NeuroImage xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

NeuroImage

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

Review

Miniaturized optical neuroimaging in unrestrained animals☆

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A R T I C L E   I N F O

Article history:
Accepted 20 February 2015
Available online xxxx

Keywords:
Optical
Imaging
Freely moving
Unanesthetized
Tetherless
Miniaturized

A B S T R A C T

The confluence of technological advances in optics, miniaturized electronic components and the availability of ever increasing and affordable computational power have ushered in a new era in functional neuroimaging, namely, an era in which neuroimaging of cortical function in unrestrained and unanesthetized rodents has become a reality. Traditional optical neuroimaging required animals to be anesthetized and restrained. This greatly limited the kinds of experiments that could be performed in vivo. Now one can assess blood flow and oxygenation changes resulting from functional activity and image functional response in disease models such as stroke and seizure, and even conduct long-term imaging of tumor physiology, all without the confounding effects of anesthetics or animal restraints. These advances are shedding new light on mammalian brain organization and function, and helping to elucidate loss of this organization or 'dysfunction' in a wide array of central nervous system disease models.

In this review, we highlight recent advances in the fabrication, characterization and application of miniaturized head-mounted optical neuroimaging systems pioneered by innovative investigators from a wide array of disciplines. We broadly classify these systems into those based on exogenous contrast agents, such as single- and two-photon microscopy systems; and those based on endogenous contrast mechanisms, such as multispectral or laser speckle contrast imaging systems. Finally, we conclude with a discussion of the strengths and weaknesses of these approaches along with a perspective on the future of this exciting new frontier in neuroimaging.

Contents

Introduction

Imaging the brain has provided unprecedented insights into its functioning as well as disruption of this function due to various neuropathologies. Noninvasive imaging techniques such as functional Magnetic Resonance Imaging (fMRI) (Heeger and Ress, 2002), Positron Emission Tomography (PET) (Nasrallah and Dubroff, 2013) and Computed

☆ Acknowledgments: Supported by NCI 1R21CA175784-01.
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http://dx.doi.org/10.1016/j.neuroimage.2015.02.070
1053-8119/

Tomography (CT) (Cianfoni et al., 2007) have been widely used for neuroimaging. However, these clinical or 'human-scale' imaging modalities often lack the resolution to spatially and temporally resolve underlying neuronal processes. Therefore, investigators circumvented this drawback by utilizing pre-clinical animal models in conjunction with imaging methods capable of high spatial and temporal resolution.

The availability of an ever-increasing spectrum of optical contrast agents (Zhang et al., 2002), and technical advances in optics (Kerr and Denk, 2008; Tye and Deisseroth, 2012), coupled with optogenetic constructs for manipulating neuro-circuitry (Tye and Deisseroth, 2012), have resulted in optical neuroimaging becoming the tool of choice for neuroscientific applications. Moreover, these optical neuroimaging techniques permit cellular-scale spatial resolution and millisecond temporal resolution (Kerr and Denk, 2008).

Much of today's optical neuroimaging is performed using sophisticated optics and cumbersome electronic hardware (Theer et al., 2003). The bulky nature of such setups requires the animal to be anesthetized and restrained stereotactically, greatly limiting the types of experiments that can be performed in vivo and at multiple time points. Additionally, the use of anesthetics has been found to alter the baseline physiology of the brain during in vivo imaging (Bonhomme et al., 2011). Therefore, miniaturization of the imaging hardware in conjunction with the ability to image the brains of awake and unanesthetized animals would circumvent these issues.

Recent advances in miniaturized optics and electronic devices (Theuwissen, 2008) paved way for the "next generation" optoelectronic systems capable of unique real-time, awake optical imaging. Fig. 1 shows the evolution of neuroimaging systems from benchtop setups to 'head-mounted' platforms. It is not always necessary to miniaturize the entire system. As shown in Fig. 1, depending on the type of experiment, individual elements of the imaging system can be modified to match the required level of animal mobility. This can range from having the animal's head affixed while the animal pedals on a moving ball (Dombeck et al., 2007), to systems that allow unrestrained animal mobility (Ghosh et al., 2011). It is worth noting that similar technical advances were also responsible for the development of 'implantable' microimagers (Ng et al., 2008a, 2008b). These implantable devices are image sensor array chips that have been packaged into 'ready-to-use' modules. Recent work has elegantly demonstrated their utility in applications ranging from neural imaging (Ng et al., 2008a, 2008b) to blood-flow imaging in freely moving rats (Haruta et al., 2014). However, the focus of the current review is on non-implantable imagers. An excellent recent review by Kerr and Nimmerjahn focused on functional imaging at the cellular level and primarily covered imaging approaches that utilized exogenous contrast agents (Kerr and Denk, 2008). In this review, we examine miniaturized neuroimaging systems that utilize exogenous contrast agents, e.g. wide-field fluorescence imaging (Ferezou et al., 2006; Flusberg et al., 2008), two-photon fluorescence imaging (Helmchen et al., 2001; Sawinski et al., 2009), as well as those that exploit intrinsic optical properties of biological tissues, e.g. multispectral imaging and blood flow based laser speckle imaging systems (Liu et al., 2013). Finally, we discuss the relative advantages and disadvantages of each approach and the exciting prospects of this technology from the micro- (i.e. cellular) to the macro-scale (i.e. whole tissue) for neuroimaging.

Miniaturized optical systems based on exogenous contrast agents

Optical contrast agents permit visualization of underlying microvasculature (Bassi et al., 2011) as well as functional cellular dynamics such as membrane potentials (Mutoh et al., 2011) and intercellular calcium concentrations (Mittmann et al., 2011). Conjugation of fluorescent dyes with genetically encoded biomarkers/target molecules (Chalfie et al., 1994), as well as their ability to shift emission spectra in response to biological perturbations (McVea et al., 2012) has enabled fluorescent imaging to be utilized in a wide range of applications (Petersen et al., 2003; Mank et al., 2008). Although variability in contrast agent delivery or unstable gene expression can affect the emitted fluorescence, an ever increasing array of fluorescent dyes with different excitation spectra, better quantum yields and extinction coefficients has greatly enhanced our ability to simultaneously monitor a multitude of targets and neurophysiologic processes.

Miniaturization of fluorescent microscopy was first attempted by using an optic fiber bundle to relay the emitted fluorescent light as well as the high intensity excitation illumination to and from a standard benchtop system (Helmchen et al., 2001). However, recent technological breakthroughs have enabled additional miniaturization of fluorescent microscopy systems as discussed below. A summary of miniaturized and mobile brain imaging platforms from the recent literature can be found in Table 1.

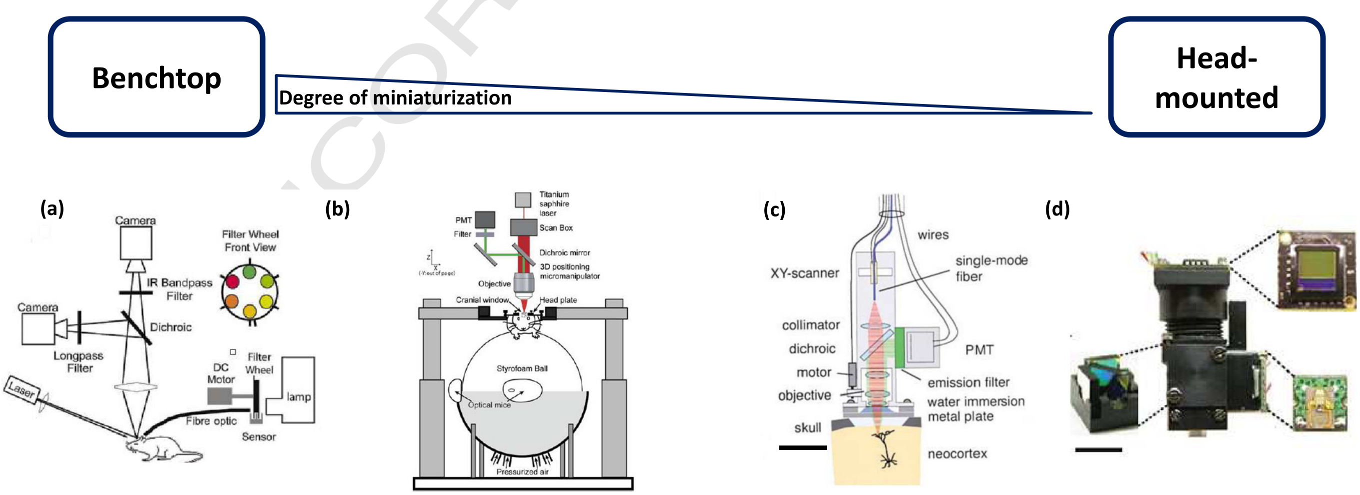

Fig. 1. Evolution of benchtop to 'head-mounted' neuroimaging systems. The degree of miniaturization increases from (a) to (d). (a) A dual modality benchtop system for simultaneous multispectral imaging and laser speckle contrast imaging in anesthetized animals (Jones et al., 2008). (b) Schematic of the system setup for imaging in head-restrained, awake mice (Dombeck et al., 2007). The head-mounted imaging system was modified from a standard two-photon microscope. The head of each mouse was restrained while the animal moved on a treadmill for behavioral testing. (c) Additionally miniaturized fiber-optics-based system (Helmchen et al., 2001), in which the photomultiplier tube (PMT) was incorporated into the head piece, wherein the excitation light was still derived from a benchtop system. The head piece was 7.5 cm long (scale bar = 23.5 mm). (d) An integrated head-mounted system (Ghosh et al., 2011), using surface mounted LEDs for exciation and a minaturized CMOS sensor for detection (scale bar = 5 mm). This self-contained system enabled experiments involving interactive and natural animal behaviors. All images have been adapted with permission of the publishers.

Please cite this article as: Yu, H., et al., Miniaturized optical neuroimaging in unrestrained animals, NeuroImage (2015), http://dx.doi.org/10.1016/j.neuroimage.2015.02.070