# Comparison of six different anesthesia protocols for phMRI

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## Introduction

Pharmacological MRI (phMRI) is a modern imaging method providing indirect information of the actions of pharmaceuticals in tissue exploiting functional MRI (fMRI). The influence of anesthesia on brain activation and blood oxygenation level dependent (BOLD) signal changes is one of the most critical factors in the experimental design. The aim of this study was to investigate the BOLD signal changes by using different anesthesia protocols with a nicotine challenge in order to find optimal experimental conditions for a phMRI study.

## Materials and methods

All animal procedures were approved by the Animal Ethics Committee of the Provincial Government of Southern Finland. Altogether 39 adult male Wistar rats (357 ± 38 g) were used to study nicotine induced BOLD changes in six groups with four different anesthetics (medetomidine 0.1 mg/kg/h i.v., thiobutabarbital 140 mg/kg i.p., urethane 1.25 g/kg i.p., and isoflurane 1.3 %). All rats were first anesthetized with isoflurane (5 % for induction and 2 % for maintenance during surgery) in $N_2/O_2$ 70/30 mixture for femoral arterial and venous cannulation. Tracheotomy was performed and pancuronium bromide (0.5 mg/kg/h i.v.) administered if ventilation (VNT) was used. After surgery anesthesia was switched to either medetomidine with VNT (n = 6), thiobutabarbital (n = 5), thiobutabarbital with VNT (n = 10), urethane (n = 6), urethane with VNT (n = 6), or isoflurane was continued with VNT (n = 6). MRI measurements were performed with 7 T Bruker PharmaScan. Functional data were acquired with single-shot SE-EPI (TR 2 s, TE 45 ms, 9 slices, 1.5 mm slice thickness, image matrix 64 x 64 and FOV 2.5 x 2.5 cm). A bolus of nicotine (hydrogen tartrate salt 0.25 mg/kg) was administered i.v. after 200 baseline images and scan was continued for 500 images. Temperature of animals was kept stable with warm water circulation. ECG and respiration were also monitored. Blood samples were taken before and after the measurement and the arterial blood gas values were within normal physiological range, except in the thiobutabarbital non-ventilated group where mean value for $pCO_2$ was 72.4 ± 5.7 mmHg. All data were analyzed in Matlab with Aedes (aedes.uku.fi) or with other in-house made Matlab code.

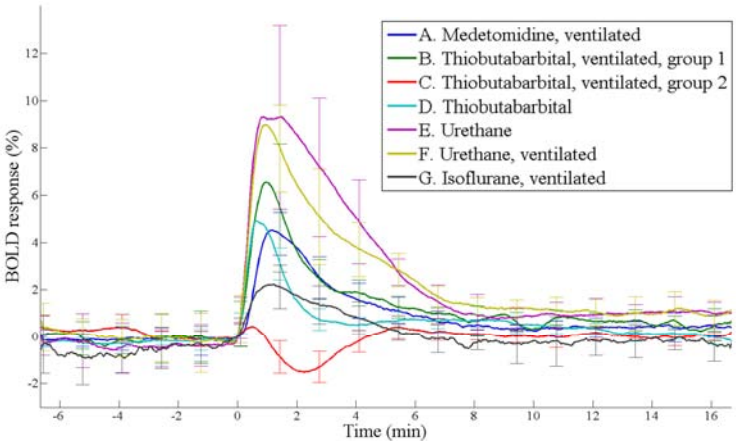


**Figure 1.** The mean BOLD responses from 175 voxels in the cortex. A bolus of nicotine (hydrogen tartrate salt 0.25 mg/kg) was injected at time zero.

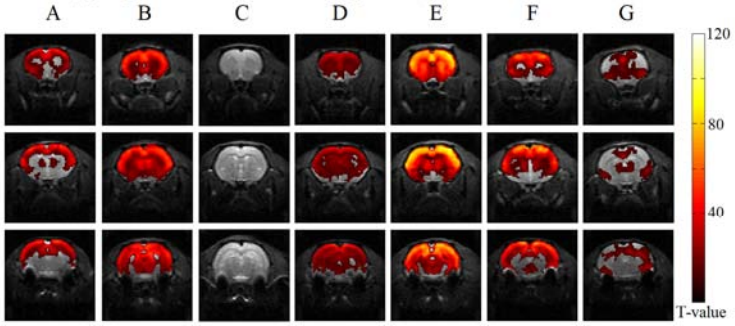


**Figure 2.** The statistical BOLD activation maps in three slices from single animals. Group letters correspond the marking in Figure 1.

## Results

Nicotine injection induced clear cortical signal changes in every rat. As Figure 1 shows, the choice of anesthetic leads to notably different BOLD responses. Observed BOLD responses were mainly positive, but in thiobutabarbital VNT group the measured rats showed two different BOLD time courses and were accordingly divided in two subgroups, group 1 (n=4) with positive (green line, Fig. 1) and group 2 (n=6) with positive and negative BOLD responses (red line, Fig. 1). Maximum BOLD changes for the groups are as follows: medetomidine VNT 4.5 ± 1.1 %, thiobutabarbital VNT first group 6.6 ± 2.5 %, thiobutabarbital VNT second group -1.5 ± 0.6 %, thiobutabarbital 4.9 ± 0.7 %, urethane 9.3 ± 2.3 %, urethane VNT 9.0 ± 1.9 %, and isoflurane VNT 2.2 ± 1.0 %. Maximum positive BOLD response was measured 58 ± 13 s after the nicotine injection. Statistical activation maps from single animals are represented in Figure 2. Saline injections did not produce activations in urethane anesthetized control animals (data not shown) and the BOLD response followed dose-dependency under urethane anesthesia (data not shown).

## Discussion

Nicotine induced BOLD changes are parallel with the known nicotinic acetylcholine receptor distribution [1]. To our knowledge this is the first time that thiobutabarbital anesthesia is used in fMRI measurements although it has been used in other rodent studies related to e.g. renal functions [2]. Rats anesthetized with thiobutabarbital had good BOLD responses, however, the spontaneously breathing animals suffered from hypercapnia. Additionally, six of the animals in thiobutabarbital VNT group produced a combination of small positive and negative BOLD responses. This might indicate that the level of thiobutabarbital anesthesia has to be carefully adjusted and maintained for brain activation studies. These results emphasize the need of carefully optimized anesthesia protocol in phMRI studies, for each pharmacological agent individually. Despite the fact that the exact mechanisms of action of anesthetics are still mostly unknown, the phMRI method with careful study design has great potential in brain mapping and characterization of drug effects.

## References

1. London et al., J Neurosci, 1988, 8:3920-8. 2. Walter et al., Q J Exp Physiol, 1989, 74:805-12.

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