Characterizing alcohol-induced changes in forebrain activity in awake rats using phMRI and assessing brain tissue alcohol pharmacokinetics using proton MR Spectroscopy

F. Luo[1], Z. Li[1], S. Treistman[1], C. F. Ferris[1]
[1]UMass, Worcester, MA, United States

Introduction.
Recently many of the technical problems associated with imaging of fully conscious animals have been resolved [1]. Studying brain function in awake animals makes it possible to follow activation of neural pathways in a variety of behavioral and neurological models ranging from sexual arousal in monkeys [2] to generalized absence seizures in rats [3]. While functional MRI in awake animals has also been used to study changes in brain activity caused by psychostimulants, there is no fMRI study on the acute and chronic effects of alcohol, though the *in vivo* pharmacokinetics of acute alcohol concentration can be monitored in real time with proton MR spectroscopy (MRS) [4]. It is known that individual differences in alcohol uptake kinetics might contribute to the development of pathological drinking behavioral. The goal of this present study is to determine EtOH-involved forebrain neurocircuitry in awake rats using high sensitivity contrast-enhanced fMRI-CBV method and simultaneously correlate EtOH-induced brain function alterations with brain tissue EtOH pharmacokinetics measured by proton MRS.

Materials and methods.
Behavioral acclimation: Prior to imaging, rats were acclimated to the restrainer and the imaging. Under 2-3% isoflurane anesthesia, rats were secured into the restrainer (Insight Neuroimaging LLC., Worcester, MA). When fully conscious, the restraining unit was placed into a black opaque tube "mock scanner" with a tape-recording of an MRI pulse sequence for 90 min in order to simulate the bore of the magnet and an imaging protocol. This procedure was repeated every other day for four days. *fMRI-CBV experiments*: five rats were used in CBV measurements. phMRI was performed on a Bruker Biospec 4.7T/40cm scanner with a 20-G/cm field gradient at the introduction of 10 mg/kg MION. A single-shot, gradient-echo EPI was acquired. The MR parameters were: FOV=2.56 cm, slice thickness=1.5 mm, image matrix=64 x 64, giving an in-plane image resolution of 400 x 400 µm, TR=2 sec, and TE=18.7 ms. Tail vein was cannulated for delivery of contrast agent and EtOH. *Drug challenges:* a threshold does of EtOH (0.75 g/kg) to disrupt behavioral tests of attentional processing and emotional reactivity in rats was chosen in the present study. EtOH was introduced 2 min into a 15 min scan. *Data analysis:* After linear detrend and motion correction using AFNI, CBV time courses were calculated based on pre- and post-MION baseline signal intensities. A differential exponential (Diff-Exp) model fitting analysis was performed based on CBV time courses. Significant activation voxels ($p<0.05$ after Bonferroni correction) from four forebrain coronal slices (interaural 11.2 -6.7 mm, far from susceptibility artifacts contamination) were chosen for characterizing brain responses to EtOH infusion. Four parameters: 1) percent area under the curve (AUC%); 2) the maximum signal change (peak-to-peak S%); 3) absorption rate (α_2), and 4) elimination rate (α_1) in cortex and striatum were employed to characterize acute EtOH-induced brain function changes. *Proton MRS:* three rats were employed in EtOH pharmacokinetics assessments under low dose isoflurane (1.0-1.2%) anesthesia with self respiration. First- and second-order shims were adjusted using FASTMAP auto-shim method. In vivo localized proton spectra were acquired from a ROI (3×3×3 mm) located on rat forebrain cortex using PRESS (TR/TE: 1500/15.5 msec) with time resolution of 2 min (64 average). The water resonance was suppressed with VAPOR. Proton spectra under normal control condition, saline infusion, 5-min post ETOH infusion, and 15-min post ETOH infusion were acquired sequentially. NMR spectra were processed using Bruker XwinNMR program. A 6 Hz Gaussian broadening was used before Fourier transformation in the chemical shift dimension. Automatic baseline subtraction was used for baseline correction of the 1D spectrum. Each spectrum was further referenced to the (NAA) peak at 2.02 ppm to compensate for small temperature variations during the exams. The most three reliable peak frequencies were identified (NAA, Cr, Cho) in addition to EtOH peak after EtOH i.v. infusion. The ratio of EtOH/NAA was calculated 5 min and 15 min after ETOH administration as NAA was selected as an internal reference for brain EtOH concentration.

Results.
Specific brain regions were activated (Fig. 1), mainly involved in dopaminergic mesolimbic pathways, including prefrontal cortex, cingulate cortex, caudate putamen, nucleus accumbens, amygdala, and sensorymotor cortex. No significant changes in CBV were detected in hippocampus, lateral parts of thalamus and hypothalamus. A uniform kinetics in CBV time course (Fig. 2, Fig. 4) was found in all regions investigated exhibiting a fast absorption rate and a slower elimination rate by Diff-Exp model fitting. The global elimination half-life of EtOH-induced CBV responses is about 2 min (estimated from elimination rate) (Fig. 3). An EtOH peak was robust and reliably measured by proton MRS reflecting a mM EtOH accumulation after EtOH infusion (Fig. 5). No significant elimination of brain EtOH concentration was found at 5 min and 15 min after EtOH administration with an EtOH/NAA ratio of 1.4 ± 0.5 and 1.3 ± 0.5, respectively.

Fig. 1

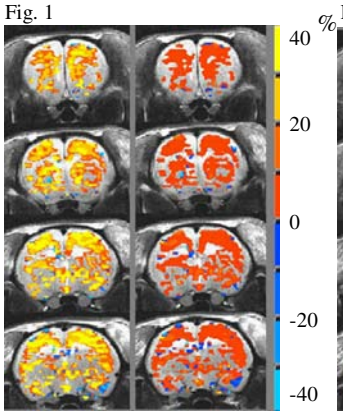


Fig. 2

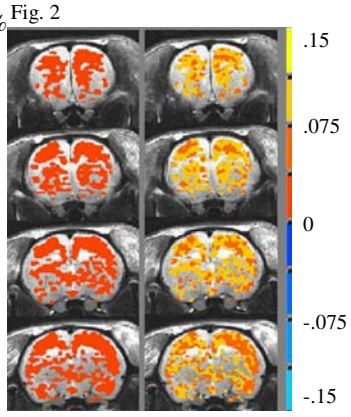


Fig. 3

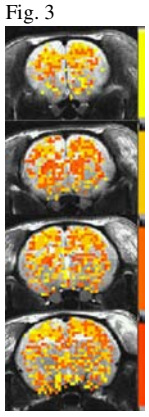


Fig. 4

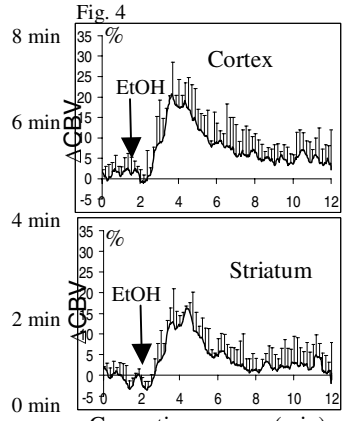
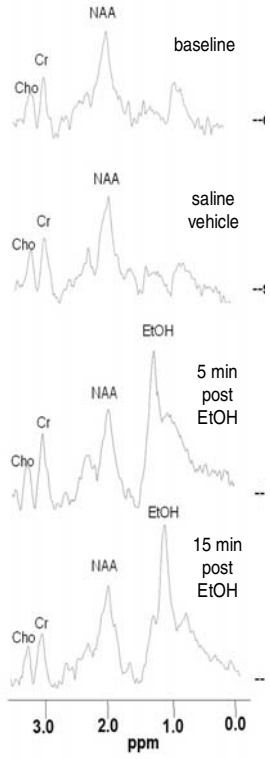


Fig. 5



Discussion.
This study demonstrates that it is feasible to use fMRI-CBV method to localize EtOH induced brain actions in the fully conscious animals, since CBV-weighted fMRI significantly improves functional sensitivity relative to BOLD, though it suffers more from susceptibility artifacts in the posterior part of the rat brain and makes it very hard to detect signals from ventral tegmental area and cerebellum. Despite the consistent findings of EtOH-induced increases in cerebral perfusion, a striking mismatch of elimination rate was found between EtOH-induced CBV changes and sustained brain EtOH concentration. Our results are corroborated by Lyons' reports that detectable perfusion increase occurred only at 5 min postinjection (i.p.) of EtOH but 15 min despite sustained blood alcohol levels at later time points [5]. This finding also correlates with changes in behavioral and subjective experiences after a single drug exposure with the underlying mechanism of acute tolerance. In summary, the present study identified discrete changes in CBV in fully conscious rat associated with EtOH administration. Discrepancies in brain tissue EtOH kinetics suggest that acute tolerance plays a role in the action of a single does of EtOH within the CNS. Such complex pharmacodynamics and pharmacokinetics induced neuroadaptation can only be understood by combing information from evaluation of brain function by phMRI with assessment of brain tissue EtOH kinetics by MRS.

References. [1] King *et al*., Neuroimage 2005 [2] Ferris et al., JMRI 2004 [3] Tenney *et al*., Epilepsia 2003 [4] Pfefferbaum, *et al.*, ISMRM 2004 [5] Lyons *et al*., Alcohol 1998