

# Increased BOLD signal in conscious rats during hypercapnia

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**Abstract:**

Functional MRI studies in animals are often conducted under anesthesia to minimize motion related artifacts that aren't present in human fMRI studies. While awake animal fMRI studies are still uncommon, some BOLD studies in awake animals have indicated a greater vascular response to evoked activity leading to a more robust change in signal. In this study, hypercapnia also produced a larger and faster increase in BOLD signal in awake animals than in animals under sedation. Awake animals also had increased contrast to noise ratios and differences in the response of subcortical regions to hypercapnia.

**Introduction:**

BOLD studies have been conducted in awake rats, rabbits, and monkeys in order to study cognitive processes that would otherwise be eliminated under anesthetized. Eliminating anesthesia also shown to increase BOLD signal. While the sequences utilized in fMRI studies are sensitive to motion, proper immobilization of the animal will greatly reduce the impact of motion artifacts. Hypercapnia is a well established and simple method of manipulating cerebral blood flow and BOLD signal. While stress might alter restrained animals in the imaging environment, pilot data showed physiologic parameter such as respiration rate remain normal with respect to awake, unrestrained conditions.

**Methods:**

Eight Male SD rats were anesthetized with IM Domitor (Pfizer) and Ketamine. The animals were placed into a rodent restraint/RF coil system (Insight Neuroimaging Systems) with a facemask and femoral artery catheter. Anesthesia was reversed with antisedan (Pfizer). Animals were monitored for pulse oximetry, capnography and respiration rate for the entire duration of the experiment and blood was drawn for analysis at intervals during the experiment.

Experiments were conducted in a Bruker 4.7T system. Anatomical, fast spin echo images were acquired with the following parameters; TR = 3s, TE = 48 msec, echo train = 8, 8 NEX, 256x256 matrix, 2.7x 2.7 cm FOV, 2 slices, 1.5 mm thick. BOLD fMRI images were acquired with a two-segment, gradient-EPI repeated 100 times for each trial with; TR = 1s, TE = 25msec, 128x128 matrix, 2.7x 2.7 cm FOV, 2 slices, 1.5 mm slice thickness. The first 50 repetitions, the rats would inhale normal air. For the next 50 repetitions, the air was premixed with 5 or 10 percent CO<sub>2</sub> (Airgas). At the end of the 100 repetitions, the CO<sub>2</sub> was turned off and the animals were given 10 minutes to recover. After 3 repetitions animals were anesthetized with 1% isoflurane, and several more scans were acquired

**Results:**

The increased CO<sub>2</sub> lead to a higher pCO<sub>2</sub>, increased PaO<sub>2</sub>, decreased pH, and increased ventilation in all rats. The awake rats increased ventilation by increasing respiratory force while maintaining a constant rate, while anesthetized rats increased their respiration rate, but not ventilatory force. The heart rate decreased in both awake and anesthetized animals during hypercapnic conditions.

All animals showed significant BOLD signal increase in response to CO<sub>2</sub> inhalation. The averaged, normalized time courses in figure 1 shows a marked and rapid increase in BOLD signal in the awake groups compared to the anesthetized animals (arrow indicates CO<sub>2</sub> inhalation). The average BOLD signal risetime, percent change, and contrast to noise values for each cohort can be found in table 1. The signal increased in awake animals as quickly as it did in the anesthetized group. BOLD signal increased twice as much in awake animals 5% CO<sub>2</sub> when compared to anesthetized animals, and nearly 3 times as much during 10% CO<sub>2</sub> challenge. The contrast to noise

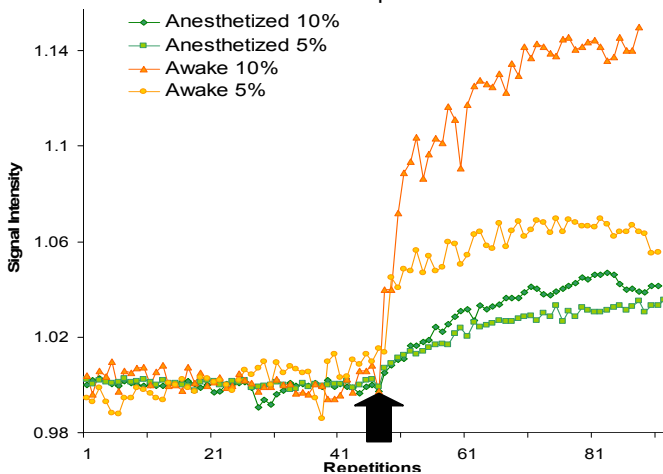


Figure 1. BOLD timecourse during hypercapnia.

ratio in the awake animals was markedly greater at 10% CO<sub>2</sub>, and similar at 5% CO<sub>2</sub>, to that of the anesthetized animals. The subcortical regions of anesthetized animals showed no difference in change in BOLD signal whether given 5 or 10% CO<sub>2</sub>, while this increase in CO<sub>2</sub> produced a marked increase in BOLD signal in the awake cohort.

	5% CO <sub>2</sub>				10% CO <sub>2</sub>			
	Cortical		Subcortical		Cortical		Subcortical	
	Awake	Anesthetized	Awake	Anesthetized	Awake	Anesthetized	Awake	Anesthetized
Risetime(s)	18.8 (13.6)	60 (28)**			28 (13.6)	64 (30)**		
% change	6.7 (2.1)	3.2 (2)**	4.2 (2.2)	2.7 (1.4)*	13.3 (5.4)	4.9(3.5)**	9.1 (2)	2.4 (2)**
CNR	6.3 (4.8)	7.3 (4)	3 (2.8)	6.4 (3.6)*	13.1 (6.2)	8.0 (4.9)*	10.8 (6.9)	5.3 (4.1)*

Table 1: Overview of BOLD signal changes in awake and anesthetized animals. p-value \*<.05, \*\*<.005.

**Conclusions:**

Awake animals had greater BOLD signal change in response to hypercapnia when compared to animals anesthetized with isoflurane. This greater signal lead to increased signal contrast despite a slightly higher baseline signal noise in awake animals. There was no detectable effects of restraint stress on the physiology, or baseline BOLD signal in the awake animals. A lack of differentiation in the vasoreactivity of anesthetized animals implies that anesthesia might alter autoregulation.