Non-invasive control of physiological status by measurement of transcutaneous CO_2 for longitudinal fMRI studies in rats

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Introduction

BOLD based fMRI studies in the rat are usually invasive, hampering the performance of longitudinal experiments: close control of arterial pCO_2 levels is essential to grant a consistent BOLD contrast.[1] For this reason, a femoral vein has to be catheterized to withdraw blood samples, being necessary to sacrifice the animals after the experiment. For small animals, the alternative of measuring end-tidal CO_2 is difficult to perform, because of the large dead volume required for this technique. In the present study we explore a simple, fully MRI compatible[2] and non-invasive technique of monitoring transcutaneous CO_2 levels ($tcCO_2$), providing the experimental platform for longitudinal fMRI studies in the rat.

Materials and Methods

Wistar rats (250-300 g) were anesthetized with 1% halothane in O_2:N_2O (3:7) during preparation and surgery.

A) Bench experiments: femoral artery and vein of a group of animals (n=5) were catheterized for blood sample withdrawal and delivery of drugs. Animals were intubated and mechanically ventilated to influence paCO2 levels during the experiments. Finally, anesthesia was switched to α-chloralose (ICN, USA; bolus:infusion: 50:36 mg/kg/h, i.v.). Arterial pCO2 values were periodically measured using a blood gas analyzer (Ciba-Corning, Germany); in parallel $tcCO_2$ values were continuously recorded using a transcutaneous CO_2 system (TCM4, Radiometer Copenhagen, Denmark). Animals were sacrificed at the end of the experiment.

B) fMRI experiments: some animals (n=2) were also intubated and ventilated, but not catheterized. In this group anesthesia was switched to medetomidine (Domitor, Pfizer, Germany; 0.15-0.3 mg/kg/h i.p. or s.c.), and only tpCO2 was measured. Needle electrodes were implanted on both forepaws for electrical stimulation. Medetomidine effects were reversed using atipamezol (Antisedan, Pfizer, Germany; 0.1 mg/kg, i.p.), and animals were completely recovered after the fMRI sessions.

fMRI experiments were conducted on a 7T scanner (Bruker BioSpin, Germany) with a 20 cm gradient insert (200 mT/m) using a 12 cm Helmholtz coil for rf irradiation and a 3 cm surface coil for signal detection. After pilot scans, SE-EPI images were acquired (FOV=2.56x2.56 cm²; 64x64 points, giving a 400 µm in-plane resolution; TE=30 ms; BW=150 kHz; 1 slice of 2 mm located at -4.7 mm from the rhinal fissure). Functional activation imaging was achieved using BOLD contrast. Forepaw stimulation was performed using rectangular pulses (1mA, 3Hz, 0.3ms) following a [5x(15 OFF + 5 ON)]+15 OFF paradigm, acquiring in total 115 images in 5min 45s (TR=3s). Activation maps were constructed with STIMULATE (University of Minnesota, USA) using a t-test ($p<0.05$).

Results and discussion

Our bench experiments showed that it is difficult to establish a general quantitative relationship between $tcCO_2$ and $paCO_2$ values for rats. However, selecting a rather homogeneous group of animals (body weight, age), a perfect linear correlation between transcutaneous and arterial CO_2 values was found (figure 1a, r=0.89). The relationship must be recalibrated when varying the experimental conditions like the age and weight (fat under the skin) of the animals, anesthesia used (which influences in different manner the peripheral blood flow) or electrode positioning and working temperature. In our situation of a well defined group of animals, continuous $tcCO_2$ measurements were found very valuable for the performance of fMRI studies. Once the electrode reached a stable value after attachment to the skin under mild anesthesia (halothane), this value was used as a baseline to measure percentage of changes in $tcCO_2$. These values permitted to assess if the animal was under hypercapnic conditions, where recording of BOLD signal becomes very complicated or impossible due to the prior vasodilation. Our data indicate a threshold for $\Delta tcCO_2$ of approx. 120%, marking the conditions in which reliable and robust BOLD signal can be obtained in fMRI experiments in the rat (Figure 1b). The non-invasive assessment of $tcCO_2$, together with the use of injectable anesthetics (with fast and efficient reversibility) enable the stable and reproducible performance of longitudinal fMRI studies in the rat.

References

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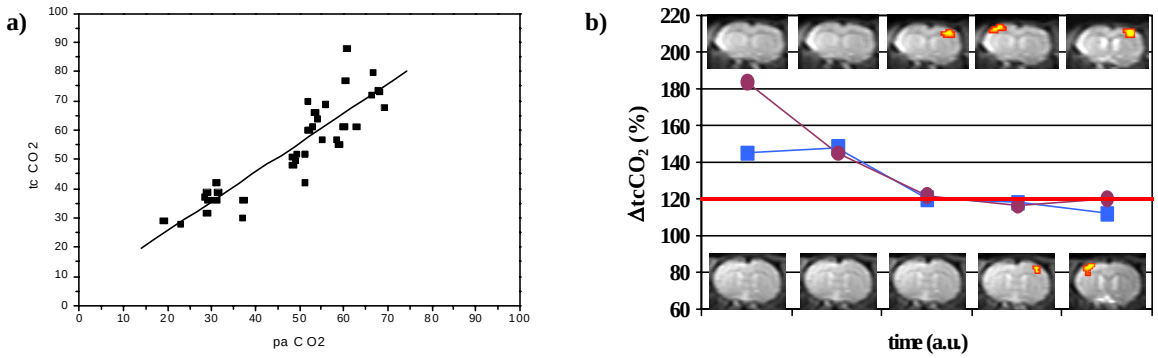

Figure 1. a) Plot of $tcCO_2$ vs. $paCO_2$ values for a homogeneous group of animals (r=0.89). **b)** $\Delta tcCO_2$ values (% respect to baseline) of two animals at different time points, together with the corresponding simultaneous fMRI experiments (top row fMRI: squares; lower row fMRI: circles). If $tcpCO_2$ values are above a certain threshold (~120% of baseline), no BOLD signal is observed.