Journal of Neuroscience Methods 205 (2012) 86–95

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Basic Neuroscience

High resolution 3 T fMRI in anesthetized monkeys

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ARTICLE INFO

Article history:
Received 28 October 2011
Received in revised form
23 December 2011
Accepted 23 December 2011

Keywords:
BOLD
3 T magnet
Anesthesia
Macaca mulatta
Visual stimulation
Methodology

ABSTRACT

Although there are numerous 3 T MRI research devices all over the world, only a few functional studies at 3 T have been done in anesthetized monkeys. In the past, anesthetized preparations were reported to be misleading when exploring cortical brain regions outside the primary sensory areas. Nonetheless, a great improvement has been achieved in the limited effect of anesthetic agents on the reactivity of the brain.

Here, we re-address the feasibility and potential applications of the brain oxygen level dependent (BOLD) fMRI signal in *Macaca mulatta* monkeys that have been lightly anesthetized with sevoflurane and curarized. The monkeys were studied with commercially available coils and sequences using a 3 T clinical magnet. We obtained sagittal T1 scout images, gray matter double inversion recovery, standard gradient echo sequences and gradient echo functional imaging sequences. Given that fMRI signals are most readily identified in the cerebral cortices, we optimized Echo Planar Imaging sequences to reproduce significant changes in the BOLD signal subsequent to a visual stimulation paradigm.

Our results provide a satisfactory signal to noise ratio with a limited standard deviation range, when compared with studies on alert macaques.

We suggest that the 3 T magnet remains a valuable tool to analyze neural pathways in the macaque brain under light anesthesia and report the use of spatially resolved fMRI in higher visual areas of anesthetized monkeys. This methodology avoids the need for time-consuming training of awake monkeys, is stable over many hours, provides reproducible data and could be applied successfully to future functional studies.

1. Introduction

Magnetic resonance imaging (MRI) techniques are widely used and contribute to the understanding of the brain in health and disease. This methodology allows the examination of the global properties of the brain in a non-invasive way.

Neuronal activation is accompanied by an increase in both energy metabolism and local cerebral blood flow (Kida and Hyder, 2006). These physiological functions permit the identification of brain activation through the use of blood oxygenation level-dependent (BOLD) contrast in functional MRI (fMRI). BOLD-fMRI is sensitive to the decrease in deoxyhemoglobin concentration during neuronal activation (Ogawa et al., 1990). On the basis of this unique property, studies with fMRI have multiplied in human and non-human primates (NHP).

fMRI studies can be conducted in animal experimental protocols, with behaving or with anesthetized preparations. Work has been performed with behaving macaque monkeys in functional studies (Goense et al., 2010) but, a major constraint on such procedures is the fact that only a very limited number of macaques per protocol can be trained and are finally used for fMRI studies (Goense et al., 2010; Joseph et al., 2006). In fact, this is the principal advantage of the use of anesthetic preparations in functional studies, but it is thought that anesthesia would greatly influence, or even invalidate, the results obtained in both basic electrophysiological

Abbreviations: BOLD, brain oxygen level dependent signal; CBF, cerebral blood flow; $CMRO_2$, cerebral metabolism; MRI, magnetic resonance imaging; DIR, double inversion recovery sequence; fMRI, functional magnetic resonance imaging; EPI, Echo Planar Imaging; FWE, corrected family-wise error rate; GLM, general linear model; GM, gray matter only images; GRE, standard gradient echo sequence; NHP, non-human primates; TE, echo time; TI, inversion time; TR, repetition time.

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doi:10.1016/j.jneumeth.2011.12.023

and MRI investigations (Sloan and Erian, 1993). While this concept may have been relevant in the past (see below), today's generation of anesthetic agents appear more suited to studies of the cerebral circulation if used at an appropriate concentration (Ishizawa, 2007).

The effects of anesthesia on the cerebral circulation and metabolism have always been of concern to physiologists. It has been well documented in humans that barbiturates depress both cerebral blood flow (CBF) and metabolism ($CMRO_2$) (Kida and Hyder, 2006), and that halogenic agents such as halothane and isoflurane can alter the reactivity of the cerebral circulation to an external challenge by, for example, hypo- or hypercapnia (Goode et al., 2009). However, the more recent generation of halogenic agents, which includes desflurane and sevoflurane, when used at concentrations that do not induce surgical anesthesia, may have a positive role to play in study of the functional reactivity of the brain to a stimulus that is repeated over time. In addition, the use of curare allows muscle relaxation without affecting the neuromuscular junction, so the neuronal circuits are preserved (Sloan and Erian, 1993).

Indeed, if it could be demonstrated that a light maintenance anesthetic regime could be employed during functional activation of the brain, and that fMRI could be used to map changes in the resulting BOLD signal, then such a methodology could be used to circumvent the problems associated with the use of awake primates (environment habituation, control of physiological and biochemical parameters, movement, experimental studies of long duration) through the use of a readily available clinical 3 T camera.

Therefore, we have re-addressed the use of anesthetized NHP in fMRI studies following a visual stimulation paradigm. To circumvent the problems associated with the use of awake primates, we have addressed the feasibility of fMRI monitoring of the BOLD signal in lightly anesthetized and curarized monkeys (*Macaca mulatta*) with a clinical 3 T camera.

Given that activity in primary cortical structures is readily recognizable from the fMRI signal, we optimized Echo Planar Imaging (EPI) sequences to identify the BOLD signal following a visual stimulation paradigm, as already published by other authors in awake monkeys. We show that the BOLD response to the visual stimulation is strongly repeatable in anesthetized monkeys and that changes in intensity are comparable with those of awake primates, as reported by other authors. Beyond reporting the feasibility of previously described spatially resolved fMRI in higher visual areas of the NHP in anesthetized *M. mulatta*, the present results may be of relevance to future functional studies of the central nervous system in anesthetized primates with available clinical 3 T magnets.

2. Methods

The study was conducted on two adult male rhesus monkeys (*M. mulatta*, 0303 and 0390, weighing 6 and 7 kg, respectively). All experiments were performed during daytime. A veterinarian skilled in the healthcare and maintenance of NHP supervised all aspects of animal care. Animals were checked at least daily by a competent person. These checks ensure that all sick or injured animals are identified and appropriate action is taken.

The experimental procedures were performed in accordance with the *European Directive on the Protection of Animals Used for Scientific Purposes* (2010/63/UE) and the recommendations of the Weatherall report "The use of non-human primates in research" (http://www.bprc.nl/BPRCE/L4/newsdownloads/The%20use%20of%20non-human%20primates%20in%20research%20-%20The%20Weatherall%20Report.pdf). The protocol was approved by the local committee on the ethics of animal experiments (Comite Regional d'Ethique en Experimentation Animale Normandie N/03-09-10/15/09-13). Non-invasive imaging of animals (e.g. MRI) with appropriate sedation or anesthesia is considered mild distress experience by the *European Directive on the Protection of Animals Used for Scientific Purposes* (2010/63/UE). Therefore, all efforts were then made to minimize suffering, as initial sedation was performed before any manipulation and general anesthesia was maintained throughout the experiment. Animals were also checked after recovery from general anesthesia.

Data were obtained from over 11 individual imaging sessions for fMRI (Table 1: 7 sessions from case 0390 and 4 sessions from case 0303). The animals were maintained in individual primate cages (at least 1 m^3 volume free per animal) on a 12 h light/12 h dark cycle. Fresh water and primate biscuits were available ad libitum and fresh fruits and vegetables were provided daily. Access to food was withdrawn at least 12 h before an experimental session. All experiments were conducted in the GIP Cyceron facilities which have accreditation for non-human primate research (Licence No. D 14118001). Regular and efficient cleaning schedule for the rooms maintained satisfactory hygienic standards.

2.1. Anesthesia and general preparation

In each experiment, the monkey was sedated initially by an intramuscular injection of ketamine (0.2 mg/kg Virbac; Carros, France). The hair on the head and hind legs was shaved and a perfusion line was placed in the saphenous vein. Following intravenous administration of atropine 0.25 mg (to prevent buccal secretions) and curare (Atracurium, 5 mg, to achieve adequate muscular relaxation) (Hospira; Meudon La Forêt, France), the monkey was intubated under 2.5% sevofluarne. Atracurium was infused continuously at a rate of 0.75 mg/kg/h throughout the experiment to minimize residual eye movements (Tootell et al., 1988), and anesthesia was maintained with sevoflurane (1.0–1.5%) in a mixture of N_2O:O_2 of 2:1 at a respiratory frequency of 18 cycles per minute with an MRI compatible ventilator (Aestiva™ 5MRI; Datex-Ohmeda Inc., Madison, USA). Heart rate, oximetry (spO_2), end-tidal CO_2 pressure ($etCO_2$) and arterial pressure were monitored continuously (Millennia 3155 MVS monitor, In Vivo Research, Orlando, Florida) and the body temperature was maintained at a constant value (38.2 ± 0.5 °C) by the use of hot water bottles.

The monkeys were placed in the sphinx position in an MRI compatible stereotaxic device (1430 M MRI, David Kopf Instruments, Tujunga, CA; USA) (Fig. 1). Care was taken to limit the pressure caused by the introduction of the ear bars into the external auditory meatus because this procedure may cause undue pain to the animal. During visual stimulation, both eyelids were kept open throughout the experiment with surgical adhesive tape, while optimal hydration of the eyes was maintained by the application of physiological saline every 15 min. The room was darkened and a visual stimulus was presented which consisted of a high-contrast black and white checkerboard pattern rotating at 16 Hz and placed 150 cm from the eyes with an angle of 10–11° (Fig. 1). The stimuli were presented in a synchronized manner towards the magnet through the use of a computer connected to a video projector (Panasonic PT-LB30NTE) which projected the image onto a screen placed at the end of the scanner bore (Fig. 1). This stimulus was presented alternately with periods of blackboard uniform intensity (background) illumination.

2.2. MRI data acquisition

Experiments were performed in a 3 T scanner (Achieva quasar dual, Philips Healthcare; Amsterdam; the Netherlands) using small and medium two element flexible surface coils (small flexible coil (FlexS) and medium flexible coil (FlexM), Philips Healthcare; Amsterdam, the Netherlands) positioned either with only one element fitting the occipital pole (FlexS) (Fig. 1) or with both elements lateral to the brain at the level of the ears when necessary (FlexM). The