Hemodynamics under pharmacological challenge in rat brain: comparison of BOLD, CBV and CBF using bicuculline

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Introduction

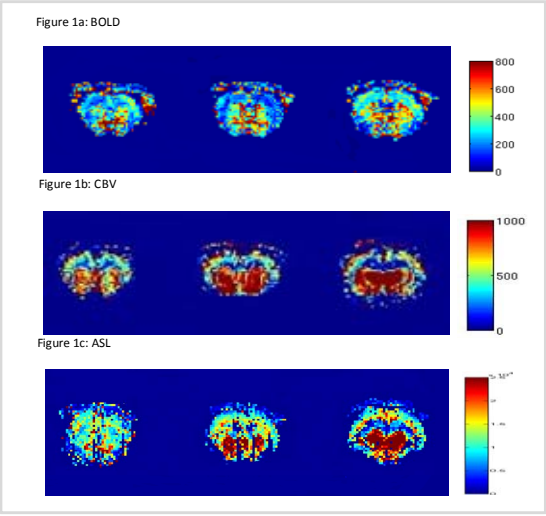
Pharmacological MRI (phMRI) allows noninvasive mapping of drug-induced brain activity and has been increasingly used as a translational endpoint for drug discovery research [1]. phMRI data has been collected using BOLD, CBV, or ASL imaging techniques. Each method appears to have its own advantages and disadvantages; ASL is quantitative but less sensitive, whereas CBV is more sensitive but requires exogenous contrast agents. More importantly, these approaches can offer complementary information of hemodynamic response produced by a pharmacological challenge and thus collectively could better characterize the CNS effect of drug actions [1-4]. The aim of our study was to characterize brain activation elicited by bicuculline ($GABA_A$ receptor antagonist) infusion using BOLD, CBV, and ASL in isoflurane-anesthetized rats. We hypothesized the distinct spatiotemporal patterns of phMRI signal revealed by these three different methods might underpin the specific neural circuitry associated with GABAergic transmissions. Additionally, technical challenges and feasibility of applying these imaging methods for phMRI studies were also assessed and compared.

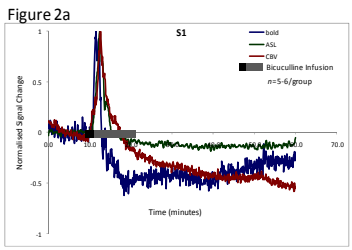
Methods

All animal studies were approved by the Institutional Animal Care and Use Committee (BMSI, A*STAR, Singapore). Male Wistar rats (300-400 g) were anesthetised, intubated, cannulated (tail vein and artery) then artificially ventilated (TOPO) under 1.25% isoflurane and air/ 47% oxygen. Animal's physiology was stabilized at pCO_2: 35-45 mmHg; pO_2: 120-280 mmHg; pH: 7.4-7.49; MABP: 80-140 mmHg. For each drug and method group, 6 different rats were used. **Experimental Design:** Total scan duration was 60 minutes: baseline [10 min], bicuculline infusion via tail vein [12 min; 0.56 mg/kg/min for 2 min, and followed by 0.056 mg/kg/min for 10 min]. **MRI:** MRI was acquired on a 9.4T/31 cm MRI system interfaced to a Varian console. **BOLD:** 2-Shot SE-EPI (TR = 2500 ms, TE = 38 ms slices= 12, FOV = 25.6 mm x 25.6 mm; matrix: 64 x 64, thickness = 1.4 mm; gap 0.1 mm). **CBV**: T2 contrast agent feraheme, (10 mg/kg; *AMAG Pharmaceuticals Inc*) was injected i.v. and acquired with 2D FLASH (TR = 78.13 ms, TE = 4 ms, flip angle = 20 degrees, slices = 12, FOV = 25.6 mm x 25.6 mm; matrix: 64 x 64, thickness = 1.4 mm; gap 0.1 mm) and data was calculated to percentage CBV change. **ASL**: An optimized Flow-sensitive Alternated Inversion Recovery with single-shot SE-EPI acquisition (TR = 3600 ms, TE = 22 ms, TI = 1300 ms, tag width = 25 mm, slice= 7, FOV=25.6 mm x 25.6 mm; matrix: 64 x 64, thickness = 2 mm; gap 0 mm) [5]. High-pass filtering method was used to extract the perfusion signal and to suppress the BOLD contamination in the ASL [6]. **Analysis:** Area under curve map was generated from the normalized signal integral during the drug infusion period. ROI analysis was conducted to derive regional mean signal changes. Furthermore, Contrast-to-Noise Ratio (CNR) was calculated from the averaged signal difference between drug infusion and baseline and divided by the standard deviation during baseline.

Results

Robust and significant activation was observed in all bicuculline injected rats and in all three methods. Prominent activation was detected in thalamus and caudate putamen. Modest and acute responses were seen in somatosensory cortex (S1) and hippocampus (Figure.1). Comparing the three hemodynamic responses in each area, CBV shows a prolonged undershoot after the drug infusion while CBF returned to baseline rapidly (Figure.2). Sensitivity was measured by calculating CNR in the thalamic region. BOLD CNR: 5.88 ± 1.64 (n=6); CBV CNR: 3.5 ± 1.25 (n=6) and ASL CNR: 8.77 ± 4.70 (n=6); BOLD *vs.* CBV: $p<0.23$ (*t*-test), BOLD *vs.* ASL: $p<0.54$, CBV *vs.* ASL*:* $p<0.28$.





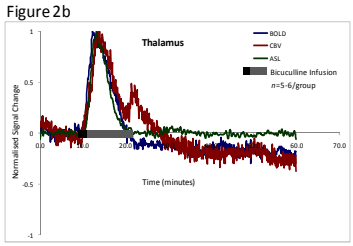


Figure 1: Area-under curve maps obtained by BOLD, CBV and ASL. **Figure 2**: Normalized signal change of BOLD, CBV, ASL in S1 and thalamus ROI.

Discussion

We demonstrated the BOLD, CBV and CBF responses under acute bicuculline challenge. The observed brain activation patterns correlate well with the known $GABA_A$ receptor distribution [7]: high density $GABA_A$ binding regions (S1, thalamus, caudate putamen) and mid-low density receptor region (hippocampus). Interestingly, temporal dynamics of phMRI signals appear to vary in a region-specific manner, where short and sharp responses were seen in the S1 and hippocampal regions and prolonged signal responses were seen in the caudate putamen and thalamus. Furthermore, despite the fact that similar response patterns can be seen in BOLD, CBV and CBF within the same region, we noticed that CBF response followed the stimulus almost immediately, whereas CBV and BOLD responses were long-lasting suggesting much slower characteristics as described by the balloon model. It has been reported that CBV signal can be larger and more sensitive than BOLD [8]. In our study, changes in CBV could be hampered by larger T2 decay at high field. Since a pharmacological challenge can exert its CNS effect via triggering vascular and neural response directly or indirectly, it is useful to collect phMRI data using all three methods where this information will provide a better understanding of the underlying link between drug-induced neuronal activity and the hemodynamic response.

References

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