Journal of Neuroscience Methods 271 (2016) 55–64

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

Two-photon imaging of cerebral hemodynamics and neural activity in awake and anesthetized marmosets

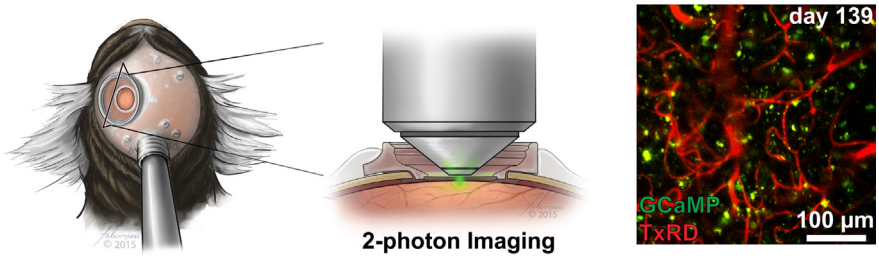
Thom P. Santisakultarm[a], Calvin J. Kersbergen[a], Daryl K. Bandy[b], David C. Ide[c], Sang-Ho Choi[a], Afonso C. Silva[a,*]

[a] Cerebral Microcirculation Section, Laboratory of Functional and Molecular Imaging, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, 20892, USA
[b] Section on Instrumentation, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, 20892, USA
[c] Section on Instrumentation, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, 20892, USA

HIGHLIGHTS

- Longitudinal 2-photon microscopy was performed in awake marmosets.
- Neurons were labeled with AAV-GCaMP to allow optical detection of neural activity.
- Neuronal and vascular responses to sensory stimulation were preserved for months.
- Vascular remodeling including increased tortuosity and branching was quantified.
- Isoflurane anesthesia modulated cerebral hemodynamics.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 22 June 2016
Accepted 3 July 2016
Available online 5 July 2016

Keywords:
Non-human primates
Two-photon laser scanning microscopy
GCaMP
Neurovascular coupling
Vascular topology
Somatosensory activation

ABSTRACT

Background: Marmosets are a powerful, emerging model for human behavior and neurological disorders. However, longitudinal imaging modalities that visualize both cellular structure and function within the cortex are not available in this animal model. Hence, we implemented an approach to quantify vascular topology, hemodynamics, and neural activity in awake marmosets using two-photon microscopy (2PM).
New method: Marmosets were acclimated to a custom stereotaxic system. AAV1-GCaMP5G was injected into somatosensory cortex to optically indicate neural activity, and a cranial chamber was implanted.
Results: Longitudinal 2PM revealed vasculature and neurons 500 μm below the cortical surface. Vascular response and neural activity during sensory stimulation were preserved over 5 and 3 months, respectively, before optical quality deteriorated. Vascular remodeling including increased tortuosity and branching was quantified. However, capillary connectivity from arterioles to venules remained unchanged. Further, behavioral assessment before and after surgery demonstrated no impact on cognitive and motor function. Immunohistochemistry confirmed minimal astrocyte activation with no focal damage. Over 6 months, total cortical depth visualized decreased. When under anesthesia, the most prominent isoflurane-induced vasodilation occurred in capillaries and smaller arterioles.
Comparison with existing method(s): These results demonstrate the capability to repeatedly observe cortical physiology in awake marmosets over months.
Conclusions: This work provides a novel and insightful technique to investigate critical mechanisms in neurological disorders in awake marmosets without introducing confounds from anesthesia.
Published by Elsevier B.V.

* Corresponding author.
E-mail address: silvaa@ninds.nih.gov (A.C. Silva).

http://dx.doi.org/10.1016/j.jneumeth.2016.07.003
0165-0270/Published by Elsevier B.V.

1. Introduction

Marmosets are a powerful, emerging model for human behavior and neurological diseases. Many disorders of higher brain function are difficult to closely model in rodents (Mansfield, 2003). In addition to having markedly different neuroanatomy, endocrinology, metabolism, and genetics, extrapolation of social and behavioral evaluation from rodents to primates can be uncertain or unavailable. Hence, the use of non-human primates in biomedical research can help neuroscientists address these concerns. Although many studies have used the rhesus macaque as a biomedical model, the common marmoset (*Calithrix jacchus*) is fast emerging as an attractive alternative. Similar to humans, marmosets have neural specializations such as face-selective areas in the visual cortex (Hung et al., 2015; Burman et al., 2015) and rich social interactions including biparental care of offspring. Compared to rhesus macaques, marmosets are smaller (350 g), reach sexual maturity faster (18 months), have a shorter gestational period (5 months), often give birth to fraternal twins or triplets, and produce up to 80 progeny during their life. A marmoset's short natural lifespan (12 years), compared to a macaque's (25 years), is also specifically beneficial for the study of age-related dysfunctions. For instance, aged marmosets (>7 years) display neurodegenerative changes, such as reduced neurogenesis and beta-amyloid deposition (Mansfield, 2003). Most importantly, marmosets are the first primates to have demonstrated germline transmission that allows for natural breeding of transgenic marmoset models of human neurological conditions, including Parkinson's disease, amyotrophic lateral sclerosis, and schizophrenia (Sasaki et al., 2009). Therefore, the use of marmosets in biomedical science has tremendous translational appeal for both basic and clinical research.

Understanding neural activity and cerebral blood flow regulation in the marmoset is an attractive step for deciphering the human brain and mechanisms of disease. Cerebral blood flow is controlled with exquisite precision to ensure that homeostasis of the delicate cellular environment is maintained at all times, including during neural activation when metabolic demand is elevated. Such relationship between neural activation and cerebral blood flow, termed neurovascular coupling, is achieved through the integrated actions of neurons, astrocytes, pericytes, and the endothelium (Girouard and Iadecola, 2006; Liu et al., 2013; Tian et al., 2010; Hall et al., 2014). Neurovascular coupling forms the basis of many neuroimaging methods, including blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) and optical intrinsic imaging. Because functional imaging techniques detect changes in cerebral blood flow, volume, or oxygenation as surrogate markers of the underlying neural activity, understanding the mechanisms of neurovascular coupling is of vital interest to neuroscientists. Additionally, dysfunction of vascular response is common to many neurological disorders, substantiating its importance in maintaining a healthy brain (Girouard and Iadecola, 2006; Santisakultarm and Schaffer, 2011). Although many neurophysiological studies use anesthetic agents to maximize experimental control, anesthesia strongly influences brain physiology, including direct effects on vasotone, neurosuppresion, and alterations of inter-hemispheric activity (Liu et al., 2013; Shtoyerman et al., 2000). Therefore, understanding these cellular processes in the awake animal model is of great importance to neuroscience.

The lack of long-term optical imaging modalities in marmosets is a hurdle that prevents biomedical scientists from taking full advantage of this animal model. Optical imaging techniques such as two-photon microscopy (2PM) allow systematic, longitudinal investigation of neural activity and hemodynamics at the cellular level in awake, behaving animals (Chen et al., 2002; Ruiz et al., 2013). Despite the limitations of depth penetration compared to MRI-based methods, optical imaging tools are invaluable to capture both structural and functional information of multiple cells and cell types simultaneously with subcellular resolution in intact animals (Hall et al., 2014; Santisakultarm et al., 2012; Kleinfeld et al., 1998; Lecoq et al., 2011). The marmoset's lissencephalic cortex also ensures optical access to most functional areas of cortical tissue. Additionally, marmosets can be trained to rest comfortably with restraint, allowing researchers to bypass anesthesia and its confounding influences on brain physiology (Liu et al., 2013). Recently, 2PM was used to image neuronal populations in anesthetized marmosets (Sadakane et al., 2015). In the present study, we aim to establish a longitudinal two-photon imaging technique in awake marmosets, providing a critical tool to visualize cortical vasculature, neurons, and their dynamics at the cellular level.

2. Methods

2.1. Experimental overview

Three adult male marmosets were used in this study. All experiments were approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke. The timeline of experimental protocols began with acclimatization to body restraint and pre-operative testing of cognition and motor skills. Next, marmosets underwent surgical procedures for viral delivery of GCaMP and implantation of a headpost and cranial chamber. Upon recovery, acclimatization to head restraint in the stereotaxic apparatus resumed along with post-operative testing of cognition and motor skills. Imaging experiments (*i.e.* awake and anesthetized 2PM) and continuous monitoring of post-operative cognition and motor skills followed in the subsequent months until euthanasia and immunohistochemistry of brain tissues.

2.2. Stereotaxic acclimatization

Animals were gradually accustomed to body restraint in the prone, sphinx position starting with 15 min of restraint on the first day of training and increasing by 15-min intervals up to 2 h at 3 times per week, over the course of 2 weeks (Silva et al., 2011). The behavioral assessment scale (Fig. S1) was used to monitor the marmoset's progress (Schultz-Darken et al., 2004). Similarly, animals were acclimatized to both body restraint and head-fixation in the custom-designed stereotaxic apparatus following implantation of the headpost (Computer-aided design files available upon request; Figs. S2 and S3). The microscope stage (MP-285, Sutter Instrument) provides translation in X, Y, and Z, and the stereotax is secured to the stage by a mini lab jack (LJ750, Thorlabs) that provides additional course translation in Z. Angular positioning of the marmoset under the objective is obtained using a high precision ball & socket mounting platform articulating base (SL20, Thorlabs) that provides 360° of rotation.

2.3. Pre- and post-operative testing of cognition and motor skills

The Hill Staircase, the Valley Staircase, and the Six-Tube Search behavioral tasks (Marshall and Ridley, 2003) were administered biweekly before any surgical procedures were performed in order to evaluate any cognitive impacts of surgery, imaging, and longitudinal cranial chamber implantation. Briefly, animals were required to reach through vertical slots in a see-through screen panel to retrieve food rewards on each step of the staircase in the Hill and Valley Staircase tasks. Marmosets were given 3 min to obtain all rewards, and the cumulative score was recorded (score 1 for retrieving the reward on the nearest step, and 5 for the 5th farthest step). In the Six-Tube Search task, a food reward was hidden randomly in one of the six tubes. The marmoset was marked successful