Spatio-temporal and dose characteristics of fMRI-BOLD signal to anesthesia in rats

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Introduction:
Most animal experiments currently used in fMRI use some type of anesthesia to immobilize the animals prior to using neurovascular perturbation (stimulus) to study changes in blood flow/BOLD/CBV signal changes in the brain. Standard statistical techniques including cross-correlation, t-test, F-test are then used between the rest (control) condition and the stimulus condition to determine regional and temporal extent of signal changes. The underlying hypothesis is the effect of anesthesia is uniform throughout the brain and does not change significantly over time. However, brain activation studies may be confounded by type of anesthesia used [1]. Anesthesia can influence baseline physiology regionally or globally and also effect the outcome of functional stimulation [1] or physiological perturbations [2] in different ways depending on their short and long term effects. In this study, the spatio-temporal characteristics of the BOLD signal were measured after application of gaseous anesthesia (Halothane or Isoflurane) at different doses above the minimal dose required for sedation. Greater levels of gaseous anesthesia were presented in a block design type of stimulus as is typically used in fMRI for stimulus presentation and for prolonged durations in separate studies.

Methods:
Male Sprague Dawley rats 250-300 g (n=16) was used for all studies and separated into two groups. Eight rats were anesthetized with Halothane and eight rats with Isoflurane. The body temperature was controlled by placing the rat on a warming pad at 38°C (Baxter K-MOD100, Gaymar Industries). The femoral arteries were cannulated with PE50 tubing for mean arterial blood pressure (MAP) measurements and blood gas sampling. Arterial blood pressure, end-tidal CO_2 and inspired oxygen concentration were continuously monitored (POET II, Criticare Systems). fMRI experiments were performed using a 9.4T/21cm horizontal bore (Magnex Scientific) using a Bruker Advance console and custom made surface-RF coil. In order to minimize motion artifacts, the rat was secured to the RF coil by a bite bar resting below the upper hard palate and over the snout along with a face-mask for the delivery of anesthetic gas. Coronal localization of slices was accomplished using an initially obtained mid-line sagittal slice and comparing it with the sagittal section from a stereotaxic rat brain atlas. All catheters for mean arterial pressure (MAP) measurement and injection and ventilation tubes was brought outside the magnet room of the MR scanner. Anatomical images were obtained before fMRI scanning using a RARE sequence with TR=1sec, TE=19msec, 256x256 matrix and FOV=3.0 cm. For fMRI-BOLD measurements, a single shot gradient EPI sequence was used to acquire multiple slices of images using a 128x128 matrix, TR/TE=2sec/15msec, FOV=3.0 cm, slice thickness=1mm. The resulting image had a spatial resolution of 0.24x 0.24 x1 mm^3.

Baseline fMRI-BOLD signals were measured at different doses of Halothane and Isoflurane sequentially from an initial level of 1% to 1.8% and 2.7% in oxygen for a prolonged period of time (30 min). Short term exposure (5 min) to higher doses of anesthesia was performed in separate group of animals.

A differential exponential model was used to characterize the anesthetic effects including onset time, absorption rate, elimination rate, etc. The difference in exponential model was processed on a voxel by voxel basis for all voxels in the imaged brain.

Figure 1

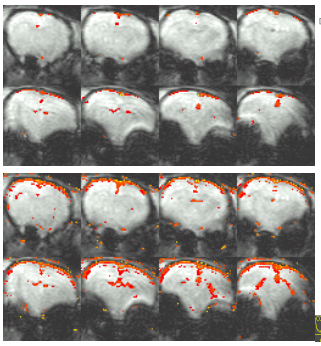
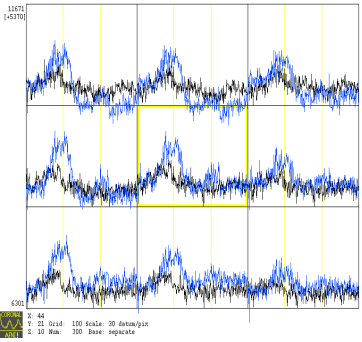


Figure 2



Results
In all rats an initial concentration of 1% halothane and 1.2% Isoflurane was maintained. All dose dependent effects were measured from the initial level of halothane anesthesia of 1%. During the infusion of higher concentration of Halothane, a dose dependent increase in the fMRI-BOLD signal was observed. The increase in BOLD signal after induction of the respective dose of anesthesia was fitted to a difference of exponential model using least squares fit. Signal parameters such as onset time t_o, time for maximum t_{max}, signal amplitude change in %, area under the curve, absorption rate and elimination rate were estimated from the model on a voxel-wise basis. Fig 1 (top) and (bottom) show the spatial extent of fMRI-BOLD signal increase in the rat brain anesthetized with 1.8% and 2.7% halothane respectively. At both doses significant BOLD signal change was observed throughout the cortex and other deeper brain structures. The anesthetic concentration was applied transiently for 5 min in this experiment.

Fig 2 shows the BOLD signal dynamics from a 3x3 voxel region of interest from the somatosensory cortex region in the rat. BOLD signal increase was observed in a dose dependent manner during inhalation of 1.8% (black) and 2.7% (blue) halothane respectively. No significant change in the onset time was observed for the two dose levels studied, however a significant increase in the amplitude of the response and area under the curve was observed with different dose. Considering the 1% halothane condition as the initial baseline signal, transient infusion of 1.8% and 2.7% halothane for 5 minutes each resulted in a peak (mean) BOLD signal increase of 56% and 90% respectively. MAP decreased significantly during inhalation of 1.8% or 2.7% halothane from the initial baseline level of 1%, however no significant change was observed in the heart rate.

In a different experimental protocol, anesthesia was maintained for a prolonged period (30 min) at a certain dose level in a different animal and the baseline BOLD signals were measured. Fig 3 shows the BOLD signal dynamics at three different concentrations of halothane namely, 1.8%, 2.7% and 3.2% respectively from the same region of interest in the somatosensory cortex as shown in fig 2 (each dose demarcated by a vertical line in the figure). It was observed that pixels from one cortical layer showed an increase in BOLD signal to increasing anesthetic concentration whereas an adjacent cortical layer below showed a decrease in BOLD signal. No such responses were observed during the transient infusion of anesthesia at different doses. Similar response was observed with isoflurane anesthesia at different doses.

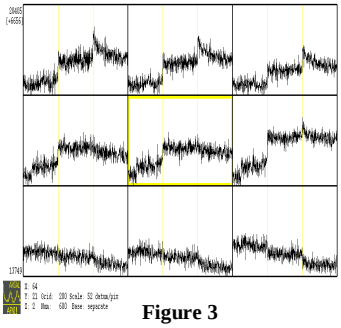


Figure 3

Discussion These results demonstrate at high spatial and temporal resolution that anesthetic effect can vary in different anatomical regions and the extent of the response can also vary. An interesting observation was that the adjacent pixels in the cortex presumably belonging to different cortical layers show opposite BOLD signal change at different anesthetic dose. Our results indicate that such a phenomena could be an effect of prolonged exposure to specific anesthetic dose.

References

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2. Kannurpatti SS, Biswal BB. Effect of anesthesia on CBF, MAP and fMRI-BOLD signal in response to apnea. Brain Res. 2004, 1011: 141-147.