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Title:

Potentials and Challenges for Arterial Spin Labeling (ASL) in Pharmacological MRI (phMRI)

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Abbreviations: Central Nervous System (CNS); Signal-to-Noise Ratio (SNR); Arterial Spin Labeled (ASL); Blood-oxygen-level-dependent (BOLD); Pharmacological Magnetic Resonance Imaging (phMRI)

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Abstract

Pharmacological MRI (phMRI) is increasingly being used in drug discovery and development to speed the translation from the laboratory to the clinic. The two primary methods in phMRI include blood-oxygen-level-dependent (BOLD) contrast and arterial spin labeled (ASL) perfusion MRI. BOLD contrast has been widely applied in existing phMRI studies. However, due to the lack of absolute quantification and poor reproducibility over time scales longer than hours or across scanning sessions, BOLD fMRI may not be suitable to track oral and other long term drug effects on baseline brain function. As an alternative method, ASL provides noninvasive, absolute quantification of cerebral blood flow both at rest and during task activation. ASL perfusion measurements have been shown to be highly reproducible over minutes, hours to days and weeks. These two characteristics make ASL an ideal tool for phMRI to study both intravenous and oral drug action as well as to understand drug effects on baseline brain function and on brain activation to cognitive or sensory processing. When ASL is combined with BOLD fMRI, drug induced changes in cerebral metabolic rate of oxygen ($CMRO_2$) may also be inferred. Representative phMRI studies using ASL perfusion MRI on caffeine, remifentanil and metoclopramide (dopamine antagonist) are reviewed here, with an emphasis on the methodologies used to control for potentially confounding vascular and systemic effects. Both the potentials and limitations of using ASL as an imaging marker of drug action are discussed.

Pharmacological MRI (phMRI)

Imaging methods are increasingly being used in drug discovery and development to speed the translation from the laboratory to the clinic (Wong et al. 2009). Imaging biomarkers of drug effects can be applied in both preclinical models and human patients, and have the potential to demonstrate a proof-of-concept of drug actions in small human cohorts. Imaging is particularly valuable for the evaluation of drug candidates for CNS disorders because of the difficulties in quantifying the human brain function in vivo. The use of radioisotope labeled ligands with positron emission tomography (PET) remains central to the assessment of drug penetration through the blood-brain barrier as well as the engagement of specific receptor targets. However, pharmacological MRI (phMRI) – the use of functional MRI to study drug effects on baseline brain function as well as drug modulation of brain activation to cognitive or sensory processing – has been gaining popularity due to its noninvasiveness, lower cost, and relatively wide availability (Wise and Tracey 2006). Although phMRI may never rival PET for demonstrating specificity at a molecular level, its complementary capabilities for assessing brain function may provide greater sensitivity than traditional clinical endpoints for early phase studies.

Blood-oxygen-level-dependent (BOLD) contrast is the most widely used approach for phMRI due to its technical simplicity and relatively high sensitivity (Iannetti and Wise 2007). The effects of a drug on baseline brain function in the absence of other modulating factors have been demonstrated by BOLD fMRI using a variety of pharmacological agents including cocaine, dopamine agonists, nicotine, opioidergic and serotonergic agents (Becerra et al. 2006, Breiter et al. 1997, Chen Y. C. et al. 1997, Leppa et al. 2006,

McKie et al. 2005, Stein et al. 1998, Upadhyay et al. 2010). However, because of the noise properties of BOLD fMRI (Aguirre et al. 2002, Wang et al. 2003) and the lack of absolute quantification, BOLD phMRI is in general poorly suited to detecting the effects of oral drugs, or drug responses over days or weeks. It is worth noting that resting BOLD fMRI measures (e.g. functional connectivity and independent component analysis) have recently been applied to characterize drug effects on baseline brain function (Li et al. 2009, Upadhyay et al. 2010).

Instead of directly studying drug induced changes in baseline brain function, more recent phMRI based on BOLD contrast has focused on pharmacological modulation of the brain response to a cognitive task or sensory stimulation. Drug effects can then be inferred from the modulation of the benchmark BOLD activation associated with task performance or sensorimotor stimulation. Modulation of the BOLD fMRI responses to a painful stimulus has been among the most widely studied application of BOLD phMRI (Wise and Tracey 2006, Wise et al. 2002), though a range of sensorimotor or cognitive task effects in response to pharmacological modulation have also been examined (Anderson et al. 2010, Jokeit et al. 2001, Laurienti et al. 2002). A limitation of this approach is that it is difficult to determine whether the modulational effects of the drug on BOLD fMRI are attributable to changes in baseline brain function, the activation state, or both. Conversely, a lack of modulational effects on BOLD fMRI does not exclude the possibility that the drug equally affects the baseline and activation states (i.e., a shift of approximately equal magnitude). These theoretical arguments may be further complicated by the fact that BOLD fMRI reflects a complex interplay between cerebral

blood flow (CBF), cerebral blood volume (CBV) and oxygen consumption. An observed drug effect on BOLD fMRI may thus be the result of changes in one or more of the above parameters at baseline either regionally or systemically (e.g., blood pressure or vascular tone). There have been several studies demonstrating an interaction between basal physiologic and metabolic states and task induced BOLD activation (Cohen et al. 2002, Hyder et al. 2002).

Arterial spin labeled (ASL) perfusion MRI – Advantages for phMRI

Arterial spin labeled (ASL) perfusion MRI provides an alternative and complementary tool to BOLD fMRI for phMRI (Detre J. A. et al. 2009). Methodologically, ASL is similar to ^{15}O-water PET. Instead of injecting the subject with ^{15}O labeled water, ASL uses radiofrequency (RF) pulses to magnetically label the water molecules in the subject's own arterial blood, usually at the base of the brain. The magnetically labeled blood water is then utilized as an endogenous tracer for the measurement of CBF, which has a half life (determined by blood T1) on the order of 1-2 seconds. The effects of ASL are measured by comparing images acquired with active or control labeling, and this signal difference can be quantified using a model that requires a few additional measured or assumed parameters (Buxton et al. 1998b). Several methodological approaches exist for ASL and include continuous ASL (CASL), pulsed ASL (PASL), and pseudo-continuous ASL (pCASL), which differ in their labeling efficiency, ease of implementation, and compatibility with other scanner hardware (Detre J. A. et al. 2009).

Although ASL MRI measurements of CBF have been shown to correlate closely with

PET CBF both at rest (Ye et al. 2000) and with task activation (Feng et al. 2004), there are also some significant differences between ^{15}O-water PET and ASL perfusion MRI. First, there is large background tissue signal in ASL, which is eliminated through pairwise acquisition and subtraction of label and control signals. This signal-processing step produces beneficial noise characteristics for phMRI studies, as shown below. Second, the arterial input function (AIF) can be explicitly defined by the duration and amplitude of the labeling RF pulses in ASL, whereas in general an arterial line has to be inserted to sample AIF in PET studies. This factor greatly facilitates the absolute quantification of CBF using ASL. Lastly, ASL is entirely noninvasive and can be repeated as often as required to track the dynamic course of pharmacological effects over time.

Compared to BOLD fMRI, ASL offers several appealing features and may be ideally suited for phMRI studies (Aguirre et al. 2005, Detre J.A. and Wang 2002). Due to the pairwise subtraction of label and control acquisitions, which acts as a high-pass filter of the temporal signal, the frequency spectrum of ASL time series is flat (or "white noise") (Aguirre et al. 2002, Wang et al. 2003). As opposed to BOLD fMRI, which has universally elevated power at low frequency, ASL is suitable for studying slow changes in brain function, including the direct pharmacological effects of drugs that may take effect over hours or days, obviating the need for measuring drug effects indirectly by their modulation of a stimulus or task response. Parenchymal perfusion or CBF (in unit of ml/100g/min) is also a well characterized physiological parameter, and may be considered a more direct marker of neuronal activity as compared to the BOLD contrast

that reflects “lumped” changes of CBF, CBV and oxygen metabolism (Buxton et al. 1998a). For task effects, ASL offers quantitative CBF measurements both at rest and during task activation, which is critical for separating drug effects on baseline brain function and task induced activation. An example can be found in Fig. 5 of (Liau et al. 2008). While both ASL and BOLD fMRI can demonstrate relative signal changes to visual activation pre and post caffeine, quantitative baseline CBF offered by ASL is valuable for calculating CBF changes in physiological units. Lastly, ASL perfusion contrast can be imaged using pulse sequences that are resistant to magnetic field inhomogeneity (susceptibility) effects (Wang et al. 2004), offering improved visualization of orbitofrontal, inferior temporal and limbic regions that are linked to major neurotransmitter systems.

These potential advantages of ASL for phMRI studies have already been recognized by the scientific community, and several review articles have noted the value of ASL for assessing drug effects on baseline brain function (Iannetti and Wise 2007, Wise and Tracey 2006). At least one early phMRI study has included both ASL and BOLD fMRI pre and post cocaine infusion (Gollub et al. 1998). However, the limited availability, SNR and image coverage of ASL techniques have remained a major obstacle for application studies. During the past decade, technical advances have greatly improved sensitivity and coverage of ASL techniques. For instance, ASL methods derive a dual benefit from high magnetic field strengths (Wang et al. 2002). In addition to increased sensitivity at high field, T1 also increases, allowing more label to accumulate in brain tissue. ASL methods also benefit from parallel imaging and array receiver coils (Wang et

al. 2005b). The most recent approach to ASL, pseudo-continuous ASL (pCASL), combines the advantages of pulsed ASL (PASL), including technical simplicity and compatibility with array receivers, and conventional continuous ASL (CASL), including a longer tagging bolus and consequently higher SNR (Dai et al. 2008, Wu et al. 2007). Pseudo-CASL can be further combined with 3D acquisitions such as GRASE (a hybrid of gradient and spin echo) readout that allows the use of background suppression (BS) of static tissue signals to improve the temporal stability (Fernandez-Seara M. A. et al. 2008, Fernandez-Seara M. A. et al. 2007). As a result, whole-brain perfusion images with isotropic 4x4x4mm^3 spatial resolution can be achieved within just a few minutes, free of susceptibility artifacts. Figure 1a displays a sample image acquired using pCASL with 3D BS GRASE, showing excellent whole-brain coverage and visualization of orbitofrontal cortex. Paralleling these technical advances, recent progresses in commercialization also facilitate the application of ASL in translational neurosciences.

Two of the key characteristics determining the viability of a translational imaging tool are its reliability (precision) and accuracy. The timescale of drug action ranges from seconds/minutes (e.g., intravenous injection), hours (e.g., oral administration) to days and weeks (e.g., treatment cycle). Various test-retest studies have been carried out using ASL across minutes, hours, days and weeks. The within-subject Coefficient of Variation (wsCV) is generally on the order of 10% for global CBF, and 10 to 15% for regional measures (Chen Y. et al. 2010, Floyd et al. 2003, Jain et al. 2010, Parkes and Tofts 2004). The variation between repeated ASL measurements increases with lengthened time interval, suggesting increased effects of fluctuations in subjects' physiological states at

long scan intervals. In terms of accuracy, global CBF measurements using ASL have been compared with phase-contrast (PC) MRI which measures the flow velocity and cross-sectional area of the carotid and vertebral arteries (Chen and Pike 2010, Jain et al. 2010). Since the product of flow velocity and cross-sectional area of the carotid and vertebral arteries provides total blood flow to the brain, PC MRI offers a practical and accurate approach (intraclass correlation coefficient/ICC=0.98 by Jain et al 2010) to calibrate the labeling efficiency of ASL between scans, which may be affected by differences in subjects' head position, shimming conditions and the efficiency of background suppression (Garcia et al. 2005). Alternatively, a phase cycling approach may be applied to calibrate the labeling efficiency of pCASL (Jung et al. 2010). Regional CBF measurements using ASL have also been validated with ^{15}O-water PET both at rest (Ye et al. 2000) and during task activation (Feng et al. 2004). Figures 1b&c show the scatter plot of test-retest pCASL CBF measurements (2-4 weeks apart) and pCASL vs. PC-MRI global CBF measurements, respectively. Both excellent precision (ICC=0.62, wsCV=7.5%) and accuracy (ICC=0.77) are achieved. These reference data also provide the benchmark for designing ASL based phMRI studies. For instance, detecting a 15% change in CBF with 90% power, given 15% variation between repeated measurements, requires approximately 20 subjects.

Arterial spin labeling (ASL) perfusion MRI – Challenges for phMRI

While PET studies may be sensitive to the distribution of radiolabeled drug ligands or to the displacement of receptor-specific ligands, phMRI based on ASL measures changes in regional brain function indirectly through the coupling between neuronal activity and

blood flow, also termed functional hyperemia. Mounting evidence suggests that phMRI can successfully detect regional changes in brain function in response to pharmacological challenge, reflecting changes in regional neural function. The physiological basis for neurovascular coupling remains incompletely understood, but is known to involve coordinated activities of the neuron, astrocyte, and the microvasculature (Iadecola and Nedergaard 2007, Jakovcevic and Harder 2007). Synaptic activity triggers an increase in the intracellular calcium concentration of adjacent astrocytes, stimulating the release of adenosine triphosphate (ATP) and glutamate. Astrocytic Ca^{2+} elevations can lead to secretion of vasodilatory substances from perivascular endfeet, such as epoxyeicosatrienoic acid (EETs), adenosine, nitric oxide (NO), and cyclooxygenase-2 (COX-2) metabolites, resulting in increased local blood flow.

For some phMRI studies, pharmacological agents may directly alter CBF, or the mediators of neurovascular coupling. For instance, both caffeine (an adenosine antagonist) and indomethacin (an inhibitor of cyclooxygenase) cause vasoconstriction and reduce baseline CBF. Estrogen increases astrocytic Ca^{2+} levels, which raises the possibility that both BOLD and ASL signals may fluctuate across phases of the menstrual cycle in female subjects (Iadecola and Nedergaard 2007). Recent data suggest that a polymorphism of COX-2 may affect the magnitude of hemodynamic responses (Hahn et al. In press). Drugs may also cause systemic cardiovascular effects, which in turn may affect cerebral circulation. For example, many neurotransmitters including dopamine, serotonin and norepinephrine have receptors distributed within the cardiovascular system. However, systemic effects on CBF are mitigated by cerebrovascular autoregulation, and

would be expected to produce global rather than focal changes, though optimal study designs would control for such effects by monitoring systemic physiology.

A promising approach to circumvent (vascular) confounding factors in phMRI is to estimate drug induced changes in cerebral metabolic rate of oxygen ($CMRO_2$) through concurrent or combined ASL and BOLD scanning. For instance, the calibrated BOLD approach (Davis et al. 1998) employs a vasoactive agent such as CO_2 that has minimal effect on $CMRO_2$ to "calibrate" the BOLD contrast against known CBF levels using ASL. The derived mathematical relationship between CBF, BOLD and $CMRO_2$ can then be applied to BOLD and CBF changes from functional data to calculate relative changes in $CMRO_2$. Alternatively, oxygen extraction fraction (OEF) can be derived from quantitative BOLD MRI scans based on the model originally proposed by Yablonskiy and Haacke (1994). The product of OEF and CBF provides an estimate of $CMRO_2$. To date, measurement of $CMRO_2$ is available through PET scanning that involves 3 boluses of radioactive tracers (Mintun et al. 1984). It is worth noting that MRI measurements of $CMRO_2$ rely on assumptions of minimal changes in $CMRO_2$ during calibration (Davis et al. 1998) and neglectable effects of diffusion (Yablonskiy and Haacke 1994) which await further validation.

The main challenge for the widespread application of ASL for phMRI is probably the relatively lower sensitivity and image coverage of existing ASL methods as compared to BOLD fMRI. The latest pCASL techniques such as the one with background suppressed 3D GRASE need to be translated into phMRI studies. Concurrent acquisitions of ASL

and BOLD fMRI offer another appealing approach to assess changes in both CBF and oxygenation associated with a drug. Recent phMRI studies have generally employed a standard double-blind randomized controlled trial (RCT) design used in clinical trials. But, while conventional clinical trials typically rely on a single primary outcome to determine the efficacy of treatment, the complexity of statistical analysis for phMRI studies is magnified when drug effects on multiple brain regions are being considered simultaneously, or when changes over the entire brain are being considered at the voxel level. Appropriate correction for multiple comparisons must be used when determining the significance of observed effects, and wherever possible a priori regions should be specified. A promising new approach is to apply multivariate analyses (that simultaneously process all brain pixels) of perfusion based pharmacological and functional MRI data, often in a data driven fashion (Wang et al. 2007). The existence of a potential hierarchical brain response to drugs – from regional CBF changes to neurotransmitter system to whole brain CBF changes to repeated measurements within the same subject – may call for more advanced statistical analysis methods and clinical trial design such as multilevel mixed effects model in conjunction with within-subject cross-over design to improve the sensitivity of ASL based phMRI (Meriaux et al. 2006).

Representative phMRI Studies using ASL

In the following section we show representative phMRI studies involving the use of ASL perfusion MRI. Since it is such a new field, many studies are still in the form of meeting abstracts. Nevertheless, we emphasize the design and methodological aspects of these

phMRI studies, in particular with respect to the controlling of potential vascular and systemic confounding factors.

Dose-Response Curve – Remifentanil and Caffeine induced CBF Responses

Measuring dose-response curve is typically used in early phase drug trials to determine the optimal dosage for safety and efficacy. Such methodology can be adapted for phMRI studies to determine the drug specific CBF response. In one study to investigate opioid effects on brain function (Kofke et al. 2007), healthy volunteers received a remifentanil (mu opioid) infusion at four sequentially increasing doses: 0, 0.05, 0.1, and 0.2 ug/kg/min while receiving 100% oxygen. Mean CBF values were measured by PASL at each dose globally and in the amygdala, cingulate, hippocampus, insula, and thalamic regions, based on an anatomical template in the SPM Marsbar toolbox (Tzourio-Mazoyer et al. 2002). Significant dose-related CBF increases were detected in all areas (see Fig. 2a&b). This global CBF increase was expected since Paco2 increases with deepened sedative states induced by remifentanil. To correct for Paco2 effects, regional CBF values were normalized by global CBF. After normalization, the remifentanil-mediated increased CBF in the cingulate persisted, with decreased flow occurring in the hippocampus and amygdala (see Fig. 2c). The authors also investigated whether the observed limbic activation was linked to the presence of the ApoE4 genotype. The results showed that all these PaCO2-corrected effects were reversed in the presence of the ApoE4 polymorphism.

A caveat in ASL phMRI studies is the potential error of CBF quantification caused by

changes in arterial transit time (ATT – the time for the labeled blood to flow into brain tissue). To address this issue, a PASL study with 3D BS GRASE readout at multiple delay times (500-2500ms with 250ms interval) was carried out to simultaneously measure ATT and CBF during remifentanil infusion (MacIntosh et al. 2008). Administration of remifentanil produced an increase in end-tidal CO2, an increase in CBF from 57±12.0 to 77±18.4mL/100g/min and a reduction in ATT from 0.73±0.073 to 0.64±0.076 secs.

Caffeine is a popular psychostimulant that improves mental performance and alertness at low to intermediate doses, but may induce negative feelings such as insomnia, anxiety and nervousness at high doses. Chen and Parrish (2009a) investigated the dose-response curve of caffeine effects on functional activation. Twenty-seven healthy subjects were assigned randomly to four different groups: saline, 1 mg/kg, 2.5 mg/kg and 5 mg/kg doses of caffeine. Simultaneous ASL/BOLD time series were collected both before and after an intravenous infusion of saline or caffeine and task-induced CBF and BOLD percent signal changes were compared. The maximum increase in BOLD response was associated with the intermediate caffeine dose of 2.5 mg/kg, while the maximum increase in CBF response was associated with the highest caffeine dose of 5 mg/kg. The authors attributed the difference in BOLD and ASL responses to a different density of A(1) and A(2A) adenosine receptors in the brain.

Metoclopramide induced Systemic and Regional CBF effects

Metoclopramide, a substituted benzamide compound with antidopaminergic action, is widely prescribed in the symptomatic treatment of nausea and vomiting, although it can cause adverse neurologic side effects. To determine its effects on brain function, cerebral perfusion changes after a single 10 mg oral dose were assessed in healthy volunteers in a placebo controlled paradigm (Fernandez-Seara M. A. et al. In press). CBF was measured using pCASL before and one hour after medication intake. PC MRI was used to measure total blood flow through the carotid and vertebral arteries, as a way to calibrate the labeling efficiency of pCASL. PC MRI showed that metoclopramide caused a significant reduction in mean blood velocity in the internal carotid arteries, which is consistent with its known hypotensive effect (MacDonald 1991). Whole brain voxel-wise statistical analysis on the CBF data showed that metoclopramide induced perfusion increases bilaterally in the striatum, consistent with its dopamine D_2 receptor antagonism. In contrast, reduced perfusion was observed in the insular cortices and anterior temporal lobes. In addition, a decrease in functional connectivity was found between the insular cortex and the dorsolateral prefrontal cortex. The authors propose that these cortical changes affecting neural circuits between high-order association areas may underlie certain neuropsychiatric conditions occasionally reported after metoclopramide administration.

Caffeine induced Metabolic and Physiologic Responses – Multimodal Neuroimaging

As an adenosine antagonist, caffeine is suspected to alter the coupling between blood flow and oxygen metabolism, and has been the research topic in many phMRI studies. In order to derive the metabolic and neuronal changes induced by caffeine intake, several

investigators have used concurrent or combined BOLD/ASL to estimate cerebral metabolic rate of oxygen ($CMRO_2$) (Chen and Parrish 2009b, Perthen et al. 2008). A calibration paradigm is carried out during hypercapnia (e.g. breathing 5% CO2), assuming no significant changes in $CMRO_2$. Using such calibrated fMRI, Perthen et al. (2008) reported that caffeine decreased CBF (-34.5%, +/-2.6) with a non-significant change in $CMRO_2$ (+5.2%, +/-6.4). The coupling between CBF and $CMRO_2$ in response to visual stimulation was significantly different during caffeine consumption. Using visual and motor cortex stimulation tasks, Chen and Parrish (2009b) showed that caffeine decreased the CBF:CMRO2 coupling ratio from 2.58 to 2.33 in motor ($p=0.006$) and from 2.45 to 2.23 in visual ($p=0.002$) areas respectively. A similar calibrated BOLD/ASL fMRI study has been carried out using indomethacin (an inhibitor of cyclooxygenase) (St Lawrence et al. 2003). While indomethacin reduced the CBF increase during activation, it did not significantly affect the $CMRO_2$ increase, suggesting $CMRO_2$ may be a closer marker of neuronal activity compared to CBF. It was later argued that indomethacin may reduce the baseline $CMRO_2$ and there is a coupled response between changes in CBF and $CMRO_2$ (approximately 2:1) with and without indomethacin (Uludag and Buxton 2004). However, such response may depend on the dose of indomethacin and developmental stages of the specie (St Lawrence et al. 2004), highlighting the complexity of phMRI studies.

In a recent study, we used CASL, quantitative BOLD MRI (a modeling technique to fit oxygen extraction fraction and venous blood volume) (An and Lin 2000, He and Yablonskiy 2007) and ^{18}FDG PET to study physiological and metabolic responses to a

200mg caffeine pill (Chen Y. et al. 2009). Figure 3a shows the mean CBF maps pre and post caffeine intake (N=4). Caffeine caused CBF reduction (-10%, p=0.001) globally (Fig. 3b) and in ROIs based on SPM Marsbar toolbox template (Tzourio-Mazoyer et al. 2002). Caffeine also decreased CMRglu (-18%, $p<0.05$) (Fig. 3c) and increased OEF (+7%, p=0.006) and the $CMRO_2$: CMRGlu ratio (+22%, p=0.003) in all regions. CMRO2, on the other hand, remained unchanged due to compensatory effects of CBF and OEF. As shown in Figure 3d, anterior cingulate and caudate show significantly different CMRO2: CMRGlu ratio (p=0.014), which may be associated with heightened alertness post caffeine intake. The results suggest that CBF may be affected by vascular agents (e.g. caffeine), and combined physiological and metabolic MRI can provide more accurate information regarding the underlying neuronal events.

Comparison of ASL and BOLD for phMRI and Future Directions

Due to the specific focus on ASL, a comprehensive comparison of ASL and BOLD phMRI studies is out of the scope of this review. Interested readers can refer to earlier reviews addressing the general properties of ASL and BOLD contrast for fMRI studies (Aguirre et al. 2005, Detre and Wang 2002) as well as reviews on existing BOLD phMRI studies (Honey and Bullmore 2004, Wise and Tracey 2006). To date, BOLD remains the main contrast for phMRI due to its relatively high sensitivity and technical simplicity. For intravenous drug action that takes place on the order of minutes or even seconds, BOLD is advantageous to track the hemodynamic responses associated with drug action especially when the design includes repeated injections of drug and saline (Leppa et al. 2006). For detecting modulation effects of drug on task performance, several BOLD

fMRI paradigms have been proven to be “robust” such as N-back working memory and sensorimotor stimulation. For instance, BOLD activation in the visual cortex has been shown not to be sensitive to baseline flow reduction (Gollub et al. 1998, Liau et al. 2008).

Existing ASL methods are generally suitable for studying (oral) drug actions on the time scale of minutes, hours to days and weeks, although the latest ASL technique allows reliable perfusion measurements within a minute (Fernandez-Seara M. A. et al. 2008). As a quantitative MRI technique, one key advantage of ASL for phMRI studies is the capability for detecting drug effects on CBF during both resting and task activation states. In clinical populations, direct assessments of baseline or resting CBF may be more advantageous than task activation fMRI studies which may be confounded by deteriorating performance as well as compensatory processes. Drug induced global CBF changes generally indicate systemic effects, and can be compensated by normalization with global mean CBF or calibrated using PC MRI. Regional CBF responses may indicate specific drug action or binding to target regions, although alternative explanations exist. In this sense, resting ASL perfusion MRI provides a noninvasive and alternative approach to PET for assessing target engagement. Indeed, recent industry sponsored trials have applied ASL CBF as the marker of a single dose of Citalopram in healthy volunteers (Chen Y. et al. In press) as well as 3 month treatment of Aricept in subjects with mild Alzheimer’s disease (Antuono et al. 2009). The capability of ASL for absolute quantification of CBF may also facilitate the uniform exercise of multi-center phMRI studies.

As a way to assess drug effects on brain function and to bridge biochemical reaction and clinical endpoints, an fMRI paradigm may be included in addition to baseline CBF measurement in phMRI studies. This fMRI paradigm can be performed with either BOLD or ASL, or both contrasts. With knowledge of drug effects on baseline CBF, the observed modulational effects on fMRI activation can be interpreted with greater accuracy. A less explored area is the use of perfusion based fMRI in phMRI studies, which involves the use of long epochs of behavioral tasks such as cue induced drug craving (Franklin et al. 2007), emotional distress (Gillihan et al. 2010) and psychological stress (Wang et al. 2005a) etc that are otherwise difficult to study using BOLD fMRI. Combined or concurrent BOLD and ASL acquisition is a promising approach in phMRI to estimate metabolic changes which are thought to be closely linked to neural activity. Repeated ASL scans can readily be carried out to track the dynamic course of pharmacological action, and regional CBF values can be correlated with the plasma drug concentration (drug exposure) to improve the specificity of ASL in phMRI studies.

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Authorship Contributions

Participated in research design: Wang, Chen, Fernández-Seara, and Detre

Conducted experiments: Wang, Chen, Fernández-Seara, and Detre

Performed data analysis: Wang, Chen, and Fernández-Seara

Wrote or contributed to the writing of the manuscript: Wang, Chen, Fernández-Seara, and Detre

Other: Wang and Detre acquired funding for the research.

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Footnote

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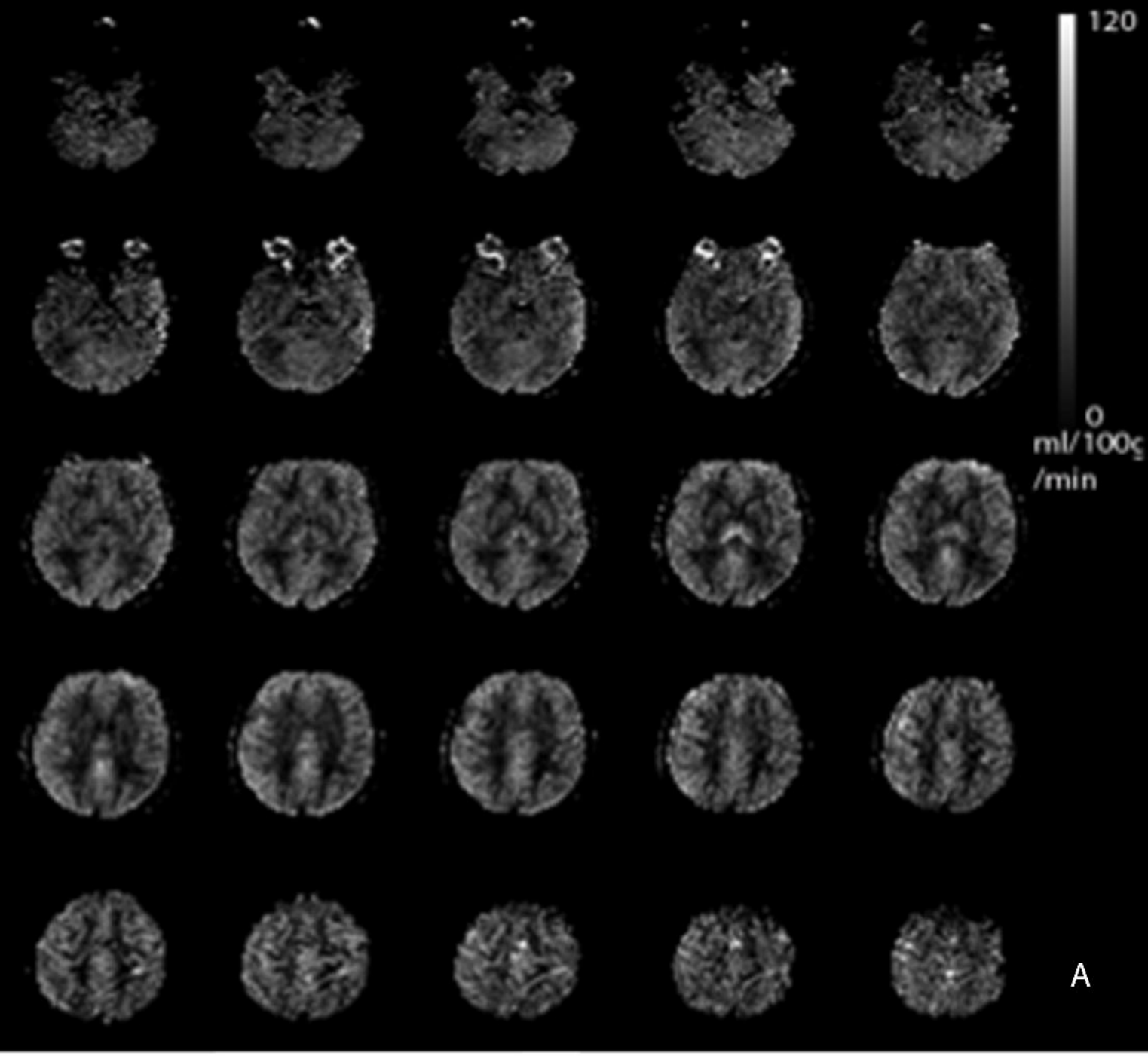
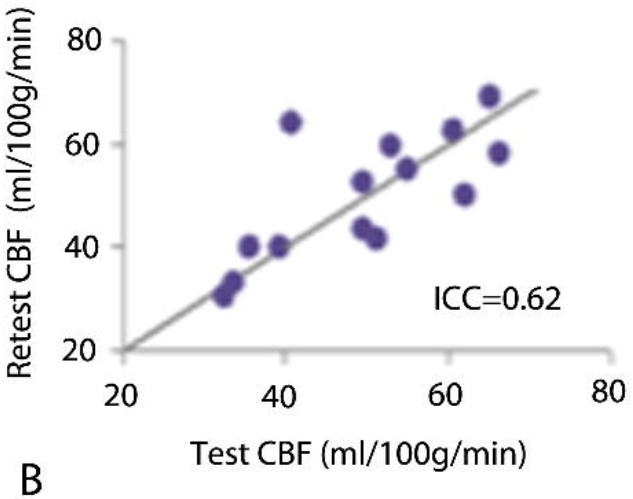
Figure Legends

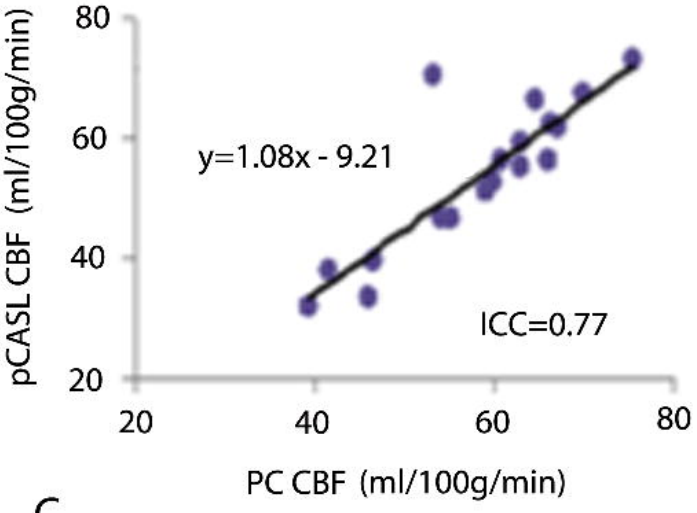
Figure 1. Representative whole brain perfusion images acquired using pCASL with 3D GRASE readout (A); scatter plots showing test-retest results (B) of pCASL perfusion measurements acquired 2-4 weeks apart, and comparison of pCASL perfusion versus global blood flow measurements using phase-contrast (PC) MRI in healthy children aged 7 to 17. A comparison of pCASL vs. conventional PASL and pCASL can be found in (Wu et al. 2007).

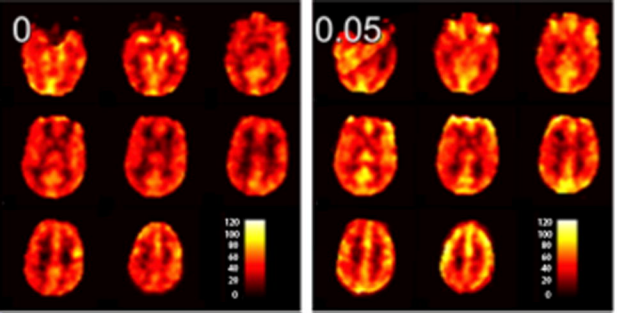
Figure 2. Mean cerebral blood flow (CBF) images for the four conditions (baseline and dose level 1–3) from a representative subject (A). Remifentanil doses noted in lower left of each figure as 0.0, 0.05, 0.1, and 0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Significant dose effect was observed using absolute CBF values in global, amygdala, cingulate, hippocampus, and insula (B). Ratio of regional to global CBF at each dose, thus normalizing for effects of $Paco_2$ shows significant dose effect in the amygdala, cingulate, and hippocampus (C). (need permission from International Anesthesia Research Society)

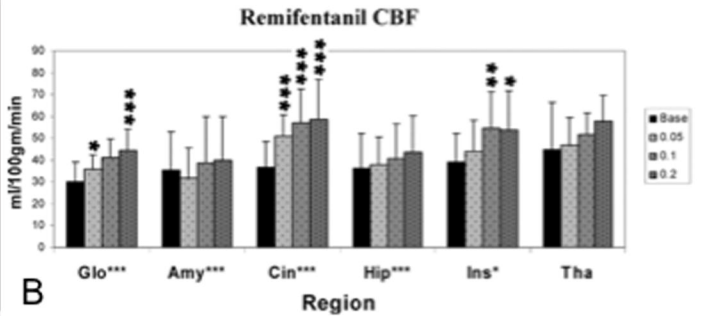
Figure 3. Mean CBF maps pre and post caffeine intake (a), and ROIs results of mean CBF (b), CMRglu (c) and CMRO2:CMRglu ratio (d). Anterior cingulate and caudate show significantly different CMRO2: CMRGlu ratio (p=0.014), which may be associated with heightened alertness post caffeine intake. Errors bars indicate standard deviation (SD) (B).

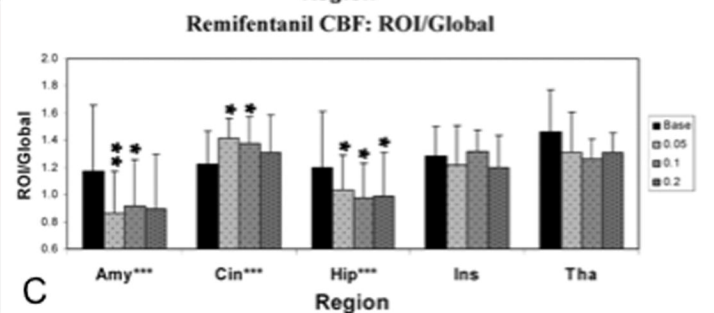

120
0
ml/100g
/min
A

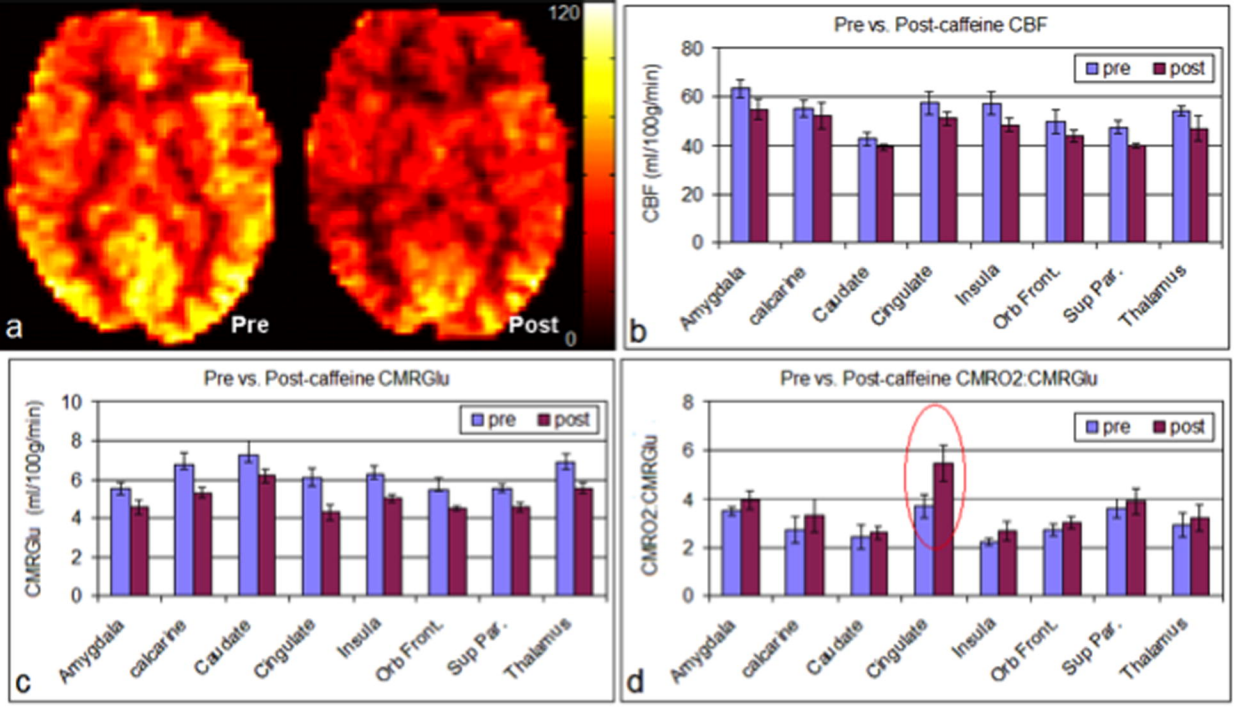

Retest CBF (ml/100g/min)
80
60
40
20
20
40
60
80
ICC=0.62
Test CBF (ml/100g/min)
B


pCASL CBF (ml/100g/min)
80
60
40
20
y=1.08x - 9.21
ICC=0.77
20
40
60
80
PC CBF (ml/100g/min)
C


0
0.05
0.1
0.2
(Remi dose ug/kg/min)
A


Remifentanil CBF
ml/100gm/min
Base
0.05
0.1
0.2
Glo***
Amy***
Cin***
Hip***
Ins*
Tha
Region
B


Remifentanil CBF: ROI/Global
ROI/Global
Base
0.05
0.1
0.2
Amy***
Cin***
Hip***
Ins
Tha
Region
C


120
0
a
Pre
Post
b
Pre vs. Post-caffeine CBF
CBF (ml/100g/min)
pre
post
Amygdala
calcarine
Caudate
Cingulate
Insula
Orb Front.
Sup Par.
Thalamus
c
Pre vs. Post-caffeine CMRGlu
CMRGlu (ml/100g/min)
d
Pre vs. Post-caffeine CMRO2:CMRGlu
CMRO2:CMRGlu