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Original article

Monitoring the stress-level of rats with different types of anesthesia: A tail-artery cannulation protocol

Dávid Z. Balla [a,*], Saskia Schwarz [a], Hannes M. Wiesner [a], Anita M. Hennige [b], Rolf Pohmann [a]

[a] High-Field MR Center, Max-Planck-Institute for Biological Cybernetics, Spemannstr. 41, 72076 Tübingen, Germany
[b] Department of Internal Medicine, Eberhard Karls University, Otfried-Müller-Str. 10, 72076 Tübingen, Germany

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ABSTRACT

Introduction: Functional MRI in rats under anesthesia can largely minimize motion artifacts and attenuate the stress of the animal. However, two issues remain to be clarified and improved. First, fMRI results obtained with different types of anesthesia during surgical preparation and imaging show a large variability, which could be caused by the variable stress level of the rodents. Second, the most common surgical procedure used for anesthesia, blood gas analysis and mean arterial blood-pressure (MABP) monitoring is the femoral vein and artery catheterization that makes longitudinal studies difficult. **Methods:** In order to examine the variability of the stress level with three different anesthesia protocols using isoflurane (Iso), medetomidine–ketamine (MK) or propofol–remifentanil (PR), we measured the plasma corticosterone (CORT) concentration with ^{125}I-radioimmunoassay in blood samples collected prior to, immediately after and 60 min after surgery. Tail-artery and vein catheterization was adapted for long-term monitoring of MABP with periodic blood sampling and is proposed as a less invasive and technically simple alternative to femoral vessel catheterization in fMRI preparation protocols. **Results:** We show that the CORT concentration depends on the anesthesia protocol with both alternatives providing more efficient stress reduction than the protocol using Iso. However, only the protocol using PR achieved a significant hormone reduction during surgery. Stress was not reliably manifested in changes in heart-rate and breathing-rate. Anesthesia and strain related changes in these two physiological parameters may be assigned to the pharmacological effects of the premedication and anesthetic agents. The results indicate also that MABP can be monitored over a long period of time (e.g. functional imaging session) through an arterial access point in the rat tail after cannulation with the proposed procedure. **Discussion and conclusion:** Animals can experience stress during fMRI preparation protocols without obvious signs in commonly monitored physiological parameters. Our results challenge the efficiency of surgical protocols using Iso as mono-anesthetic agent, even when extended with topical analgesia. It was demonstrated that the CORT-based stress-level measurement through tail-artery cannulation can be used for developing anesthesia protocols (i.e. the presented PR protocol) when setting up future fMRI studies. The proposed surgical method for the tail is expected to facilitate longitudinal fMRI studies with permanent arterial access.

1. Introduction

The analysis of complex physiological processes in the brain of animal models, like stimulus responses in functional studies, relies on extensive monitoring of basic physiological parameters (Sanganahalli, Bailey, Herman, & Hyder, 2009; Yu et al., 2010). The mean arterial blood pressure (MABP) and the partial pressure of blood gases are sensitive indicators of physiological changes in the cardiovascular system. In functional magnetic resonance imaging (fMRI) studies, systemic changes in these parameters can have significant impact on the results (Silva & Stefanovic, 2008). Therefore, continuous monitoring of MABP and, in case of small animal experiments, periodic arterial blood gas analysis are part of the most fMRI protocols (Sanganahalli et al., 2009; Silva & Stefanovic, 2008; Yu et al., 2010, 2012).

For ethical and scientific reasons, anesthesia with loss of consciousness, at least during surgical preparation, and sufficient surgical and post-operative analgesia are pivotal for an efficient imaging protocol (Fish, Brown, Danneman, & Karas, 2008; Hildebrandt, Su, & Weber, 2008). Especially in an fMRI study, a reproducible baseline physiological state of the resting brain shortly after surgery is required. However, since homeostasis is a dynamic equilibrium with regulatory processes activated by various stressors, the physiological state of the brain can

* Corresponding author at: Max-Planck-Institute for Biological Cybernetics, Spemannstr. 41, 72076 Tübingen, Germany. Tel.: +49 7071 601724.
E-mail address: david.balla@tuebingen.mpg.de (D.Z. Balla).

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be affected by preceding procedures, like the handling of the animal before anesthesia or pain during or after surgery. Especially pain, which enhances the blood corticosterone (CORT) level and induces numerous physiological changes (e.g. in the cardiovascular system and brain metabolism), has direct impact on the physiological state even without any visible sign like muscle contraction, increased heart or breathing rate (Borsook & Becerra, 2011; Ferris & Stolberg, 2010; Fish et al., 2008). Since the half-life of free plasma CORT concentration is approximately 25 min (Sainio, Lehtola, & Roininen, 1988), trauma during surgery or insufficiently suppressed post-operative pain during fMRI can lead to continuously changing or constantly biased stress-hormone level during the entire experiment (Ferris & Stolberg, 2010). If functional imaging itself is performed under anesthesia, the pharmacological effects of the fMRI anesthetic agent on the already modulated or biased physiological state are difficult to predict (Borsook & Becerra, 2011; Sanganahalli et al., 2009). Thus, homeostatic regulatory mechanisms as a reaction to stressors, like unsuppressed pain, and the pharmacological effects of anesthetic agents used during surgical preparation (Lestage et al., 1985) can confound the relevance, reproducibility and comparability of the fMRI results preceded by artery catheterization.

In this study, the analgesic efficiencies of three different anesthetic protocols during a minimally invasive tail artery catheterization in rats were investigated. Catheterization of the rat-tail was preferred (Guo & Zhou, 2003), because it is reported to cause less tissue damage and less stress for the animals than femoral and iliac artery cannulation, although the latter methods are established for fMRI studies (Silva & Stefanovic, 2008). As the main parameter for assessing the rat's stress-level, and thus the analgesic efficiency, plasma CORT concentrations were quantified with ^{125}I-radioimmunoassay (RIA) for plasma collected at different time-points of the three anesthesia protocols. The first investigated agent, isoflurane as inhalation gas, is commonly used in fMRI experiments for induction of the anesthesia and during surgical preparation (Masamoto, Kim, Fukuda, Wang, & Kim, 2007; Silva & Stefanovic, 2008; Yu et al., 2010, 2012). The second, medetomidine–ketamine as intraperitoneal (i.p.) bolus injection, has a broad spectrum of applications for long lasting surgical anesthesia (Fish et al., 2008), but has so far only rarely been used in animal preparation protocols for fMRI in rats. The third alternative, propofol-remifentanil as intravenous (i.v.) infusion, is a modern combination for general anesthesia with surgical analgesia used in clinical practice (Vuyk, Mertens, Olofsen, Burm, & Bovill, 1997). Since several studies report on rat strain differences in physiology and behavior (Staples & McGregor, 2006; Webb, Gowribai, & Muir, 2003), differences in the level of CORT due to differences in adrenal steroid receptor occupancy and corticosteroid-binding globulin levels in plasma during resting and stress conditions (Dhabhar, McEwen, & Spencer, 1993; Dhabhar, Miller, McEwen, & Spencer, 1995) and on strain dependent susceptibility to anesthetic and analgesic drugs (Avsaroglu, Van der Sar, Van Lith, Van Zutphen, & Hellebrekers, 2007; Yoon, Lee, Lee, Chung, & Chung, 1999), we analyzed three rat strains, Sprague–Dawley, Wistar and Fischer 344, which are often involved in fMRI projects running in our lab.

2. Materials and methods

2.1. Animal handling

The study protocol was approved by the local authorities and the experiments were in accordance with the European directive for the handling of laboratory animals (86/609/EEC). Six healthy male CD (Sprague–Dawley), seven Wistar and nine Fischer 344 rats were delivered with 220–250 g (Charles River Laboratories, Germany) and housed individually until the experiment, but not longer than 3 weeks. In the animal housing, lights were switched on for 12 h at 20:00, temperature was regulated to 22 °C and humidity to 40–60%. Food and water were provided ad libitum. Body weight of the animals immediately before the experiment was in the range of 250–380 g, depending on the strain.

2.2. Protocol and monitoring

2.2.1. Preparation

After induction of anesthesia, which was preceded by premedication for two of the protocols (see Section 2.2.4), the rat was transported to the surgery table and the head was placed in an inhalation mask with continuous supply of a 1/4 v/v O_2 and air mixture. During the following interventions, body temperature, breathing rate (BR), heart rate (HR) and blood oxygen saturation (SpO_2) were monitored continuously. Body temperature was kept at 37.5 °C by a feedback guided system composed of a rectal thermo-probe, an electric heating blanket and a controlling unit (Harvard Apparatus, UK). Throughout the experiment, plethysmography was performed with an air-filled pneumatic sensor connected to a piezoelectric transducer, and the electrocardiogram was recorded using subcutaneous (s.c.) needle electrodes (13 × 0.4 mm, 27 gauges) in a forepaw and the contra-lateral hind paw (Rapid Biomedical, Germany). SpO_2, BR, HR, breath and pulse distention were measured with an infrared sensor clamped on the free hind paw and connected to a receiver system that amplified, filtered and isolated individual physiological signals (MouseOx, Starr Life Sciences, USA). The O_2 concentration of the inhaled gas mixture was adjusted to maintain a SpO_2 above 90%. Signals from different receivers were digitally recorded and visualized with a computer-based system (PowerLab & LabChart, ADInstruments, Australia). Tail vein cannulation was started after sensors were mounted, physiological parameters were found to be in the normal range and paw withdrawal (PWR) and tail flick reflexes (TFR) were tested negative. The rat was placed into the right lateral decubitus position and a 24 G neonate catheter (BD Insyte-N, Becton Dickinson Infusion Therapy Systems, USA) was introduced into the lateral tail vein. For the first plasma sample, 0.2–0.5 ml blood was collected and fluid loss was immediately compensated by injection of a balanced electrolyte solution (Jonosteril, Fresenius Kabi, Germany).

2.2.2. Surgery

The rat was positioned dorsally and the tail was placed on a custom-made holder made of a solid foam-substrate with a central strait for the tail, covered with aluminum foil to facilitate surface disinfection and reuse (see Supplementary material). A swab soaked with lidocaine (Licocainhydrochlorid 2%, 20 mg/ml, Bela Pharm, Germany) was placed for a few minutes on the site of surgery for topical pain suppression. A cranial–caudal 1–1.5 cm long incision through the skin was made approximately 2 mm right of the midline of the tail (adapted from (Guo & Zhou, 2003)). The skin was separated from the intact tissue underneath and was fixed with four needles to the foam-substrate. The artery was carefully prepared and two sutures were made, one cranially (open) and another caudally (closed, see Supplementary material for a depictive sketch). Closely below the cranial suture, a small artery clamp (S&T Vascular Clamps, Fine science tools, Germany) was attached to reduce bleeding during the following step. A 24 G neonate catheter was used to puncture the artery between the clamp and the caudal suture. Subsequently, the clamp was opened and the catheter was moved inside the artery 2 cm in the cranial direction. The cranial suture was closed to fixate the catheter in the actual position and the second blood sample was collected. The arterial catheter was then flushed with 0.1 ml heparinized (16 IU/ml) balanced electrolyte solution. Fluid loss was again compensated via the venous catheter. A pressure transducer (MLT0670, ADInstruments, Germany) was connected to the artery catheter through a PE-50 infusion-line filled with heparinized electrolyte solution (16 IU/ml) and the recording of MABP was initiated. Finally, the wound was covered with a swab soaked with saline solution to avoid tissue damage by desiccation.

2.2.3. Rest and euthanasia

During the next 60 min post-surgical period, physiological parameters were recorded. After 1 h of rest, the third blood sample was