BOLD and CBF post-stimulus undershoots are correlated with post-stimulus neuronal activity in humans.

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Introduction: The post-stimulus undershoot is a well recognised component of the BOLD response [1]. However, the BOLD response originates from a complex interaction between cerebral blood flow (CBF), cerebral blood volume (CBV) and the metabolic rate of oxygen consumption ($CMRO_2$), making the physiological origin of the post-stimulus undershoot unclear. Proposed mechanisms for the undershoot include: (i) elevated CBV once CBF and $CMRO_2$ have returned to baseline, (ii) an uncoupling of CBV/CBF from $CMRO_2$ (with CBV/CBF returning to baseline whilst $CMRO_2$ remains elevated) or (iii) a reduction in $CMRO_2$ below baseline, accompanied by a greater reduction in CBF [2]. In monkeys, the BOLD undershoot has been shown to be accompanied by a decrease in neural activity (LFPs and MUA) [3], suggesting mechanism (iii). Here, we use simultaneous EEG-BOLD-ASL during median nerve stimulation (MNS) to investigate the correlation between the undershoot and neuronal activity in humans for the first time. A consistent EEG feature on cessation of MNS is a rebound of the mu (8-13Hz) and beta (15-30Hz) frequency bands above pre-stimulus levels. This post-stimulus event-related synchronisation (PERS) [4] typically lasts a few seconds; we suggest that the PERS reflects changes in neuronal activity which modulates the BOLD post-stimulus undershoot. Whilst close spatial agreement between beta PERS and the positive BOLD response has been reported in motor cortex [5], the temporal relationship between PERS power and the amplitude of the BOLD undershoot is unknown.

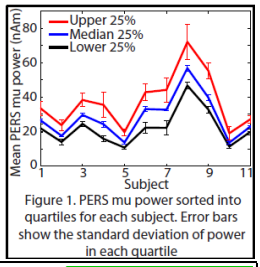

Figure 1. PERS mu power sorted into quartiles for each subject. Error bars show the standard deviation of power in each quartile

Hypothesis: We postulate a correlation between the magnitude of the PERS mu power and the BOLD post-stimulus undershoot, which would provide new evidence of a neural component to the BOLD undershoot.

Methods: fMRI and EEG data were acquired simultaneously using a Philips Achieva 3T MR scanner and a 64-channel EEG system (Brain Products). A GE-EPI BOLD localiser scan was used to position 10 contiguous axial slices encompassing the primary somatosensory cortex (S1). A FAIR Double Acquisition Background Suppression (DABS) [6] sequence was used for simultaneous acquisition of background suppressed ASL and BOLD data (TR=2.6s, TE=13/33ms [ASL/BOLD]), label delay=1400ms, 3x3x5mm^3 voxels, 212mm FOV, SENSE factor 2; background suppression at TI1/TI2=340ms/560ms). Data were acquired on 18 right-handed subjects (age=27±3yrs). MNS was applied to the right wrist (2Hz, 0.5ms duration pulses, Digitimer DS7A) at each individual's motor threshold for thumb distension. Data were recorded over 40 blocks (10s/20s MNS/rest).

Analysis: *EEG:* Gradient and pulse artefacts were corrected (Brain Vision Analyzer2 [7-8]). 7 subjects were excluded from further analysis due to insufficient data quality (>3mm or stimulus-locked movement in 5 subjects, degraded mu activity in 2). In the remaining 11 subjects, noisy channels and/or blocks were rejected; data were down-sampled to 600Hz, re-referenced to an average of non-noisy channels and filtered 8-13Hz. A regularised, scalar beamformer was used to localise the mu response to MNS, based on digitised electrode positions and a spherical head model [9]. Virtual electrode timecourses of electrical activity were extracted from the peak pseudo T-stat location in contralateral sensory-motor (S1/M1) cortex (active/passive window: 0-9.5s/20-29.5s). For each block, the mean stimulus response (0-9.5s), PERS (10.5-20s), and control window (25-29.5s) mu power values were calculated. For each subject, trials were sorted into lower (0-25%), median (37.5-62.5%) and upper (75-100%) quartiles based on: PERS, stimulus response or control window mu power.

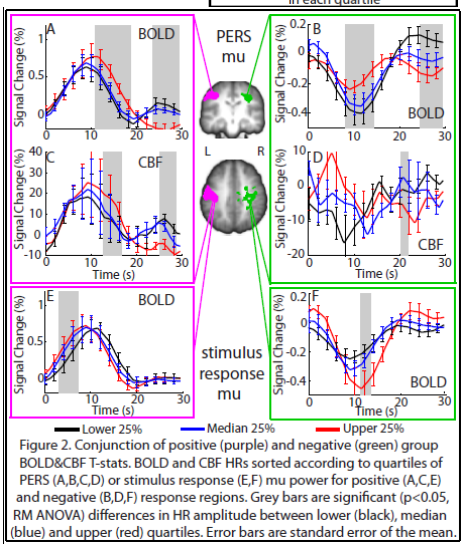

Figure 2. Conjunction of positive (purple) and negative (green) group BOLD&CBF T-stats. BOLD and CBF HRs sorted according to quartiles of PERS (A,B,C,D) or stimulus response (E,F) mu power for positive (A,C,E) and negative (B,D,F) response regions. Grey bars are significant ($p<0.05$, RM ANOVA) differences in HR amplitude between lower (black), median (blue) and upper (red) quartiles. Error bars are standard error of the mean.

fMRI: fMRI data were motion corrected using FLIRT (FSL), and BOLD data were physiologically corrected using RETROICOR. Data were interpolated to an effective TR of 2.6s, and ASL tag-control pairs subtracted to create perfusion-weighted images. BOLD and ASL datasets were normalised to the standard MNI template and spatially smoothed (5mm). A GLM analysis was performed in SPM using a boxcar regressor of the stimulation period convolved with the canonical HRF. A second level, group fixed-effects analysis was performed. Areas of significant positive/negative correlation of BOLD ($p<0.05$, FWE corrected) and CBF ($p<0.001$, uncorrected) data with the boxcar model were identified in contralateral/ipsilateral sensorimotor cortex (S1/M1). The group-level conjunction of the significant BOLD and CBF regions was used to mask individual's BOLD T-stat maps. Subject-specific, cubic regions of interest (ROIs, 3x3x3 voxels) were centred on the peak BOLD voxel in both the positive (contralateral) and negative (ipsilateral) regions. BOLD and CBF single-block haemodynamic responses (HRs) were extracted for each block from the BOLD ROIs; allowing direct comparison between CBF and BOLD responses. HRs were sorted into quartiles according to either the PERS, stimulus response or control window mu power. HRs were then converted to percentage change relative to the final 6s of the individual's mean block HR, and averaged over subjects.

Results: *EEG:* Figure 1 shows the significant difference in PERS mu power between the three quartiles and the variability across subjects. No significant correlation between PERS mu power and either stimulus response or control window mu power was observed. *fMRI:* The main BOLD/CBF signal response (at ~ 10 s) to MNS was positive in contralateral (Fig. 2 purple) and negative in ipsilateral (Fig. 2 green) S1/M1. In both the positive and negative BOLD/CBF regions the post-stimulus undershoot amplitude was negatively correlated with PERS mu power. Two-way RM ANOVA (Factors: Sorted quartile x time) showed a significant ($p<0.05$) interaction between HRs sorted according to PERS quartile mu power and time for positive and negative BOLD and positive ASL timecourses. For each HR time point a one-way RM ANOVA was used to test for significant variation in amplitude with PERS mu power. In the undershoot period, a significant effect of PERS power was observed in contralateral (positive) BOLD (23.5-29.5s) and CBF (25-28.5s) amplitude (Fig 2 A&C). For ipsilateral S1/M1, a significant difference in undershoot between quartiles was also observed for the negative BOLD timecourse (24.5-29.5s) (Fig 2B), but not for negative CBF (Fig 2D). We also found an increase in the peak HR amplitudes and a significant difference in the lag of the falling edge of the BOLD/CBF response that was linked to PERS mu power. Sorting BOLD responses based on stimulus mu power showed no significant differences in the post-stimulus undershoot or HR lag for the contralateral ROI (Fig 2E) and only a small time window where a significant lag effect was observed for the ipsilateral ROI (Fig 2F); with no sorting effects found in the CBF data. No significant effects on BOLD or CBF responses were found when sorting according to control mu power. ROIs from CBF peak T-stats showed similar effects of sorting on the undershoot (data not shown).

Discussion: Here, we provide the first evidence that the BOLD post-stimulus undershoot (>20s) is linked to post-stimulus changes in electrical oscillatory activity (10-20s) in humans. MNS blocks with higher post-stimulus mu power exhibited more negative BOLD/CBF undershoots in both positive and negative BOLD regions. This correlation was not observed when sorting data according to stimulus or control mu power, providing further evidence that our findings truly reflect correlation of the post-stimulus neuronal and fMRI signals. The difference in the HR lag and peak amplitudes observed when HRs were sorted by PERS mu power is not believed to be caused by the neuronal activity occurring between 10-20s (time window of PERS mu), as the haemodynamic response delay must be considered. Instead we postulate that differences in PERS mu power are a consequence of differences in stimulus-driven (0-10s) neuronal activity across frequency bands other than mu and that this underlies the differences in the main peak BOLD/CBF responses and lag. Since mu ERS is commonly believed to reflect inhibitory neuronal activity [10], we hypothesize that a change in the balance of excitatory/inhibitory activity in sensory-motor cortex upon termination of stimulation causes a change in $CMRO_2$. We show a concordant effect on BOLD and CBF signals post-stimulus which may be triggered by such changes in neuronal activity [3]. The observed BOLD undershoot would require changes in CBF to be greater than those of the $CMRO_2$, as proposed in mechanism (iii) and similar to the mechanism of the main BOLD response.

References [1] Kwong *et al*, PNAS **89**:5675-5679; 1992 **[2]** Sadaghiani *et al,* MRI **27**:1030-1038; 2009 **[3]** Shmuel *et al* Nat Neurosci. **9**;2006. **[4]** Pfurtscheller EEG & Clin. Neurophys. **51**:253-264; 1981 **[5]** Stevenson *et al,* HBM **32**:182-197; 2011 **[6]** Wesolowski *et al.* Proc. ISMRM, 6132;2009. **[7]** Allen *et al* NeuroImage **8**;1998 **[8]** Allen *et al* NeuroImage **12**;2000 **[9]** Brookes *et al* NeuroImage **40**;2008 **[10]** Pfurtscheller *et al* Clin. Neurophys. **110**:1842-1857; 1999.