Behavioural Brain Research 317 (2017) 327–331

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

# Prefrontal cortical activity associated with visual stimulus categorization in non-human primates measured with near-infrared spectroscopy

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## HIGHLIGHTS

- NIRS can be applied to investigate brain activity in non-human primates.
- NIRS can be a useful tool for direct comparison between animals and humans.
- The prefrontal cortex exhibits neural activity associated with visual categorization.

## ARTICLE INFO

*Article history:*
Received 31 May 2016
Received in revised form
28 September 2016
Accepted 30 September 2016
Available online 1 October 2016

*Keywords:*
Animal model
Psychiatric disorder
Biomarker
Cognitive function
Prefrontal cortex

## ABSTRACT

In biomedical research of brain dysfunction in psychiatric disorders, utilization of animal models is essential. However, translation of findings in animal models into the realm of human clinical conditions requires reliable biomarkers that are assessed with the methods mutually employed in animal models and human patients. Near-infrared spectroscopy (NIRS) is a functional neuroimaging technique that has now been widely utilized in human basic and clinical research. However, its application to animal models has been barely conducted. In this study, we developed the method to measure neural activity in the cortex of Japanese macaques using NIRS, and examined cortical responses to presentation of a set of visual stimuli that were categorized into four different groups (flower, monkey, snake, food). Prefrontal cortical (PFC) oxy- and deoxy-hemoglobin changes were found to reliably distinguish the categories of these visual stimuli. The results suggest that cortical activity measurement with NIRS in primates can be a valuable model for identifying biomarkers associated with psychiatric disorders.



## 1. Introduction

Although various animal models have been available for understanding biological mechanisms underpinning psychiatric disorders [1,2], findings achieved with such animal models often have problems in translating into the realm of human psychiatric patients, as to what extents the findings in the animal models accurately reflect the pathogenesis and pathophysiology of human psychiatric conditions are unclear in many cases. Thus, identification and detection of biomarkers that can be achieved both in animals and humans, using the identical methods and conditions are crucial in further progress of biomedical research [3].

Near-infrared spectroscopy (NIRS) is a technique that measures oxygenation/deoxygenation of blood hemoglobins, which is correlated with neural activity, in the cortex of the brain [4,5]. Although this technique still faces with several major limitations such as it can measure neural activity only in the cortical surfaces with low time resolution, because of its relative easiness of use and safety, which is even applicable to small children and infants [6,7], NIRS has now been widely used in both basic and clinical research in human subjects. With these facts, measurements of neural activity using NIRS are thought to be promising biomarkers for diagnosis of psychiatric disorders and translation of animal studies into the realm of human clinical setting. On the other hand, there are very few studies that have applied NIRS in animal models.

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http://dx.doi.org/10.1016/j.bbr.2016.09.068
0166-4328/

In this study, as an initial step for establishment of biomarkers that are assessed with the methods mutually employed in animal models and human patients, we have developed the method to apply NIRS in non-human primates and measured cortical activity associated with categorization of visual stimuli, which contained emotionally provoking ones. Indeed, disruption of affective processing has also been suggested in a number of psychiatric disorders [8–11], and thereby investigations with the emotionally provoking images could yield important insights. The electrophysiological studies in primates that have shown that prefrontal cortical (PFC) neurons exhibit neural activity associated with categorization [12]. In addition, the PFC also processes affective significance of the stimuli, which has been demonstrated using NIRS [13–15]. Thus, we hypothesized that such neural activity associated with categorization of visual stimuli could also be detected in the PFC area using NIRS.

## 2. Material and methods

### 2.1. Subjects

All experiments were conducted in accordance with the *Science Council of Japan Guidelines for Proper Conduct of Animal Experiments* and approved by the Kyoto University Primate Research Institute Animal Experiment Committee. NIRS were conducted in three adult female Japanese macaques aged at 3 to 5 years old. Monkeys were trained to accustom to sit on a monkey chair, and stay quiet for up to 30 min per day.

### 2.2. NIRS

The setup (Hitachi ETG-100), which that has originally been developed for human use, was used for recordings in primates. We designed the custom-made NIRS probe holder that fit to the skull of each monkey (Fig. 1A, B), with 9 probes allowing 12 points of measurements (Fig. 1B). Considering a macaque brain size, which is substantially smaller than that of a human brain, the distance between an emitter and a detector was set to 1.5 cm. Oxygenated (oxy-Hb), deoxygenated (deoxy-Hb), and total hemoglobin concentrations were recorded in the PFC area (pf1-2; Fig. 1B), (2) anterior frontal cortical area (f1-5; Fig. 1B), and (3) posterior frontal – anterior parietal area (fp1-5; Fig. 1B).

We determined the distance of 1.5 cm between sources and detectors based on the previous study from our institute showing that the distance of 1 cm was sufficiently able to measure cortical activity [16], in which case, it has been estimated that recordings were obtained up to 5 mm below the scalp based on the model by Okada et al. [17]. Thus, cortical activity we measured in this study has been estimated to be even deeper than 5 mm below the scalp.

Adjustments of laser power and detection threshold as well as confirmation of whether detection was at an appropriate optical level were achieved by the program implemented in the machine. Using this program, we confirmed that optical signals were not saturated with the distance of 1.5 cm between sources and detectors.

Since the best strategy to prevent this problem was to minimize physical movements of subjects as much as possible, we trained the monkeys not to move in the monkey chair, and keep focusing on the monitor beforehand of recordings. During recordings, we continuously monitored movements of monkeys with video-cameras, and even if there was any slight movements of heads, bodies, and mouths, these recordings were discarded, and additional recordings were conducted. In addition to this, to further ensure for elimination of potential motion artifacts, a high pass filter at the frequency of 0.01 Hz was applied at off-line analysis, as we observed that motion artifacts were reflected as a large drift of basal level of signals, rather than fast signal changes.

### 2.3. Visual stimuli

Cortical responses to presentations of visual stimuli were recorded with NIRS. Visual stimuli were divided into 4 different categories; snakes, monkeys, foods, flowers, with each category consisting of 6 different photos. These photos were semi-randomly presented to the subjects, in the 14" LCD monitor that was placed approximately 50 cm apart in front of the subject's eyes. During visual stimulus presentation and NIRS recordings, the room light was off, and experimenters controlled the equipments from outside of the room. Whether the subjects gazed on the LCD screen was continuously monitored with the CCD camera. First, the subjects were trained for attaching NIRS probes and maintaining to gaze the LCD screen for a minimum of 5 min, with intermittent rewards (drops of apple juice) during this period. Then, once the subjects were able to keep focusing on the monitor, visual stimuli were presented. Each trial consisted of 20 s of the gaze fixing stimulus (a red circle in the center of the LCD screen) followed by 20 s of photo presentation, and then 20 s of the resting period. One session consisting of four trial was administered per day. A photo in each trial of a session was picked up from each category (thus, one photo each of snake, flower, food, and monkey), and these photos were presented in random order for each session. No subjects had presentation of the same photographs more than twice.

### 2.4. Data analysis

All data analyses were conducted off-line. Binary data of the recordings were generated and used for statistical analyses using Origin Pro ver9.0 and Statistica ver7.0 software.

## 3. Results

Although we obtained NIRS recordings from the PFC and other cortical areas, we specifically focused on analyzing the data from the left and right PFC (pf1-2 in Fig. 1B). Fig. 1C and D are examples of oxy-Hb and deoxy-Hb responses in the left PFC, showing clear increases and decreases of oxy-Hb and deoxy-Hb, respectively, were evoked upon presentations of snake photos.

The left and right PFC exhibited distinct oxy-Hb and deoxy-Hb changes in responses to visual stimuli in different categories. Fig. 2A and B show average traces of oxy-Hb and deoxy-Hb changes, respectively, in the PFC (left and right hemispheres combined) in responses to all visual stimuli in each category obtained from all subjects. Significant difference in oxy-Hb ($F_{3,68} = 4.34$, $p = 0.007$) and m deoxy-Hb ($F_{3,68} = 4.12$, $p = 0.010$) changes were found between different categories of visual stimuli. Post-hoc Tukey analyses have revealed that oxy-Hb increase in response to snake was significantly larger than those to food ($p = 0.019$; Fig. 2C) and monkey ($p = 0.015$; Fig. 2C), whereas deoxy-Hb decrease in response to food was significantly larger than flower ($p = 0.006$; Fig. 2D).

Oxy-Hb and deoxy-Hb signals have been shown generally correlated negatively when these signals were associated with cortical activity [18]. Thus, linear regression analyses between oxy- and deoxy-Hb changes in each category of visual stimuli were examined. These analyses unveiled a marginally significant negative correlation ($r = -0.463$, $p = 0.053$; Fig. 3D) in flower images, whereas a significant positive correlation was observed ($r = 0.608$, $p = 0.007$; Fig. 3A) in snake images. No correlation was observed in food ($r = -0.056$, $p = 0.825$; Fig. 3B) and monkey ($r = 0.025$, $p = 0.921$; Fig. 3C) images.