

# In Vivo Imaging of Biophoton Emission in the Whole Brain of Mice

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## ABSTRACT

In recent years, studies have demonstrated that biophoton is a medium for the transmission and processing of neural information. However, such studies were mainly carried out by using brain slices combined with biophoton imaging technology, while there are few reports on *in vivo* brain biophoton imaging. In this study, the ultraweak biophoton imaging system (UBIS) was employed to carry out an *in vivo* biophoton imaging for the whole brain of mice. It was found that the biophoton emission of whole brain in the slightly anesthetized mice was significantly higher than that of the background, suggesting that the brain of living mouse emits a certain intensity of stable biophotons. The biophoton imaging established in this study for the *in vivo* mouse whole brain may provide a new technical method for further study of the relationship between the biophoton and brain functions, and give new ideas for developing diagnostic method of neuropsychiatric diseases.

## 1. INTRODUCTION

Biophoton is the short term of ultraweak photon emission (UPE), which is the light quantum radiated from the organisms spontaneously, and widely exists in animals, plants, microorganisms and other living organisms [1-3]. There have been many reports [4, 5] on the early use of photomultiplier tubes (PMT) as detection technology to detect biophoton in different tissues, organs and cells of animals and plants, but the study of the relationship between biophoton and brain functions had been hampered by technical limitations, and a systematic study of biophoton related to brain functions was not possible until the *in situ* biophoton autoradiography and ultraweak biophoton imaging techniques were established [6-15]. However, current techniques can only be used at the *in vitro* level with perfused brain slices, and it has been technically difficult to detect biophoton in the brain of living animal. Therefore, the development of *in vi-*

*in vivo* biophoton detection technology is necessary to the understanding of the relationship between the biophoton and the high functions of nervous system. In this study, we report here that real-time dynamic imaging, detection and analysis of the biophotons emitted from the whole brain of slightly anesthetized mice can be implemented by using modified ultraweak biophoton imaging system (UBIS).

## 2. MATERIALS AND METHODS

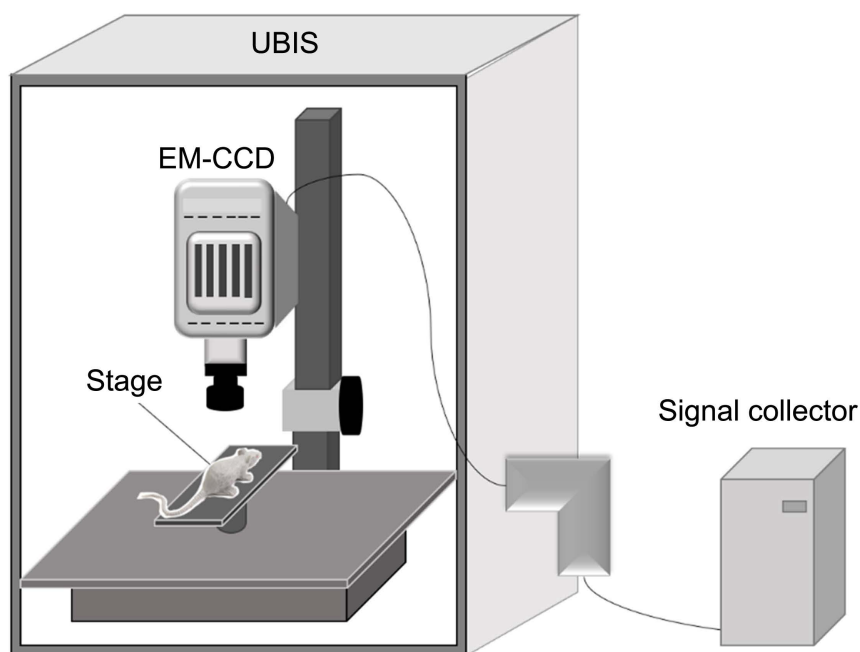
### 2.1. Mouse Craniotomy

Kunming male mice, 2 - 3 months old, were purchased from Hubei Provincial Laboratory Animal Public Service Center (Wuhan, China) and housed in a room with a 12 h light/dark cycle (lights on at 7:00 AM) with access to food and water *ad libitum*. The study protocol was approved by the Committee on the Ethics of Experimental Animals and Biomedicine of South-Central University for Nationalities.

The mice were weighed and anesthetized by intraperitoneal injection of sodium pentobarbital solution. The animals were then placed on a mouse brain stereotactic device and the hair on the top of the mouse head was shaved with a razor to expose the scalp, which then was disinfected with 75% alcohol and cut along the midline with surgical scissors. After carefully separating the subcutaneous tissue and the musculature of the skull, the fascia on the surface of the skull was removed with 8% hydrogen peroxide solution. A small hole was carefully made in one side of the skull with a skull drill, and then bilateral parietal bones were removed with a bone-biting forceps to fully expose the cerebral cortex of the mouse from the part of the olfactory cortex to the anterior part of the cerebellum.

### 2.2. *In Vivo* Biophoton Imaging of Whole Brain

The whole brain of mouse was imaged with an appropriately modified UBIS according to the previous report (Figure 1) [7]. The mouse with the head fixed on a mouse brain stereotactic device was put into the dark box of UBIS and then the mouse whole brain was imaged with an EM-CCD (Hamamatsu) as the imaging camera. The specific imaging parameters were as follows: 1) EM-CCD cooling temperature is  $-95^{\circ}\text{C}$ ; 2) 1200 $\times$  gain; 3) the exposure time is 900 s for each frame of image; 4) imaging time course is 2 h.



**Figure 1.** Schematic drawing of the *in vivo* biophoton imaging system for mouse whole brain. This system was appropriately modified according to the previous report [7].

### 2.3. Image Processing and Data Analysis

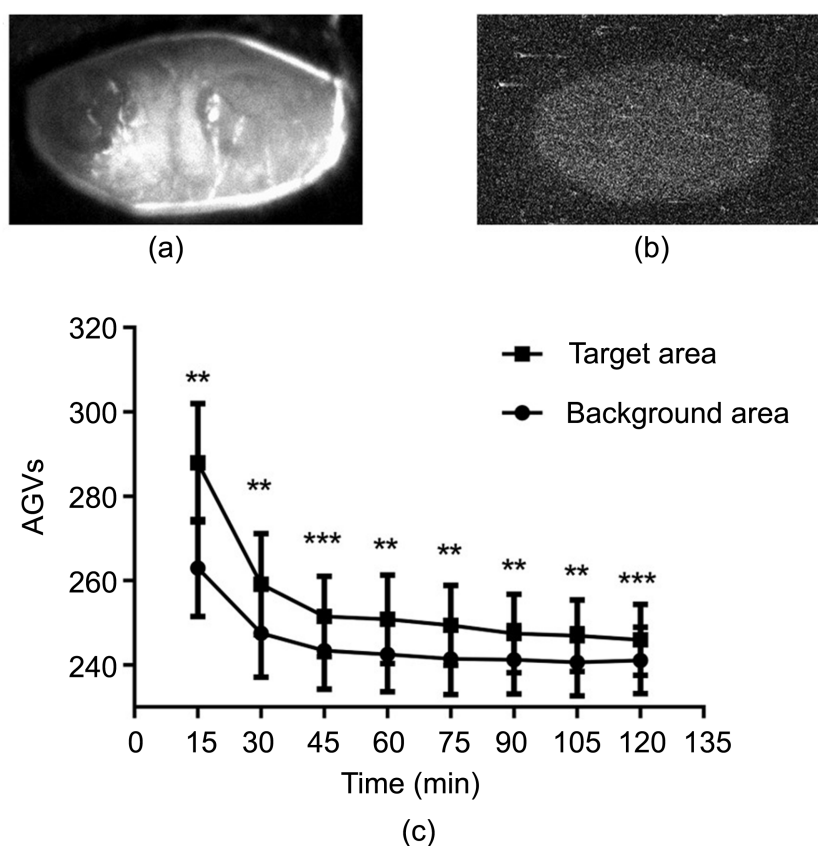
A series of images were processed using the previously reported methods [7], including the removal of bright spots caused by the cosmic radiation, and the extraction of gray values from the whole brain region and non-brain region of the images. The data was stored in MS-Excel files for further analysis.

### 2.4. Statistical Analysis

Statistical analyses were performed using Microsoft Excel and two-tailed paired T-test was used to compare the differences between the whole brain area and the background area at the different time points.

## 3. RESULTS

The biophoton emissions from the mouse whole brain were detected very sensitively by using the modified UBIS. During the first 30 minutes of imaging, the biophoton emissions decreased rapidly, and remained at a relatively stable level after 45 minutes until the end of imaging. The biophoton emissions of whole brain area at the different time points were significantly higher than that of the background region (Figure 2, Table 1,  $n = 7$ ). These results suggest that a certain amount of biophotons were emitted from the *in vivo* mouse whole brain.



**Figure 2.** Biophoton imaging of mouse whole brain. (a) A representative regular image of imaging area of mouse whole brain; (b) A representative image of biophoton imaging, showing the whole brain area (bright area) and background area (dark area) (exposure time = 900 s); (c) The dynamic changes of biophoton emissions from the whole brain area (target area) and the background area at the different time points. AGVs: average gray values, \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ .

**Table 1.** Comparison of gray values between the mouse whole brain area (target area) and background area at the different time points.

| Time (min) | Target area    | Background area | p value    |
|------------|----------------|-----------------|------------|
| 15         | 287.91 ± 37.19 | 262.96 ± 30.43  | 0.0020**   |
| 30         | 259.12 ± 31.75 | 247.55 ± 27.59  | 0.0017**   |
| 45         | 251.46 ± 25.26 | 243.42 ± 24.23  | 0.00097*** |
| 60         | 250.79 ± 27.82 | 242.47 ± 23.41  | 0.0033**   |
| 75         | 249.40 ± 24.95 | 241.46 ± 22.54  | 0.0025**   |
| 90         | 247.50 ± 24.75 | 241.23 ± 21.48  | 0.0051**   |
| 105        | 246.95 ± 22.46 | 240.61 ± 21.12  | 0.0062**   |
| 120        | 245.96 ± 22.37 | 241.03 ± 20.97  | 0.00053*** |

Asterisks indicate a significant difference between target area and background area, \*\*p < 0.01; \*\*\*p < 0.001.

#### 4. DISCUSSION

In this study, we have obtained the imaging signals of biophoton emissions from the mouse whole brain under the condition of slight anesthesia with the modified UBIS, indicating that the *in vivo* mouse whole brain can emit obvious biophotons, which originate highly likely from the cerebral cortex although the contribution of subcortical areas cannot be excluded. These findings may provide an important foundation and technical means for further study of the relationship between biophoton and brain functions.

It was also found that, within 30 minutes after the start of imaging, the biophoton emissions showed a rapid decline. The reason for such a change was that the delayed luminescence from the dark box of UBIS may contribute to most imaging signals, which were also detected in our previous study [7]. Therefore, the data of first 30 minutes was usually eliminated for further analysis if the imaging process was immediately started after closing the dark box of UBIS, otherwise, an imaging process was started after approximately 30 minutes of waiting. In addition, the technique developed in this study can only perform a two-dimensional image of a mouse whole brain, and it is not yet possible to construct a three-dimensional image.

By using different techniques, including the UBIS, we have demonstrated that the biophotonic activity can be transmitted along neural circuits and is associated with higher brain functions [7-15]; however, such findings based on the studies on *in vitro* brain tissues have not been further validated at the *in vivo* level, mainly due to the limitation of technology. Therefore, the establishment of this technique provides a new method for us to further study the biophoton emissions of *in vivo* brain related to the perception, transmission and integration of information, and it also provides a basis for the implementation of multi-modal real-time biophoton monitoring of animal brain *in vivo*. In addition, the development of three-dimensional biophoton imaging of the whole brain is of great significance in the diagnosis of neuropsychiatric diseases.

#### ACKNOWLEDGEMENTS

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## CONFLICTS OF INTEREST

The authors declare no competing interests.

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