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Abstract. Photoacoustic (PA) imaging was applied to detect the neuronal activity in the motor cortex of an awake, behaving monkey during forelimb movement. An adult macaque monkey was trained to perform a reach-to-grasp task while PA images were acquired through a 30-mm diameter implanted cranial chamber. Increased PA signal amplitude results from an increase in regional blood volume and is interpreted as increased neuronal activity. Additionally, depth-resolved PA signals enabled the study of functional responses in deep cortical areas. The results demonstrate the feasibility of utilizing PA imaging for studies of functional activation of cerebral cortex in awake monkeys performing behavioral tasks. [DOI: 10.1117/1.JBO.17.11.110503]

Keywords: photoacoustic imaging; monkey; motor cortex; muscle; functional imaging.

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1 Introduction

The spatiotemporal features of brain activation during various motor and cognitive tasks have emerged as an important research area in neuroscience over the past several decades. Numerous animal models have been used for this research. Nonhuman primates represent one of the best research models because of the high similarity between human and nonhuman primate brains.[1–4] Functional magnetic resonance imaging (fMRI) has been used to detect functional changes in the primate brain; however, this technique is high cost and has poor temporal and spatial resolution when collecting functional information. Optical brain imaging, which can provide complementary information to fMRI and provide a low-cost alternative in many cases, has also been used to detect functional domains in the cortex of behaviorally active monkeys.[5,6] However, due to strong light scattering, high-resolution optical imaging can only monitor the surface of the exposed cortex, and depth information is very limited.

In this study, we applied photoacoustic (PA) imaging to detect activation of cortical areas in an awake monkey performing a reach and grasp task. PA imaging is based on the PA effect, and provides structural and functional images.[7] During PA imaging, the light energy is absorbed by the tissue and converted to heat energy which causes the cells to expand instantaneously. Then, the ultrasound emission generated by the thermal expansion can be detected by an ultrasonic sensor. By using the detected ultrasound signals, PA images with ultrasound depth resolution and optical contrast are constructed. PA imaging can be used to acquire functional information, such as changes in the relative total concentration of hemoglobin (HbT), and the hemoglobin oxygen saturation (sO_2).[8–11] In the brain, these hemodynamic changes can be related to the brain activity. Therefore, PA imaging can be used to detect task-related brain activation.

To the authors' knowledge, this study represents the first time that PA imaging has been applied to detect brain activation in an awake monkey associated with voluntary movements of the arm and hand. In our experiments, a customized PA imaging system was attached to a chronic recording chamber implanted over motor cortex of the left hemisphere. The system included a 20-MHz ultrasound transducer that was used to detect the hemodynamic changes in the motor cortex during performance of a voluntary reaching task. Brain activation was monitored by detecting changes in regional total concentration of hemoglobin. This study demonstrates the feasibility of using functional PA imaging for brain research in nonhuman primates.

2 Materials and Methods

For this study, a 9-kg, adult rhesus monkey (*Macaca mulatta*) was used. The monkey was seated in a custom built primate chair inside a sound-attenuating chamber. A reach-to-grasp task was performed with the right arm while the left forearm was restrained. The monkey initiated the task by placing its right hand on a pressure detecting plate located directly in front of the monkey at waist level. The monkey pressed the plate for 2 to 3 s after which a food pellet dropped into a small cylindrical food cup. The monkey then retrieved the food pellet with its right hand, carried the pellet to its mouth, and returned to the pressure plate to initiate a new trial. During continuous performance of this task, the brain was scanned using PA imaging.

The monkey was first chair adapted and then trained on the reach-to-grasp task for several months. After behavioral performance reached an acceptable level, an aseptic surgery was performed to implant a 30-mm inside diameter titanium chamber over the central sulcus at the level of forelimb primary motor cortex leaving the dura intact.[4] The chamber was centered at anterior 16-mm lateral and 18-mm lateral to the midline at an angle of 30 deg to the midsagittal plane. It was anchored to the skull with 12 titanium screws and dental acrylic. Surgeries were performed under isoflurane anesthesia and sterile conditions. Postoperatively, monkeys were given an analgesic (buprenorphine 0.5 mg/kg every 12 h for three to four days) and antibiotics (penicillin G, benzathaine/procaine combination, 40,000 IU/kg every three days). All procedures were in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and the Guide for the Care and Use of Laboratory Animals, published by the US Department of Health and Human Services and the National Institutes

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of Health. The study was approved by the University of Kansas Institutional Animal Care and Use Committees (IACUC).

A schematic of the PA imaging system with the chamber on the monkey's head is shown in Fig. 1. During the imaging process, the cover of the chamber was removed to expose the dural surface, and saline solution was used to fill the chamber. The detection surface of PA detector was then immersed in saline solution. The illumination light source was a Q-switched 30-Hz pulsed Nd:YAG laser (Surelite OPO PLUS; Continuum, Santa Clara, CA) producing light at a 532-nm wavelength. The light was delivered through an optical fiber to the dural surface overlying motor cortex. The incident energy density of the laser that reached the dural surface was less than 5 $\mathrm{mJ/cm^2}$, which complies with the safety limit for human skin exposure.[12] The PA signal from the cortex was collected with a 20-MHz focused ultrasound transducer (V316, focal distance: 0.5 in., Olympus-NDT, Waltham, MA). The detected PA signal was amplified (5072PR, Olympus-NDT, Waltham, MA) and collected using a data acquisition board (CS21G8, Gage, Lockport, IL). Two automated stepper motors [Fig. 1(a)] were used to allow two-dimensional scanning of the brain. The scanning step size was 100 μm for both directions in the scanning area. A third manual translation stage was used to adjust the height of the sensor and laser source above the dural surface. At each scan position, A-line data (2000 samples, 250-MHz sampling rate) was acquired.

In each imaging session, the PA sensor scanned a 16×8 mm ovoid-shaped region inside the cranial chamber using the computer controlled two-axis motorized stage. A reference image with the behavioral task off was always obtained first. Then, the same brain area was scanned while the monkey performed the reach-to-grasp task with its right arm/hand. It took 7 min for each scanning. The PA data were acquired continuously during the task-off for about 15 min to get two reference images for averaging. Then the monkey performed the same task repeatedly for about 15 min and another two PA images were taken for averaging during this process. Because the experiment used 532-nm laser light, which is an isospectral point of hemoglobin absorption, the amplitude of the detected PA signal was proportional to the concentration of total hemoglobin. Therefore, an increase in imaging intensity indicated an increase in the concentration of total hemoglobin.

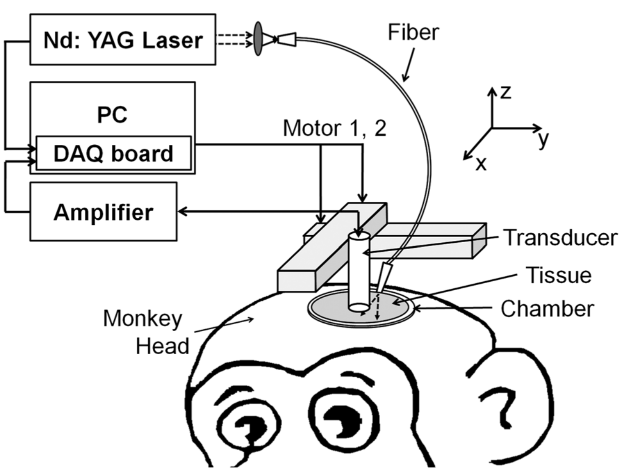

Fig. 1 Schematic of the PA system.

3 Results

The region of interest in the left hemisphere is shown in Fig. 2. Figure 2(a) is the reference image obtained with the behavioral task off, and Fig. 2(b) is the image acquired when the monkey was performing the reach-to-grasp task with the contralateral arm. The two images [Fig. 2(a) and 2(b)] are maximum-amplitude-projection (MAP) PA images, where the maximal amplitudes of detected PA signals along the depth direction are projected to the imaging plane. Comparing Fig. 2(a) and 2(b), regional changes in the amplitude of PA signals can be observed. These regional changes indicate changes in brain activity associated with task performance. Primary motor cortex (M1) is activated in association with motor execution and primary somatosensory cortex (SI) is activated in association with stimulation of various sensory receptors by the movement itself and also by grasping the food pellet. An overlay of the PA image and the magnetic resonance imaging (MRI) structural image is shown in Fig. 2(c). From the MRI image, M1 and SI cortex can easily be identified. The average increases in PA signal amplitude in M1 and SI cortex during movement compared to the task off condition are given in Fig. 2(f). The PA signal amplitude increased 28% in M1 and 27% in SI compared with the task off condition. These results were averaged over the area indicated by the dotted lines in Fig. 2(a) and 2(b). Note that the area encompassed by the dotted line is largely M1 but includes a small part of premotor cortex anteriorly. An increase in the signal amplitude indicates an increase in total hemoglobin concentration. Increased total hemoglobin concentration is associated with increased blood flow related to the additional energy requirement of increased neuronal activity.

The task off condition shows activation of a region that is largely within the M1 representation although it has a different

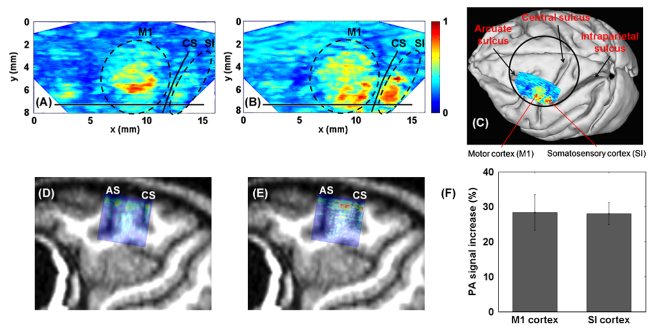

Fig. 2 PA images of functional activity in primary motor cortex and primary somatosensory cortex of a macaque monkey. Color scale indicates the amplitude of the detected PA signal with "1" indicating the maximal value. (a) Reference image acquired with the behavioral task off. Primary motor cortex (M1), primary somatosensory cortex (SI), and the location of the central sulcus (CS) are identified. (b) Image obtained over the same region of the cortex while the monkey was performing a reach-to-grasp task with its contralateral forelimb. (c) Overlay of the PA image obtained during movement on an MRI image of the monkey's brain. The dark line circle shows the position of the rim of the cranial chamber. (d) PA B-mode functional image superimposed on an MRI cross-section through the central sulcus. The black line in Fig. 2(a) shows the location and orientation of the section. The B-mode image was acquired with the task off. The dimension of the PA image is 12×12 mm. (e) The same image as (d) but obtained during performance of the reach-to-grasp task. (f) Changes in the average PA signal obtained from M1 and SI cortex averaged over the regions outlined by the dotted lines in (a) and (b). Note that the area encompassed by the dotted line is largely M1 but includes a small part of premotor cortex anteriorly.

spatial localization than the activation observed during actual task performance. The origin of the signal during the task off condition is not clear. The monkey was largely at rest but over the period of 7 min required for the scan there were small movements or isometric contractions or low level muscle activation that would not be readily observable. It is also possible that even though the task was off, the monkey might have been anticipating a return to active task performance and the activation observed in this condition was related to anticipation.

Depth-resolved images are shown in Fig. 2(d) and 2(e). PA B-mode images are overlaid on a parasagittal section taken from the reconstructed MRI of the brain. The structural MRI image shows a cross-section through the arcuate sulcus (AS) and central sulcus (CS) at the level marked by the black line in Fig. 2(a) and 2(b). Figure 2(d) is a reference image acquired with the monkey at rest and Fig. 2(e) is the image acquired from the same location during performance of the reach-to-grasp task. During performance of the reaching task, PA signal amplitude in motor cortex anterior to the central sulcus (CS) increased. In this image, average PA signal amplitude in M1 increased by 45.8% and by 35% in SI. The level shown in the figure was selected because it encompassed an area of motor cortex that was relatively quiet in the task off condition but activated during active task performance. Clearly, the specific increase observed will depend on the level of the parasagittal section.

4 Discussion and Conclusions

The images shown are averaged images obtained on the same day within 1 h. We also performed the experiment in different days and observed similar results, which further confirmed the feasibility of the technique. Several problems have to be overcome in applying PA imaging to the awake monkey performing a behavioral task. Motion artifacts due to the head movement of the monkey induce blurring of PA images. To maintain comfort of the monkey, our head fixation system was not totally rigid but allowed small movements in all directions. These movements may induce motion artifacts and reduce the spatial resolution. These artifacts were largely eliminated by designing an adaptor for the scanning device that attached directly to the recording chamber implanted on the monkey's skull. In addition, because the recording sessions were time restricted, the numbers of scans for averaging were also limited.

In the future, an important improvement in our approach could be achieved using an ultrasonic array transducer. Using a multi-channel system would reduce scanning time and provide quantitative data for various functional images in real-time. Real-time imaging will provide excellent temporal resolution and significantly reduce motion artifacts caused by monkey head movement. In addition, another future work would be using multiple laser wavelengths to measure blood oxygenation saturation in the brain,[13] and it should allow us to gain more information about the brain functions.

Another possible future improvement will be performing transcranial PA imaging of monkey brain. Several research results have shown the feasibility of transcranial PA imaging for monkey brain.[14–16] At the current stage, the imaging resolution and imaging depth is still limited because of the skull effect. However, as more advanced algorithms become available, the above mentioned limitations could be resolved.

In conclusion, we have shown that PA imaging can be used for mapping brain functional activity in awake, behaviorally performing monkeys. PA imaging detects changes in hemodynamics associated with neuronal activation within the brain. We demonstrated activation of primary motor cortex and primary somatosensory cortex during performance of a task involving reaching and grasping using the contralateral forelimb. Our results establish the feasibility of using PA imaging as a valid method for identifying and mapping task related areas of cortical activation in nonhuman primates.

Acknowledgments

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