Neuroscience Research 75 (2013) 65–68

Contents lists available at SciVerse ScienceDirect

Neuroscience Research

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

Technical note

# A simple optogenetic system for behavioral analysis of freely moving small animals

Yuya Kawazoe[a], Hiromu Yawo[b], Koutarou D. Kimura[a,*]

[a] Department of Biological Sciences, Osaka University, Osaka 560-0043, Japan
[b] Department of Developmental Biology and Neuroscience, Tohoku University Graduate School of Life Sciences, Sendai 980-8577, Japan

ARTICLE INFO

*Article history:*
Received 12 March 2012
Received in revised form 3 April 2012
Accepted 10 April 2012
Available online 18 May 2012

*Keywords:*
Optogenetics
Behavior
LED
Channelrhodopsin
*C. elegans*
CCD camera

ABSTRACT

We present a new and simple optogenetic system for the behavioral analysis of small animals. This system includes a strong LED ring array, a high-resolution CCD camera, and the improved channelrhodopsin ChRGR. We used the system for behavioral analysis with the nematode *Caenorhabditis elegans* as a model, and we found that it can stimulate ChRGR expressed in the body wall muscles of the animals to modulate the behavior. Our results indicate that this system may be suitable for optogenetic behavioral analysis of freely moving small animals under various conditions to understand the principles underlying brain functions.



## 1. Introduction

Optogenetic techniques are extremely useful for proving that a particular neuronal activity pattern is the causal reason for a specific brain function (Nagel et al., 2005; Zhang et al., 2007a). In optogenetic analysis, a light-driven ion channel or ion pump is expressed in a particular neuron such that exposure to light causes depolarization or hyperpolarization of the neuron on demand.

The nematode *Caenorhabditis elegans* is one of the best models for optogenetic analysis of neural function because of its fully characterized neuronal wiring, its transparent body, and the availability of sophisticated genetic engineering techniques for this animal (Nagel et al., 2005; Zhang et al., 2007b). Standard systems for optogenetic analysis of *C. elegans* behavior include a microscope with a strong light source and a computer-controlled motorized stage to keep an animal in the field of view (Kuhara et al., 2011; Nagel et al., 2005; Stirman et al., 2011; Zhang et al., 2007b). However, such systems have some disadvantages: they limit observation to a single animal per assay, and the motorized stage is not suitable for behavioral analysis of animals under a delicate signal gradient.

To overcome these limitations, we established a new simple system for optogenetic behavioral analysis, involving a high-resolution CCD camera for image acquisition, a strong LED ring array for photostimulation, and an improved channelrhodopsin, ChRGR (Wen et al., 2010). The system developed in this study can stimulate ChRGR expressed in the body wall muscles of *C. elegans* and monitor the effects of the channelrhodopsin with proper temporal and spatial resolution. Because of its simplicity, this system can be easily applied to optogenetic behavioral analysis in small animals that move in 2D space, such as *C. elegans*, *Drosophila* larvae, and zebrafish in a shallow water space, under various experimental conditions, for example, (1) under a delicate gradient of signals such as odor and temperature, (2) interactions among multiple animals, and (3) parallel long-term or large-scale analyses.

## 2. Materials and methods

### 2.1. Strains

The techniques used for culturing and handling *C. elegans* were as described previously (Brenner, 1974). Wild-type *C. elegans* variety Bristol strain (N2) was obtained from the Caenorhabditis Genetics Center (University of Minnesota, USA).

* Corresponding author at: Department of Biological Sciences, Osaka University, 1-1 Machikaneyama, Toyonaka, Osaka 560-0043, Japan. Tel.: +81 6 6850 6706; fax: +81 6 6850 6769.
*E-mail address:* kokimura-lab@umin.ac.jp (K.D. Kimura).

0168-0102/$ – see front matter 
http://dx.doi.org/10.1016/j.neures.2012.04.011

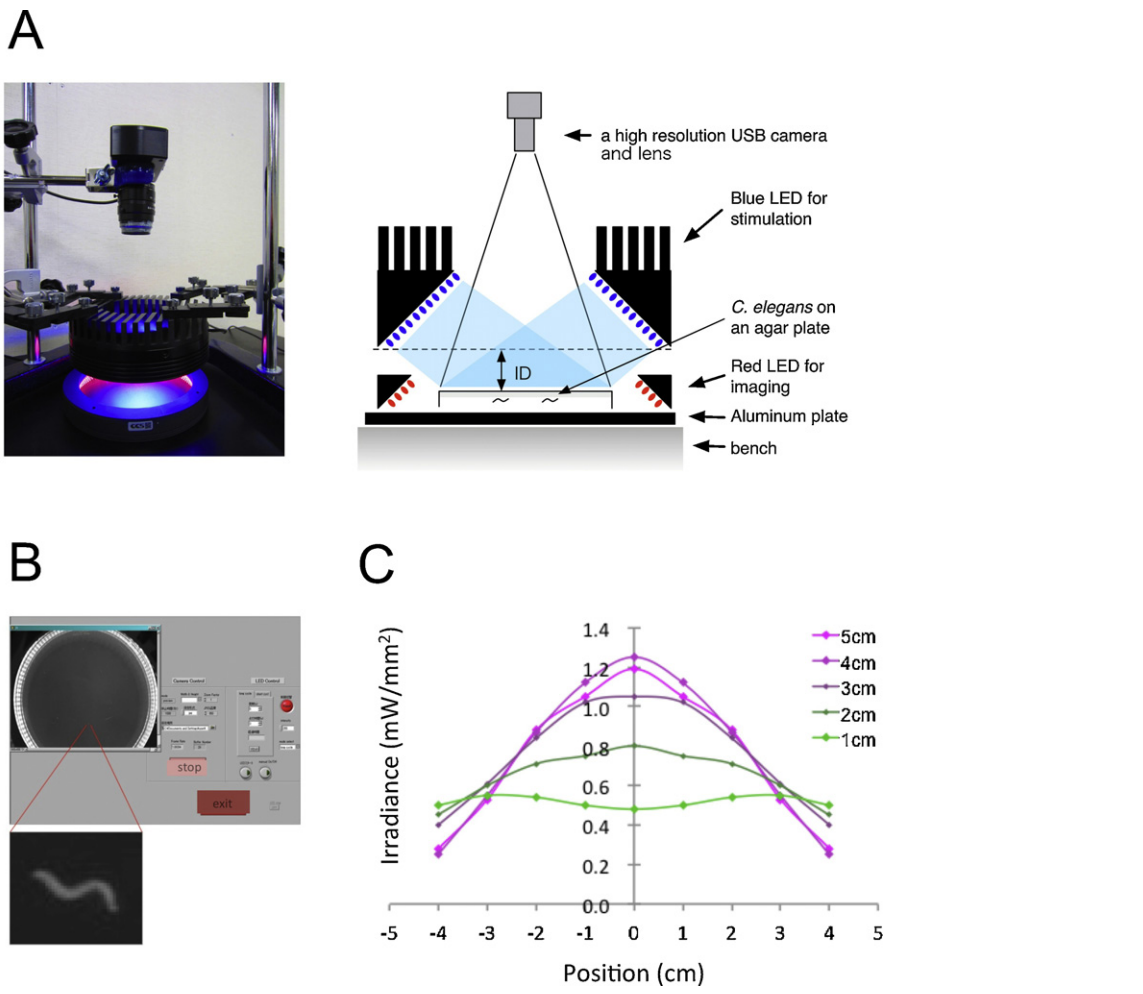


**Fig. 1.** (A) Photograph (left) and schematic drawing (right) of the tracking system with a strong blue LED array. The red LED array at the bottom is for visualization (Kimura et al., 2010). The irradiation distance (ID) is the distance between the bottom of the blue LED ring array and the surface of the agar plate. (B) An image of a worm captured using the system. A part of the acquired image was magnified. (C) Irradiance property of the blue LED. At ID = 1 cm, most of the 9-cm plate area is illuminated at 0.5 mW/mm$^2$. At ID = 3 cm, a circle of diameter 2 cm is illuminated at >1 mW/mm$^2$. Note that a regular LED ring array, such as the one shown at the bottom of the panel, can expose a 9-cm plate at a maximum of 0.1 mW/mm$^2$.

### 2.2. Molecular biology and germline transformation

ChopGR-Venus cDNA (Wen et al., 2010) was amplified from pchopGR using PCR and inserted into the pPD49.26 vector along with the *myo-3* promoter from pPD115.44 to generate pKDK394; the *myo-3* promoter was used for expression in the body wall muscles. The pPD vectors were kind gifts from Dr. A. Fire (Stanford University, USA). Germline transformation was performed using microinjection (Mello et al., 1991). The injection solution contained 30 or 50 ng/μL of pKDK394, 10 ng/μL of *lin-44p::GFP* (a kind gift from Dr. M. Koga at Kyushu University, Japan) as a marker, and *Pvu*II-cut N2 genomic DNA or pBluescript plasmid DNA as a carrier, for a total DNA concentration of 100 ng/μL. This transformation yielded 2 independent transgenic lines—OSK13477 and OSK40378—that were used in the study.

### 2.3. Behavioral analysis

Because all-*trans*-retinal (ATR) should be added exogenously for *C. elegans*, ATR was included in the standard nematode growth medium (NGM) agar plates, in accordance with a previous report (Nagel et al., 2005) with the following modifications. Concentrated *Escherichia coli* OP50 (grown in 100 mL of LB medium overnight at 37 °C and then spun down and suspended in 5 mL of $dH_2O$) was mixed with 20 mM ATR dissolved in EtOH to a final concentration of 100 μM. An OP50 suspension lacking ATR was also prepared in a similar manner. Next, 10 mg/mL streptomycin was added at a final concentration of 250 μM to prevent contamination by other bacteria. Then, 200 μL of the OP50 solution with or without ATR was poured on each NGM plate, and the plates were dried for several hours in the dark. Transgenic marker-positive animals cultivated on regular NGM plates with OP50 were transferred to the plates seeded with OP50 with or without ATR and incubated at 20 °C for 18 h in the dark. On the next day, 3 animals per assay were transferred from the plate to a 9-cm NGM plate (2.5% agar) without OP50 or ATR. Each animal's behavior was recorded using our optogenetic system (see below) for 40 s as follows: no blue light stimulation for the first 20 s followed by blue light stimulation for 20 s. Then, the *X*–*Y* coordinates of the centroids of the animals in each image were measured off-line using the Move-tr/2D software (Library Inc., Japan). The linear distance between the start and end points of the animal's location was regarded as the distance the animal had migrated. Statistically significant differences in the migratory distances between the blue-light stimulated group and the non-stimulated control group were evaluated using the Mann–Whitney *U* test with Prism ver. 5.0 for Mac OSX (GraphPad Software, USA).