Confounding effects of volatile anesthesia on CBV assessment in rodent forebrain following acute alcohol challenge: a study of isoflurane and halothane anesthesia

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Introduction. Currently most applications of pharmacological MRI (phMRI) studies in animals are done under generalized anesthesia to help improve image quality by reducing motion artifact and stress, though many technical problems in awake animal studies have been overcome [1]. While the anesthetized nervous system differs from the conscious in its baseline metabolism, sensitivity to stimuli and functional integration, it has proven useful in some fMRI studies focused on the pharmacology of psychostimulants. For example, anesthetized and conscious rats show a similar activation pattern in the dopaminergic mesocorticolimbic system in response to cocaine. While there are differences, there are also enough similarities between the anesthetized and conscious preparations to support the use of either experimental approach depending upon the question being asked. In contrast, drugs that stimulate the nicotinic cholinergic system produce a dose-dependent change in fMRI signal in discrete brain areas in conscious rats that is obscured by the use of anesthesia [2]. Disparate fMRI results were also reported when comparing dopamine receptor activation between anesthetized and conscious rhesus monkeys challenged with apomorphine [3]. The purpose of this study was to characterize the responsiveness of the anesthetized and conscious animal to an acute dose of EtOH.

Materials and methods. *Behavioral acclimation:* Prior to imaging, rats were acclimated to the restrainer and imaging protocol. 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*: relative CBV measurements were 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 using Insight Neuroimaging coil systems with a small animal restrainer. 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. Fifteen rats were assigned to fMRI-CBV experiments under isoflurane (n=5), halothane (n=5) anesthesia, and fully conscious conditions (n=5) following an acute dose of EtOH challenge. With low concentration of isoflurane (1.0-1.2%), animals maintained normal respiration without the aid of artificial ventilation [4]. Comparable minimum alveolar concentration (MAC) halothane (0.8%) which preserved dopamine function was employed as the second volatile anesthesia slightly enriched with oxygen (33%) in air to maintain normal physiological status [5]. Body temperature was maintained at 37 ^{o}C with a water circulated heat pad during two volatile anesthesia preparations. The right femoral vein was cannulated in isoflurane anesthetized rats for i.v. delivery of contrast agent and EtOH. Tail vein was cannulated for delivery of contrast and EtOH under halothane anesthesia and awake preparations. *Drug challenges:* 0.75 g/kg EtOH was employed in the present study as a threshold does to disrupt behavioral tests of attentional processing, emotional reactivity in rats [6]. Drug was introduced 2 min into a 15 min fMRI-CBV experiments. *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 two forebrain coronal slices (interaural 11.2 -9.7 mm) (attention-related areas) 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 compare acute EtOH-induced MRI signals between the conscious and anesthetized groups. In order to check the system stability, SNR and CNR in cortex were measured under three different preparations. *Physiological studies:* Another nine rats were allocated into bench studies to collect physiological parameters for different conditions.

Results.

No significant differences of mean arterial blood pressure, heart rate, respiration rate, PCO_2, SO_2%, and pH were found before and 15-min after EtOH administration within each group or between groups, except PO_2 was significant higher in the halothane preparation whereas 33% oxygen was introduced. No significant SNR and CNR differences were found among three conditions. Two different brain activation patterns in terms of AUC% changes was found between two volatile anesthesia preparations and fully conscious condition (Fig.1, Fig. 2). Isoflurane and halothane significantly suppressed brain activation in cortex more than that of striatum, while an equivalent CBV increases were found under fully conscious condition after acute EtOH challenge (Fig. 2). At comparable MAC, halothane anesthesia produced a significant higher hemodynamic response (AUC%) following EtOH administration in striatum than that of under isoflurane anesthesia and awake conditions (Fig. 3). A significantly slower absorption rate was found in forebrain cortex and striatum under halothane anesthesia than that of isoflurane anesthesia and awake conditions (Fig. 3).

Fig 1

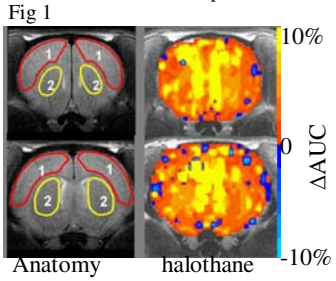
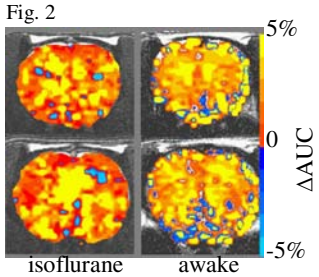
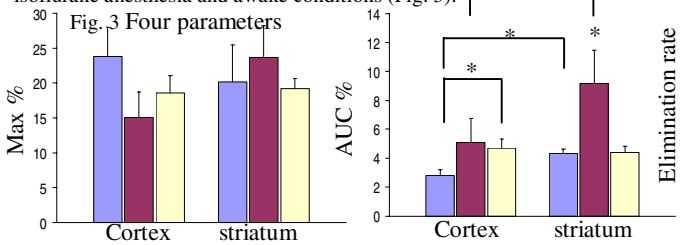


Fig. 2



Composite functional images (n=5, without threshold) in each group overlaid on corresponding anatomical images.

Fig. 3 Four parameters



Discussion. Several fMRI studies have reported that use of anesthesia blunts the functional response to different physiological stimuli as compared to data obtained from fully conscious animals. This is not unexpected since general anesthetics suppress neuronal activity and perturb hemodynamic coupling. In addition, commonly used anesthetics for electrophysiology, such as chloral hydrate and urethane, have been found to block cocaine-induced immediate early gene expression [7]. Consequently, it is uncertain whether the data collected on drug-induced changes in brain activity in anesthetized animals is physiologically relevant and interpretable in the context of understanding brain function from human imaging studies. In the present acute EtOH phMRI studies, two widely used volatile anesthetics in clinics with equivocal MAC were employed to examine how anesthesia affects phMRI signals. Halothane as a potent vessel dilator significantly enhanced EtOH-induced hemodynamic responses. In addition, isoflurane and halothane produced a heterogeneous perfusion background with region specific suppression in cortex while augmenting CBV/CBF in striatum. This observation was consistent with reports of cerebral perfusion changes using arterial spin-labeling techniques under isoflurane anesthesia [8]. Although the mechanism of how volatile anesthetics produce a heterogeneous perfusion background is unclear, isofurane induces widespread inhibition of sodium channel-dependent glutamate release in cortex [9] and might explain the cortical suppression. Nevertheless, the difference between the anesthetized and conscious condition in response to EtOH underscores the need to pursue future imaging studies in fully conscious animals.

References. [1] King *et al.*, Neuroimage 2005 [2] Skoubis et al., Neurosci 2005 [3] Zhang *et al.*, Brain Res 2000 [4] Liu, *et al.*, MRM 2004 [5] Schwarz *et al.*, Neuroimag 2004 [6] Jones, *et al.*, Psychopharmacology 2000 [7] Kreuter, *et al.,* Neurosci 2004 [8] Hendrich, *et al.,* MRM 2001 [9] Lingamaneni, *et al.*, Anesthesiology 2001