Propofol Infusion with Physiological Monitoring Improves Success for BOLD fMRI in Rats

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Introduction:

Controlled anesthesia in rats combined with a robust physiological monitoring protocol can improve success rates for BOLD fMRI and help explore perception under differing levels of awareness. Most previous fMRI studies in rats involved the use of bolus injections of the drug alpha-chloralose.[1, 2] This anesthetic is not used clinically nor has the ability to control the level of anesthesia. Also alpha-chloralose disrupts homeostasis, and is inappropriate for survival studies for reasons described elsewhere.[3] Propofol is an anesthetic that gained clinical acceptance in the last fifteen years and has many advantages. First, the drug is a true anesthetic and is widely used clinically in both animals and humans. Second, Propofol has a very short half-life and a fast clearance time allowing for controlled infusion and differing depths of anesthesia. Finally, the drug has few adverse effects including low toxicity, little effect on physiological parameters such as blood pressure, and extremely fast wake up after infusion removal.

Recently the developments of MRI compatible infusion pumps have allowed the pump to be pushed up to the bore of the magnet and have eliminated the need for long tubing to deliver i.v. drugs. This has provided better computerized control and the elimination of concentration gradients when using lengthy tubing. Also MRI compatible physiological monitors for animals have become available commercially. By monitoring such parameters as pO_2, in the tissue and expired CO_2, we can protect the animal from such things as hypercapnia, which can attenuate the BOLD signal. Improving monitoring protocols is also more humane and can extend the length of time an animal can be used for an fMRI protocol, reducing the number of animals needed for a given experiment. In this study we describe a procedure for using infused Propofol combined with a strong physiological examination to describe the fMRI BOLD signal at two different depths of anesthesia.

Materials and Methods:

Three male Sprague-Dawley Rats (275-350g) from Charles River Laboratories (Wilmington, MA) were anesthetized with induction of 4% isoflurane mixed with air. The animals were maintained with 1.5% isoflurane and the right femoral vein and artery were surgically canulated. A ventilation tube was inserted surgically into the trachea, the isoflurane discontinued, and the animal was switched to 0.8 mg/kg/min Propofol infused through the femoral artery using an MRI compatible infusion pump. (Harvard Apparatus / Model PHD 22/2000 Remote) All incisions were stitched closed and covered with lidocaine to minimize pain. The animals were then brought to the Bruker 3T Biospec 30/60 scanner. The surgery was continually optimized to minimize surgical time.

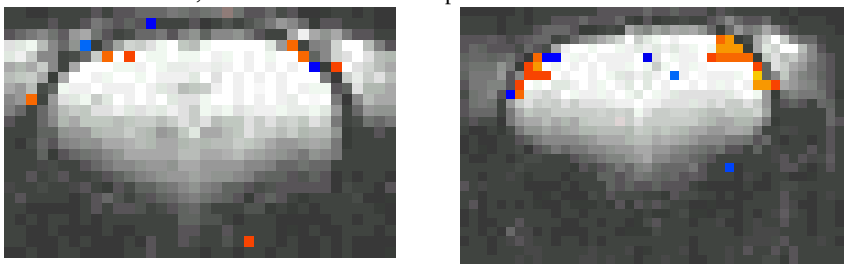
The animals were loaded into a whisker barrel stimulator/coil apparatus described elsewhere.[4] Temperature was maintained at 37.5°C through the use of a rectal thermometer connected to an MRI compatible circulator and warming blanket. (Kent Scientific / Model TP500) The animals were maintained through the use of a ventilator (Harvard Apparatus / Model 683) using a mixture of 70% air and 30% oxygen, a 3cc volume, and a rate of 60 bpm. All inspired and expired gases were monitored by the use of a POET IQ2 Agent Gas Monitor (Criticare Systems / Model 8500Q) set to the neonatal parameters. Expired CO_2 was kept below 25-35mmHg and oxygen consumption was maintained at 3-5% by adjusting the ventilation accordingly. Gallamine (80mg) was given hourly to prevent the animal from fighting the vent and causing motion interference. The rat's pulse and pO_2 was observed using an MRI compatible pulse oximeter. (Kent Scientific / Model OX8600MV) The pO_2 was maintained between 92-96% and the pulse between 350-450 bpm depending on the depth of anesthesia. Blood pressure was measured invasively (Kent Scientific / Model DBP1001) and systolic pressure was maintained between 120-160mmHg. All monitoring probes were run through a filter to prevent inference with the MRI signal.

The rats were imaged using a standard EPI sequence with a TE=27.2ms, TR=2000ms, FOV=3.5cm, 2mm slice thickness, and a matrix of 64X64. The rats were stimulated using a simple block design with five off and four on periods and 12Hz stimulation frequency.[4] Three repeat sequences were performed and then three sequences using a 6Hz stimulation frequency. The infusion was then lowered to 0.4mg/kg/min and the animal was allowed to equilibrate for twenty minutes. The series of six sequences were repeated. The animals were then sacrificed with an overdose of Sodium Pentobarbital.

Results & Discussion:

A differential response in the BOLD signal was observed between the higher 0.8 mg/kg/min titration and the lower 0.4 mg/kg/min titration in both the same animal and across multiple animals. Figure 1 shows a statistical parametric map displaying activated voxels using an F-t test with a p-value threshold of 0.001 for activated voxels under both conditions with 12Hz stimulation frequency. The percent signal changes of the activated voxels in the whisker barrel region under the 0.4 mg/kg/min were on the order of 2%, which is consistent with previous studies on the whisker barrel cortex using alpha-chloralose anesthesia.[4]

By combining infused Propofol with a strong physiological monitoring regime, repeatable differential responses where observed under different steady state depths of anesthesia. Success relies upon maintaining the rat's homeostasis especially by controlling expired CO_2 levels. This protocol improves upon earlier work by utilizing a better, controllable, and more clinically relevant anesthetic. The results were in line with earlier work performed in the whisker barrel system. Further work needs to be done with other drugs to further characterize BOLD response under lightly anesthetized conditions.



A **B**

Figure 1 Statistical parametric maps showing activated voxels under deeper A.) 0.8 mg/kg/min and lighter B.) 0.4 mg/kg/min Propofol anesthesia.

References: 1.) Van der Linden A et al. MRI 2001;19:821-26. 2.) Shulman R et al. PNAS 2002;99:10771-76 3.) Heerschap A et al. Proc. Intl. Mag. Reson. Med. 2002;10:393 4.) Lu H et al. MRM 2003;50:1215-22