CBV fMRI in conscious animals using USPIO: Development of a tool for measuring pharmacodynamic activity in drug development

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INTRODUCTION: Anesthesia effects on physiology have long been a concern in conducting fMRI. Anesthetic mechanisms remain poorly understood and may cause changes in cerebral metabolism, blood flow, and blood volume (CBV), which can obscure or alter the pharmacologic- or sensory- stimulated hemodynamic changes detected with fMRI. The complications of anesthesia in conducting these studies have prompted the development of techniques for fMRI in awake animals, requiring special MR restraint and coil systems designed to image without motion artifacts. The ability to assess drug-induced cerebral activity in awake animals creates an opportunity to investigate neurologic processes under true physiologic conditions, and provides more clinically relevant information during the drug development process. A combined restraint and coil system is evaluated for conducting fMRI measurements in conscious rats. To improve sensitivity, the blood pool contrast agent USPIO is assessed for monitoring pharmocologic-induced CBV changes. USPIO has successfully been used in fMRI protocols consisting of a block paradigm stimulation (1). However, in a single-event activation, such as that elicited by a pharmacologic stimulus, the washout effect of USPIO has to be carefully removed by post-processing in order to extract subtle changes due to pharmacological effects. For development of this technique in conscious animals, we benchmark CBV changes elicited by administration of the carbonic anhydrase inhibitor, acetazolamide (ACZ), and compare it to isoflurane-anesthetized rats.

METHODS: Scanning was conducted on a Bruker Biospec 7T/30 and all animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee. For conscious animal imaging, female Sprague Dawley (SD) rats (290-340g) were conditioned for one hour to the restraint apparatus (Insight Neuroimaging LLC) and exposed to recorded scanner noise for 2-3 consecutive days prior to the day of imaging (2). For isoflurane-anesthetized imaging, female SD rats (290-340g) received 2% isoflurane via nose cone. Body temperature was regulated with a warm water circulator.

Conscious imaging was conducted using an Insight Neuroimaging resonator with a receive surface coil. Cerebral blood volume (CBV) measurements were made using a GE EPI sequence with 6s/11ms TR/TE, 3cm FOV, 64x64 matrix, and 1.2mm slice thickness. USPIO contrast agent (15mpk Fe) was injected via tail vein 10 minutes into scanning, followed by 15mpk ACZ 20 minutes into scanning. Anatomic images were acquired with a 2D RARE sequence, 5s/14ms TR/TE, 256x256 matrix, Nechoes=8, NA=2, and 3cm FOV. Movement correction was performed with FSL/MCFLIRT. Relative CBV change was calculated by standardizing to the signal drop from pre-USPIO (S0) to post-USPIO (S1) using relCBV change = ln(Si/S1)/ln(S0/S1) (Si=signal at time ti, post-USPIO). All time curves were linearly interpolated into time segments of -10 to 20 minutes with respect to ACZ injection time (0.5 min steps). Signal recovery due to washout of USPIO was corrected by subtracting the signal from an offset mono-exponential decay function least-square fitted to the baseline period (between USPIO and ACZ injection) of the scan.

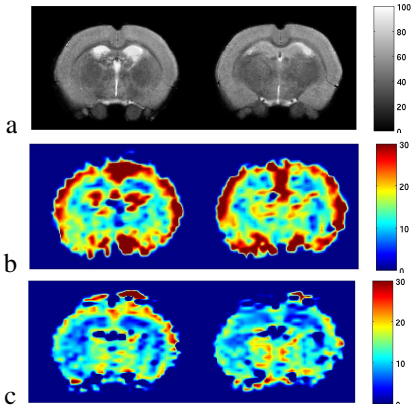
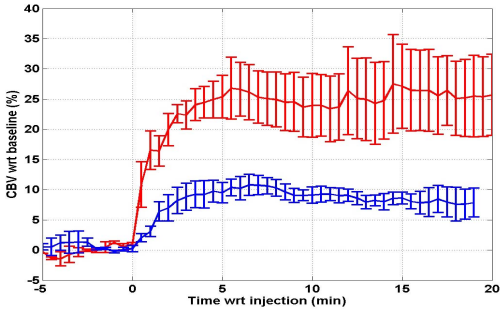


Figure 1. a) Anatomic RARE images acquired in conscious animal post-USPIO. b) CBV map in conscious animal following ACZ administration. c) CBV map in the same isoflurane-anesthetized animal following ACZ administration.



CBV % change from baseline

rat	Conscious	Isoflurane
1	30.40	11.17
2	33.12	7.17
3	12.71	6.48
mean	**25.41**	**8.27**
SE	6.40	1.46

Figure 2. Percent change from baseline for CBV following 15 mpk ACZ in n=3 conscious rats (red line) vs n=3 isoflurane-anesthetized rats (blue line).

RESULTS/DISCUSSION: Visual inspection of images and motion correction parameters revealed minimal motion during scans. Figure 1a shows anatomic RARE images in a conscious rat with no discernible motion artifact. Increased CBV following administration of ACZ was significantly ($p<0.05$) greater in conscious animals compared to isoflurane-anesthetized animals. Global CBV increased 15.7±4.1% and 6.5±1.4% from baseline in conscious and anesthetized rats respectively (figure 2). Figure 1b and 1c show CBV maps in the same rat after ACZ administration, with and without anesthesia, illustrating the higher CBV in the awake vs anesthetized state.

fMRI in conscious rats has been performed assessing brain activity in response to hypercapnia, sensory stimulation, and more recently ethanol (2,3,4), Previous fMRI studies on the affects of ACZ in anesthetized rats have been reported, but to our knowledge this is the first study to report ACZ-induced CBV changes in awake rats. We also demonstrate that a blood pool contrast agent such as USPIO can be used to monitor pharmacologically induced changes in CBV. Here CBV is increased using ACZ a known vasodilator shown to have no adverse effects on cerebral oxygen metabolism, neurovascular coupling or blood pressure, making it clinically useful to assess cerebrovascular reserve capacity in hemodynamically compromised patients(6). The greater CBV increase shown here in awake rats suggests a higher cerebrovascular reserve capacity in the awake vs anesthetized state. This may have important implications when using MRI to assess pathophysiology in some disease states, such as stroke, or to detect very small pharmacologic-induced hemodynamic changes in brain activity. The ACZ-induced CBV changes with isoflurane anesthesia in these experiments are consistent with ACZ-induced BOLD signal increases reported by Mukherjee et al in propofol-anesthetized rats. This group showed increases of 4.9-9.5% from baseline in various brain regions following 15mpk ACZ in rats under propofol anesthesia, which is structurally and mechanistically very different from isoflurane anesthesia .

The experiments described here demonstrate the ability to collect fMRI data with minimal motion artifact in awake rats. Improved sensitivity is achieved with USPIO-contrasted CBV fMRI and signal changes due to its washout have successfully been detrended. ACZ administration produces a greater CBV response in awake animals compared to anesthetized animals. Thus, conducting fMRI in conscious animals may allow for an increased dynamic range to detect small stimulus-induced changes in activity. Application of fMRI in fully conscious animals is not only feasible, but also provides a valuable tool to potentially characterize novel psycho-active therapeutics under true physiologic conditions. Most importantly, conducting such studies in the absence of anesthesia is directly translatable to the clinic.

References: 1. Zhao et al, Neuroimage, 30:1149-60, 2006; 2. King JA, et al, J Neurosci Methods, 148(2):154-60, 2005; 3. Luo F, et al, J Magn Reson Imaging, 26:557-63, 2007; 4. Lahti K, et al, Magn Reson Med, 41:412-16, 1999; 5. Sicard K, et al, J Cereb Blood Flow Metab, 23:472-81, 2003; 6. Martin C, et al, Euro J Neurosci, 24:2601-10, 2006; 7. Mukherjee B, et al, Mag Reson Imaging, 23:907-20, 2005.