Photoacoustics 17 (2020) 100153

Contents lists available at ScienceDirect

Photoacoustics

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

Visualizing cortical response to optogenetic stimulation and sensory inputs using multispectral handheld optoacoustic imaging

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ARTICLE INFO

Keywords:
Neuroimaging
Hemoglobin gradients
Brain
Optogenetic stimulation
Photoacoustic
Handheld probe
Barrel cortex

ABSTRACT

To date, the vast majority of intra-vital neuroimaging systems applied in clinic and diagnostics is stationary with a rigid scanning element, requires specialized facilities and costly infrastructure. Here, we describe a simple yet radical approach for optoacoustic (photoacoustic) brain imaging *in vivo* using a light-weight handheld probe. It enables multispectral video-rate visualization of hemoglobin gradient changes in the cortex of adult rats induced by whisker and forelimb sensory inputs, as well as by optogenetic stimulation of intra-cortical connections. With superb penetration and molecular specificity, described here in method holds major promises for future applications in research, routine ambulatory neuroimaging, and diagnostics.

Localized changes in perfusion and metabolic activity in the brain are closely linked to its functions and provide a contrast mechanism for imaging. Since the first demonstration of increased pulsation in the brain of subjects engaged in mathematical tasks [1], the field of functional neuroimaging has made drastic advances in basic and translational domains. As the largest consumer of glucose and oxygen, the mammalian and especially human brain absorbs excessively large amount of energy [2–4]. Over ~80 % of this energy is used for maintaining baseline electrochemical processes, with remaining ~20 % accounts for processing sensory inputs and higher integrative functions and driving motor activity. While moderate, the transitory changes in energy demands and fluctuations in tissue perfusion present a highly valuable source of information about physiological and pathological processes in the brain [5–7]. Based on blood-oxygenation level-dependent (BOLD) signal, for instance, major breakthroughs have been made by functional magnetic resonance imaging (fMRI) while the ability to sense small increments in metabolic activity renders positron emission tomography (PET) a powerful tool for molecular neuroimaging [8,9]. Although providing excellent amenities to neuroscience research and diagnostics, these methods fall short in yielding high-resolution data, come at poor scanning flexibility, very high costs and risks related to the use of radioactive tracers.

Optical imaging has been traditionally viewed as a low-cost alternative, yielding excellent spatial and temporal resolutions, but at the expense of limited penetration, due to scattering of the light by the brain tissue and skull [10,11]. As an inherently hybrid method, optoacoustic (photoacoustic) imaging capitalizes on excellent resolution and molecular absorption of light as a source of contrast, while emitted non-radiative decay broadband ultrasound (US) is used for image formation [12–14]. Such useful combination of superb contrast and molecular specificity of optical imaging with penetration and resolution of US render this method highly instructive for a wide range of biomedical applications. With added multiplexing and volumetric capabilities, recently, a multispectral optoacoustic tomography (MSOT) has been introduced, capable of imaging at unprecedented molecular specificity, resolution, and depth [14–17]. Unlike stationed fMRI and PET neuroimaging platforms, optoacoustic approaches offer superb flexibility, with a portable scanning element applied for translational research and diagnostics [18–20]. Up until now, however, neuroimaging applications of optoacoustic methods have been limited to the use of fixed

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https://doi.org/10.1016/j.pacs.2019.100153
Received 25 July 2019; Received in revised form 28 November 2019; Accepted 5 December 2019
Available online 26 December 2019

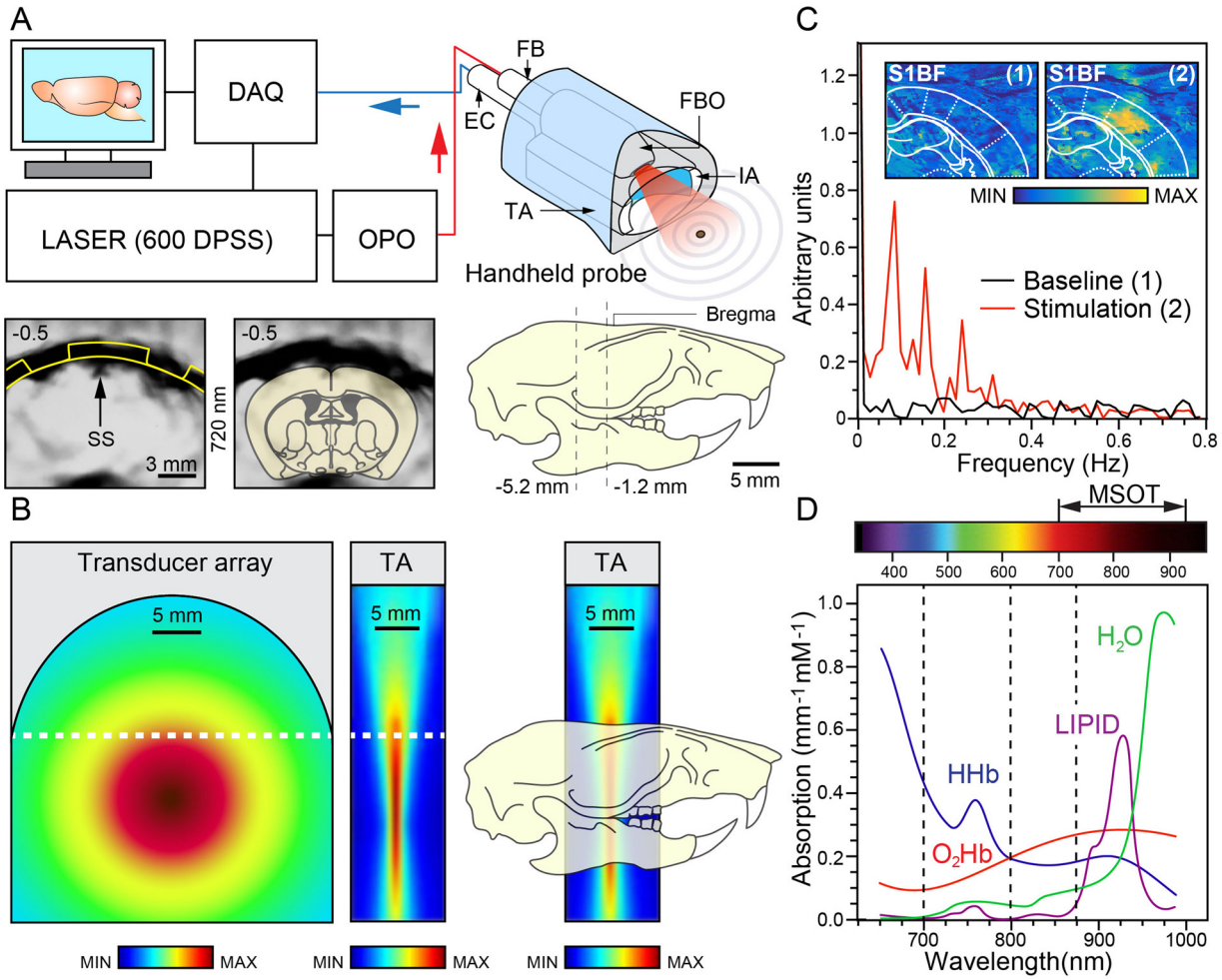

Fig. 1. (A) A schematic of the MSOT system with a portable handheld probe (top) employed for functional imaging of rat brain, with anatomical images captured at 720 nm without and with a superposition of the brain map at −0.5 mm Bregma (bottom, left and middle). Imaging planes containing S1FL and S1BF (−2.1 and 0.5 mm, respectively, bottom right) have been captured for current analysis. FB - fiber bundle, EC - electrical cables, FBO - fiber bundle output, TA - transducer array; IA - imaging aperture. (B) Simulated sensitivity distribution of the array within detection plane (left, middle) overlaid with the rat skull (right). TA – transducer array. (C) Fast Fourier transform graph of reconstructed data at 720 nm from the S1BF before and after stimulation of the whiskers input (1 and 2, respectively). Insets show frequency maps of imaging planes superimposed with schematic anatomical representations, with color-coded deoxy-HB gradients under rest (baseline) and stimulation. (D) Normalized absorptivity graphs of four key absorbers in the brain within the wavelength range used for this study, with three wavelengths (dashed vertical lines) selected in current analysis.

detectors, or their move with defined geometry [16,21,22]. Attempts of developing wearable detectors for imaging freely behaving animals have been made recently, using sensors rigidly mounted on the animal head with permanent implants [23], an approach that has dubious clinical prospects.

In this study, we implemented for the first time a portable MSOT for sensing hemodynamic response induced by two independent sensory inputs, and optogenetic stimulation of intra-cortical connections within the barrel field of adult anesthetized rats *in vivo*. Fig. 1(A) depicts a schematic diagram of the system with description of main components of the handheld probe, and anatomical image of the brain acquired at 720 nm, while (B) shows simulated sensitivity distribution field of the probe and detection band, and compares it with the size of the adult rat skull. Procedures on animal preparation were similar to those detailed elsewhere [24], and are provided in detail in Supplementary Materials and Methods.

To ensure optimal access to brain tissue, anesthetized with isoflurane rats were fixed in a stereotactic frame with two cranial windows (diameter =5 mm) opened over the S1BF and S1FL fields [25], which were covered with medical Vaseline for protection, and shielded by transparent polyethylene (PE) foil. The acoustic gel was applied to ensure coupling between the brain surface and the detector membrane of the scanner. A custom-designed elastic handle was used for supporting the probe, to prevent excessive mechanical pressure on the brain tissue during measurements. After surgery, rats were given a bolus of α-chloralose (80 mg/kg i.v.) and isoflurane was discontinued. The infusion of α-chloralose was set at a rate of 26.5 mg/kg per hour. Pancuronium bromide (2 mg/kg/h i.v.) was delivered to achieve muscle relaxation and to minimize motion artifacts.

To stimulate the forepaw and whisker representations in the cortex, incrementing electrical pulses were delivered through bipolar electrodes to the forepaw and whisker area. Pulse sequence-based trigger and stimulation control were established using Bio-Pac system (MP 150 system, Goleta, USA). The baseline and stimulated activity responses were collected at 720 and 900 nm, which correspond to optimal absorption for oxyhemoglobin and deoxyhemoglobin (Deoxy-HB), while 800 nm was used for measurements of changes in the perfusion rate. Fast Fourier Transform (FFT) analysis was applied to calculate frequency components of collected signals before and after stimulation (Fig. 1C, D), with reconstructed hemoglobin gradient maps showing activated region by plotting frequency components of response corresponding to the maximal frequency distribution of each pixel of raw

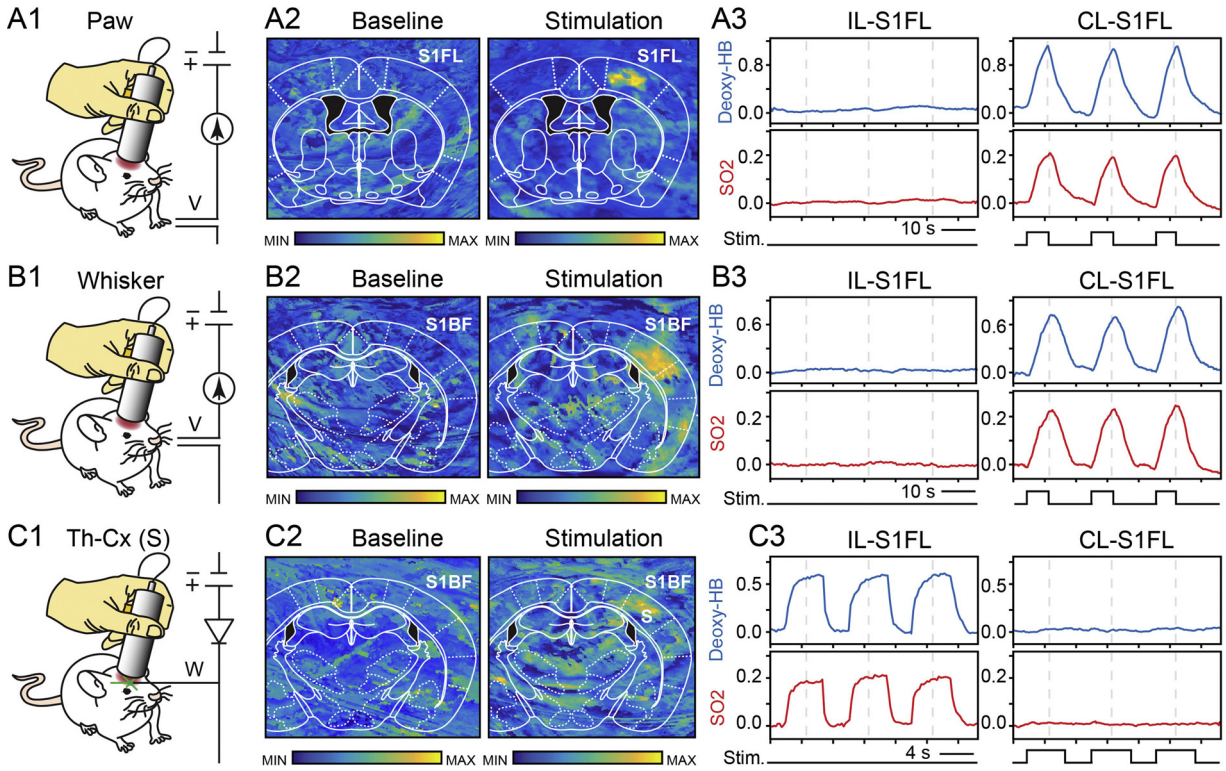

Fig. 2. (A1-C1) Schematic representaion of the experimental setup used for recording the activation response in S1FL and S1BF by electrical stimulation of the forepaw and whisker pad area (A1 and B1), and by optogenetic stimulation of the S1BF (C1). (A2-C2) FFT maps of imaging planes superimposed with schematic of anatomical representation of corresponding planes, with color-coded deoxy-HB gradients shown under rest and stimulation. Note that for optical stimulation, the fiber was inserted directly into the barrel cortex of the stimulated side. S – Stimulation site. (A3-C3) Representative traces of deoxy-HB signals and calculated oxygen saturation (SO2) under rest and stimulation (top) with corresponding stimulation protocols (bottom). IL-ipsilateral; CL-contralateral.

images. As illustrated in Fig. 2(A1–3, B1–3), electrical stimulation of the forepaw or whisker area by three consecutive trains of stimuli induced rapid onset and localized surge in deoxyhemoglobin in the frontlimb and barrel field area of the contralateral somatosensory cortex (S1FL and S1BF). These transient changes in hemoglobin gradients were paralleled by remarkable increase in tissue oxygenation (SO2) within activated regions, which showed close temporal characteristics to deoxy-HB signals. As can be seen, evoked hemoglobin gradient and SO2 changes displayed a fast on- and off-rates locked in time with stimulation trains sequences (Fig. 2A3, B3). In total 68 trials in three independent rats, these evoked responses were highly reproducible and could be triggered by relatively low stimulus intensities. Imaging of anatomically matching ipsilateral cortex revealed no changes in hemoglobin gradients, confirming the specificity of evoked signals.

To find out if hemodynamic changes associated with cortical activation could be induced by optogenetic stimulation of intercortical circuits, ~1-month-old Sprague-Dawley rats were injected with vectors (AAV5.CaMKIIa.hChR2 (H134R)-eYFP) in S1BF as described previously [24]. After 4–6 weeks of recovery time and hChR2 expression in neurons, a fine optic fiber (diameter = 0.2 mm) was inserted into the rat brain to stimulate cortical circuits using a 473 nm laser (CNI, China) with a built-in FC/PC coupler to deliver light pulses to the transfected area (Suppl. Video 1). As illustrated in Fig. 2C1–3, similar to electrical stimulation, activation of cortical circuits with light-induced a rapid hemodynamic response, which at low stimulus intensities was limited to the activation site, which was locked in time with light stimulation (Fig. 2C1–3). Stronger stimulations, however, induced a spreading response over the neighboring areas and extended in time beyond the stimulation period, suggesting induction of generalized seizure.

Taken together, presented herein data demonstrate the feasibility of mapping hemodynamic changes related to processing somatosensory input in the cerebral cortex using a handheld MSOT. To the best of our knowledge, this is the first report of multispectral functional neuroimaging using a hand-held optoacoustic probe, validating the possible use of portable brain imaging probes for research, potentially also extendable to the clinic and ambulatory settings. The principal advantage of our approach *versus* reported earlier wearable optoacoustic imaging is that it affords flexibility for a multispectral interrogation with excellent sensitivity, whereas, with wearable approach, only limited number of wavelengths is available. Also, the wearable optoacoustic imaging is more invasive as it involves removal of the skull with hard mounting of the transducer array to the skull. Finally, the presented portable approach here offers a wide range of potential translational applications in humans, particularly in infants where optical and acoustic properties of tissue afford the possibility for mapping hemoglobin gradients and their accurate quantification. Given the superb penetration of optoacoustic imaging with excellent contrast and specificity [15,26,27], further optimization of described approach is expected to facilitate routine use of handheld probe for clinical neuroimaging with MSOT. Detection of vascular and metabolic processes in human brain, as well as sensing hemorrhage, stroke, and brain tumors, are of particular interest given that they are associated with localized changes of hemoglobin gradients. Successful implementations of non-invasive optoacoustic neuroimaging in larger animals, including sheep and monkey [28,29], and relatively lower costs render handheld MSOT attractive for future use in biomedical research and healthcare benefits.

Funding source

This work was supported by G.W. Leibnitz Prize DFG (2013) to V.N., PRISAR grant (EU) to V.N., Helmholtz Association Developmental Grant to S.V.O.

Declaration of Competing Interest

Dr. Thomas C.P. Sardella and Dr. Neal C. Burton work as imaging and application specialists at iThera Medical GmbH.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pacs.2019.100153.

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