The relationship between GABA concentrations and cerebral hemodynamics is spatially heterogeneous

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INTRODUCTION: Three recent studies have found negative correlations between the BOLD response amplitude to visual stimulation and baseline GABA concentrations in the human visual cortex [1-3]. Although the BOLD amplitude is generally thought to correlate with the extent of functional hyperemia [4], one of the three studies [2] reported that, in addition to the negative GABA-BOLD correlations, resting CBF correlated positively with GABA, while activity-related CBF changes tended towards a positive relationship with GABA. Another study [3] did not find any significant GABA-CBF relationship. These studies used arterial spin labeling (ASL) to measure CBF, which implies potential bias due to tissue specific transit time differences and BOLD contamination. In addition, the studies extracted hemodynamic parameters from tissue volumes with varying relative gray:white matter ratios and varying degrees of overlap with the magnetic resonance spectroscopy (MRS) voxel. As GABA exists in gray matter, white matter and CSF, we hypothesized that correction for partial volume effects within the large MRS voxel and comparison to hemodynamic values from gray matter only, would clarify the correlation between GABA, CBF and BOLD responses. With CBF values measured using gold-standard positron emission tomography (PET), we examined the correlation between GABA concentrations, BOLD responses and CBF changes in three ROIs: (1) The area of BOLD activation in visual cortex, (2) the intersection of the BOLD activation with a gray matter (GM) mask and the MRS voxel mask, and (3) the area of the peak BOLD response within this intersection.

METHODS: **Experiment**: 14 healthy, male subjects (22-37 years) gave informed consent and were scanned on a 3T (Siemens) MR scanner and a High Resolution Research Tomograph (HRRT, CPS Innovation). The functional paradigm was a black-white, visual checkerboard reversing at 4Hz. Incomplete data acquisition for 4 subjects precluded them from further analyses. MRS MEGA-PRESS: Baseline edited GABA spectra were acquired before functional imaging. TR/TE=2500/68ms, 192 averages, 3x3x3cm^3. Editing was achieved with a 14ms dual banded Gaussian pulse with water suppression band centred at 4.7ppm. The GABA editing band alternated between 1.9ppm and 7.5ppm in even and odd acquisitions. BOLD EPI: TR/TE=2500/40ms, α=90°, voxel=2.35x2.35x4mm. MPRAGE: TR/TE=2420/3.7ms, 1mm isotropic resolution. PET: Dynamic imaging was conducted with a fixed 21-frame structure (12x5s, 6x10s, 3x20s) lasting 3 minutes, following intravenous injection of 600 MBq [15O]-H2O. Two scans were acquired for each subject (baseline and stimulation). The radioactivity concentration of arterial blood from the left radial artery was recorded continuously using an automated blood sampling system. **Analyses**: MRS: All spectra were apodized with a 5 Hz filter. Zero and 1st order phase corrections were applied. GABA levels relative to NAA were measured using AMARES [5] within jMRUI. Creatine was measured from summed subspectra. An institutional unit (i.u.) GABA:Creatine ratio was determined. MPRAGE: Volume images were segmented using SPM8 to provide individual GM masks. BOLD EPI: Using SPM8, images were corrected for motion and drift. Visually activated areas were determined by t-tests (FWE, p≤0.05). Maps were co-registered to individual MPRAGE images. PET: Scans were co-registered to individual MPRAGE images and further co-registered to a MNI template with affine transformations. Parametric maps of CBF were estimated using a linearized two-compartment model [6,7]. CBF maps were smoothed with 6mm 3D FWHM Gaussian kernel and normalized to individual mean cortex values. CBF values were averaged within the 3 areas of interest. Correlations using Spearman's rho were performed, given the non normal distributions of each metric.

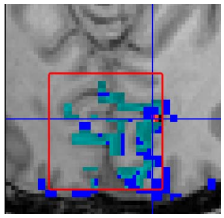

Fig. 1: Example of the 3 ROIs: Full BOLD activation area (blue+green+red); Intersection of BOLD activation with GM and MRS voxel masks (green+red); Area of peak BOLD response (red). Red boundary denotes border of MRS voxel.

RESULTS & DISCUSSION: The correlations reported below were consistent when using GABA/Cr, GABA/NAA and correction for gray matter fraction within the MRS voxel. We therefore report results for GABA/Cr to facilitate comparison with the other studies [1-3]. Fig. 1 illustrates the ROIs used. Table 1 summarizes the significance of the correlations in the 3 areas of interest, while Fig. 2 illustrates the negative correlation between GABA/Cr and the peak BOLD response. Intriguingly, we did *not* find significant negative correlations of GABA levels with BOLD responses, except in the case of the peak BOLD response. The latter result was also observed in [1,3], although study [3] also reported a negative correlation when using a larger ROI that included the medial visual cortex. In [2], the ROI used was defined by an area displaying common activation in the BOLD, ASL and VASO scans. However, it is unclear if the larger ROIs used in these studies fell within the MRS voxel location. To avoid bias due to tissue specific GABA concentrations, we compared hemodynamic values taken from gray matter within the MRS voxel location. If we instead analyzed the entire BOLD activated area in the visual cortex, we still found no clear relationship with GABA. In all 3 areas of interest, GABA levels did not show significant relationships with baseline CBF nor activity-related CBF changes. While a non significant result cannot disprove a link between CBF and GABA, it adds to the existing non significant finding in study [3]. The PET CBF measurements used here provide gold-standard, quantitative CBF and avoids potential bias of ASL CBF values. It is not clear how GABA levels *per se* influence hemodynamics [2], therefore it would be of great interest to eventually be able to combine such measurements with estimates of glutamate levels, in order to assess whether the balance of local excitatory-inhibitory neurotransmitter levels affect local hemodynamics. A possible bias in this study is that the ROIs were based on the BOLD response, and it would be interesting to consider other areas, e.g. the CBF activated area. Finally, it was encouraging to note that PET CBF changes and BOLD responses had a clear, positive relationship in gray matter (Table 1, Fig. 3), further supporting the idea of neurovascular coupling. Other than a previous study [8] that assessed PET CBF and BOLD correlations in the motor cortex, this represents the first confirmation of such correlations within the visual cortex. The lack of significant correlation with the BOLD response in the full activation area was less surprising, given the known effects of BOLD changes in neighboring veins. Correlations within the small, peak BOLD response area depend on a good co-registration between the PET CBF and BOLD images. Assuming appropriate co-registration, the lack of a correlation points to the possibility that within the peak BOLD response area, changes in CBV and/or $CMRO_2$ are stronger influences on the BOLD response than CBF, as the data from [2] also seem to suggest. To conclude, correlations between GABA and BOLD depend on the tissue under investigation. In areas showing significant GABA-BOLD correlations, this does not appear to depend on parallel changes in CBF.

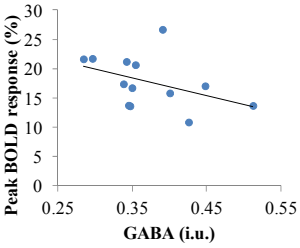

Fig. 2: GABA relationship with the peak BOLD response (r=-0.65).

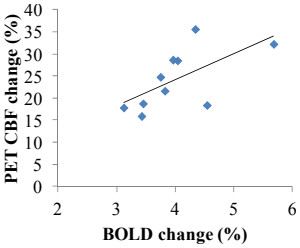

Fig. 3: Relationship of PET CBF change with the BOLD response (r=0.69) in occipital GM.

REFERENCES: [1] Muthukumaraswamy SD, '09, PNAS 106:8356; [2] Donahue M, '10, Neuroimage 53:392; [3] Muthukumaraswamy SD, '11, HBM 18:4467; [4] Davis TL, '98 PNAS 95:1834; [5] Provencher SW, '93, MRM 30(6):672; [6] Ohta S, '92, JCBFM 12:179; [7] Blomqvist G, '84, JCBFM 4:629; [8] Ito H, '05, JCBFM 25:371

	Entire BOLD activation area in visual cortex	Intersection of BOLD activation area, gray matter mask and MRS voxel mask	Peak BOLD response area within intersection
GABA (i.u.) vs BOLD change (%)	r = -0.19, p = 0.60	r = 0.43, p = 0.21	r = -0.65, p = 0.043 *
GABA (i.u.) vs CBF change (%)	r = 0.12, p = 0.75	r = 0.25, p = 0.49	r = -0.21, p = 0.56
GABA (i.u.) vs CBF baseline	r = 0.43, p = 0.21	r = 0.15, p = 0.68	r = -0.19, p = 0.60
CBF change vs BOLD change	r = -0.33, p = 0.35	r = 0.69, p = 0.029 *	r = 0.32, p = 0.37

Table 1: Summary of correlation statistics for each ROI. Asterisks (*) denote statistically significant correlations.