recording of EDs elicited by bicuculline injection is feasible over extended periods of time, but requires careful control of animal physiology. Eliciting robust EDs at low spike frequencies is difficult with this protocol, hampering the estimation of response kinetics. Despite this fact, physiologically plausible mean response dynamics are obtained for BOLD and CBV. The CBF HRF obtained with the current simple processing methods is much less robust, due to lower SNR and longer TR, and may require more sophisticated methods such as regularized Bayesian joint detection-estimation.

REFERENCES [1] Hirase H et. al. (2004) Neuroscience. 128:209-216