A simple head-mountable LED device for chronic stimulation of optogenetic molecules in freely moving mice.

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Abstract

We describe a low-cost, small, remotely triggerable LED device for wireless control of transcranial optical stimulation of cortical neurons, for use in freely moving mice. The device is easily mountable on the head of a mouse with a high-polymer block. Using the Thy1-ChR2-YFP transgenic mice, we demonstrate that the device is capable of remotely triggering muscle twitches upon activation of the primary motor cortex in freely moving conditions.

Introduction

Activation or silencing of a brain region or cell population is a promising approach for deciphering brain functions in experimental animals (Crick, 1979). Traditionally, activation of a brain area has been achieved by electrical stimulation through a stimulation electrode, and silencing of a brain area has been performed by a physical lesion. With the advent of optogenetic techniques, activation and silencing of neurons can be accomplished by illuminating neurons that express light-activated ion channels (e.g. channelrhodopisin-2, ChR2) or pumps (halorhodopsin) (for a review see, Zhang et al., 2007). Illumination of target neurons is generally achieved using optic fibers (Gradinaru et al., 2007) or a focused laser beam through a microscope (Ayling et al., 2009; Hira et al., 2009). Although these methods permit accurate positioning of the illumination spot, the behavior of experimental subjects is compromised *in vivo* conditions. To control neural activity in freely moving mice that express ChR2, we designed a simple, wireless, and head-mountable light-emitting diode (LED) module. We demonstrate that our device targeted to the primary motor cortex (M1) can trigger muscle twitches in anesthetized and awake Thy1-ChR2-YFP transgenic mice (Arenkiel et al., 2007; Wang et al., 2007).

Methods and results

The wireless stimulation system consists of two units: a transmitter and a receiver. The transmitter (Fig. 1A) is composed of an oscillatory circuit which outputs alternating TTL signals at 38 kHz (OSC), a push button module for manual triggering (MAN), a TTL input module for external triggering (TRIG), and an infra-red (IR)-LED output module (OUT). The MAN module is equipped with a variable resistor to control the pulse width between 20 and 60 ms. The OSC, TRIG, and MAN are combined via an AND logic gate and the output is used to trigger the OUT module through a field effect transistor (2SK2414-AZ, NEC). The transmitter is powered by four standard AA batteries in series. The OUT module has an extra output port for additional IR-LEDs (Fig 1B). The port allows an extension cable with a multiple number of IR-LEDs to surround an experimental apparatus, such that the IR transmission signal can reach the receiver unit at any angle.

The receiver unit is intended to be small in size so that it can be mounted on the skull of a freely moving mouse (Fig 1C, D, Supplementary Video 1). The receiver unit is composed of a low-power consumption IR reception module (GP1UD271XK, Sharp), an analog switch (ADG702BRTZ, Analog Devices), and a high-intensity 3 mm diameter blue LED (NSPB300B, Nichia). In addition, bypass-capacitors (100 ~~pF~~nF) are placed for semiconductor parts and a current limiting resistor (120 Ω) is placed in series with the LED (Fig. 1C). The device is designed to be operated with a supply

voltage of between 5 V and 6 V. In practice, power is supplied by two serially connected CR1025 lithium batteries (1.4 g, Panasonic) in a custom-designed housing (0.8 g). Together, the total mass of the receiver is 3.1 g.

We confirmed that the receiver unit can receive the IR-transmitter signal from at least two meters away and emit a bright blue light with a delay of 170−180 μs (detected by a Schmitt-trigger IC SFH 5440, OSRAM). Using a signal generator (Master-8, A.M.P.I.), we sent TTL pulses with arbitrary duration (300 μs − 300 ms) to the TRIG module and found that the receiver reliably emitted corresponding blue LED pulses. To evaluate the durability of the receiver, we examined use-dependent changes in the emission power of the blue LED (Fig. 2A). We triggered 10,000 pulses of 25 ms width with an interval of 50 ms and measured the emission power of 300 ms pulses (PM100D, Thorlabs). The initial average power supplied by a new set of batteries was 7.1 mW. The LED output power remained above 7.0 mW until 30,000 pulses, after which the average power gradually dropped (Fig. 2 A). These results show that the battery life is sufficient for standard behavioral experiments using rodents. At 100,000 pulses, the average power dropped down to 5.5 mW. To estimate whether the emission power is adjustable by pulse width modulation (PWM), we triggered 400 μs LED pulses with variable intervals (600 μs − 4 ms) during 300 ms. The average power linearly increased with the duty cycle (Fig. 2B).

We next sought to verify whether the device works in experimental animals. To this end, we anesthetized a heterozygote Thy1-ChR2-YFP mouse (10 months old, male) from a transgenic mouse line previously demonstrated to exhibit muscle movements upon optical stimulation in M1 (Hira et al., 2009), with a ketamine cocktail (dosage: ketamine 70mg/kg and xylazine 10mg/kg, i.p.). The mouse was rigidly fixed in a stereotaxic frame (Narishige), and a scalp incision was made to expose the skull. Using a dental drill, the skull above M1 (AP -0.5 mm ML 0.7 mm) was thinned to the extent that surface blood vessels became visible. The skull was wetted with physiological saline (0.9% NaCl) to maintain transparency of the skull. A polymer optical fiber (2 mm diameter, Eska, Mitsubishi Rayon) coupled with a high-power blue LED (Luxeon K-2 series, driven with 700 mA current provided by an AudioQ AQPD-700 power LED driver) was placed just above the thinned skull (~0.5 mm gap distance). A brief flash of 50 ms of blue light (average power: 4.0 mW) was enough to cause a visible muscle twitch in the contralateral side of the body, including the hindlimb. The hind limb muscle movement was monitored by electromyography (EMG) using an amplifier (ELC-03XS, NPI) as shown in Fig. 3A. Next, we replaced the optic fiber with the receiver and positioned the light-emitting part above the thinned skull in a similar manner. Both manual- and TTL-driven remote transmission of a brief IR pulse (20 − 100 ms) from the transmitter was able to trigger the bright LED and a subsequent muscle twitch (Fig. 3B). In such an ~~this~~ experiment, we could trigger a muscle twitch in all 39 trials.

Next, we implanted the receiver on two Thy1-ChR2-YFP mice (> 3 months old) for use in freely

moving conditions. The mice were anesthetized with isoflurane (1.5 – 2.0 %). Thinning of the skull above M1 was performed as described above. The exposed skull was treated with a surface activator (Super-Bond C&B, Sun Medical). A small block (7 × 7 × 5 mm, width × length × height) of a high-polymer eraser (ZEH05 regular type, Pentel) with a 2.8 mm hole in the middle was attached with a fast acting adhesive (Aronalpha Extra, Konishi) so that the hole was aligned to the thinned skull area. Two days after the surgery, the LED portion of the receiver was inserted to the hole of the implanted mounting block. We placed the animal in an open environment (47.5 × 47.5 cm). After 30 minutes, the animal intermittently stopped exploring the environment. When we remotely triggered the blue LED (60 ms pulse width), the animal had a visible whisker movement and started exploring (data not shown). In the other mouse, we restricted its movement by placing it on a raised platform (16 cm high, 6.5 × 6.5 cm platform). After 30 minutes of habituation, the animal became immobile. When blue LED flashes were remotely triggered (100 ms × 2), we could initiate whole body muscle twitches (supplementary video). In this experiment, we could trigger whole body movements in 74 out of 114 trials (65% success rate). In 28 occasions, we could detect visible whisker movements that did not accompany whole body movements after LED flashes. Including these events, the probability that a visible movement occurred due to optical stimulation was close to 90%. We note that elicited responses were more visible when the mouse was at rest, and less evident during motor movements. All experimental protocols were approved by the RIKEN Institutional Animal Care and Use Committee.

Discussion

We designed a simple, wireless, head-mountable device for use in optogenetics experiments in freely moving mice. Although the device is readily used in experiments, there is room for improvement. First, a further reduction in weight of the receiver is desired. The current design employs two CR1025 batteries (~0.7 g each) to achieve a supply voltage of above 5 V. Indeed, the battery unit contributes the largest weight. The operating voltage of 5 V was chosen as we initially intended to use a higher power LED (for instance, a Luxeon K2, which has a forward voltage of ~3.4 V). It turned out that the smaller LED (NSPB300B) emitted brighter light in the battery-operated condition. As all the listed semiconductor parts can operate with voltage < 3 V, it should be feasible to power the receiver unit with one 3V battery using appropriate resistors. Second, a further reduction in physical size can be realized. The receiver circuit in Fig. 1C is soldered by hand. Miniaturizing the receiver can be achieved by introducing a printed circuit board (PCB) design with surface mount devices. Indeed, smaller LEDs are available as surface mount devices. To this end, we are currently in the process of developing a PCB design. Third, the use of multi-color LEDs may allow for the use of transgenic mice with different optogenetic proteins and different activation

wavelengths. Such improvements could facilitate the use of multiple receivers in experimental animals and allow more sophisticated chronic behavioral experiments to be conducted.

Acknowledgements

Footnotes

YI, HO, MH, and HH declare no financial conflict of interest with regard to this manuscript. SH is an employee of Yatoro Electronics who can accept commercial orders of the receivers and transmitters.
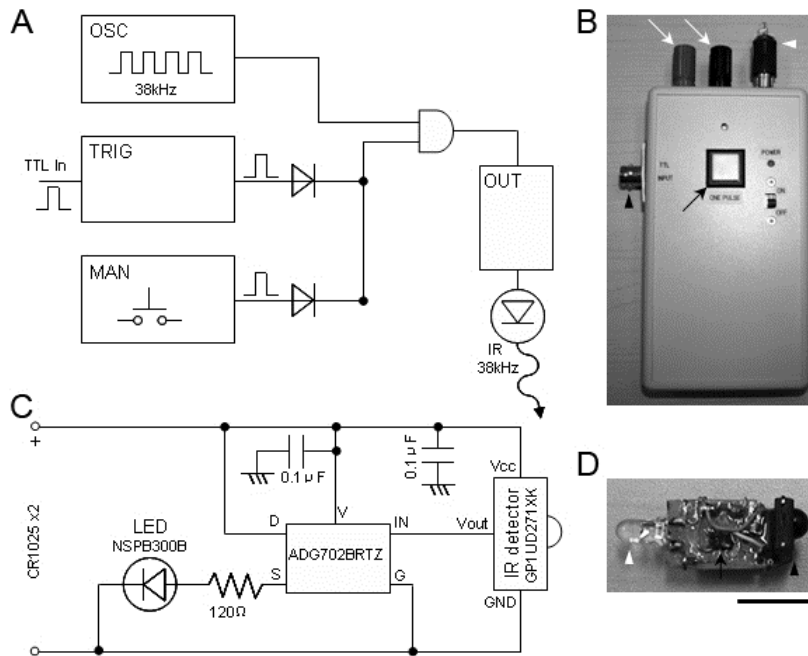
Figures and legends

Figure 1. Remotely triggerable LED device for wireless optogenetic stimulation. (A) Schematic diagram of the transmitter circuit. The 38 kHz TTL signal (OSC) is enabled by either a TTL input (TRIG) or a manual button input (MAN) to generate the IR transmission output via OUT. (B) Photograph of the transmitter. Black arrowhead and arrow indicate the TTL input (TRIG) and push button (MAN), while white arrowhead and arrows point at the output port with IR LED and a pair of sockets that comprises the extra output port respectively. (C) Schematic diagram of the receiver circuit. An IR detector (GP1UD271XK) drives a blue LED (NSPB300B) via an analog switch (ADG702BRTZ). The power is supplied by two serially connected CR1025 batteries. (D) Photograph of the receiver. Black arrowhead, black arrow and white arrowhead indicate the IR detector, analog switch and blue LED, respectively. Scale bar is 36 mm for (B) and 10 mm for (D).
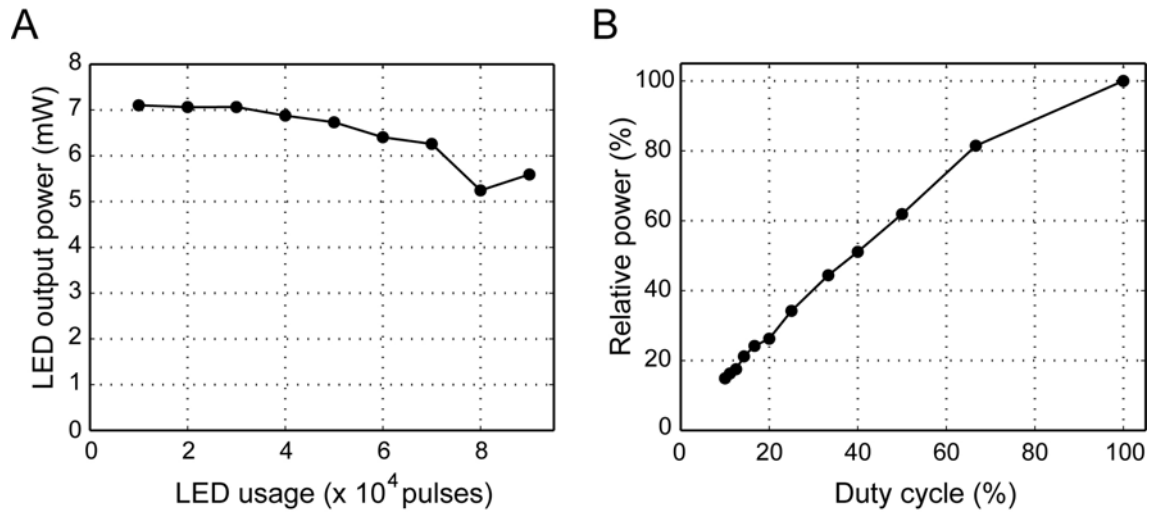
Figure 2. Assessment of battery life and adjustability of blue LED power. (A) Black circles indicate the averaged output powers of the LED after multiple 10,000 pulses of 25 ms duration. The output power remains relatively stable for the first 30,000 pulses. (B) Each black circle indicates the relative average LED power modulated by the frequency of 400 µs pulses within 300 ms. The output power linearly increases with the duty cycle.
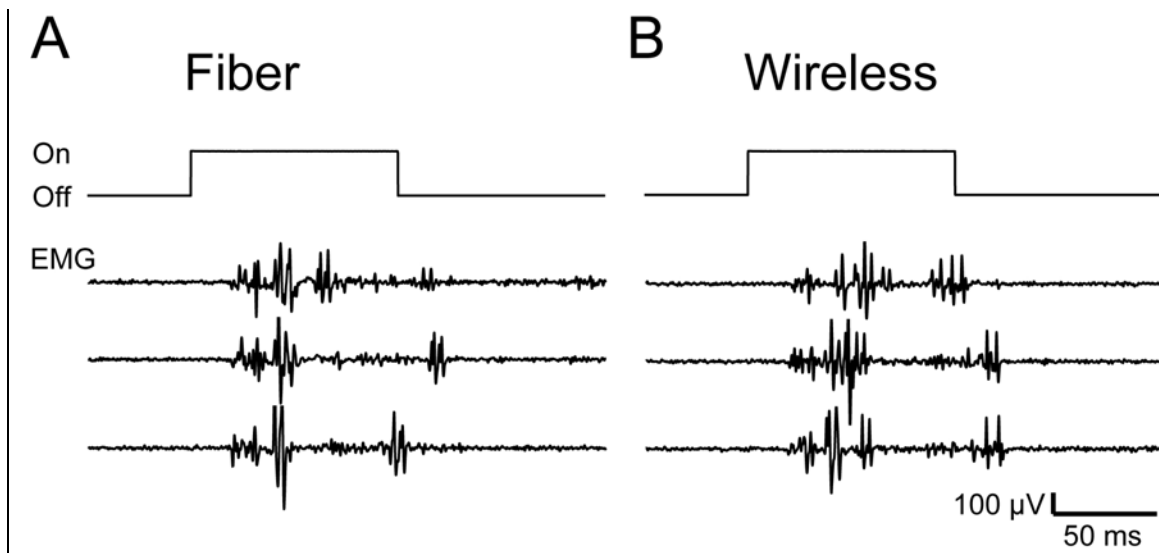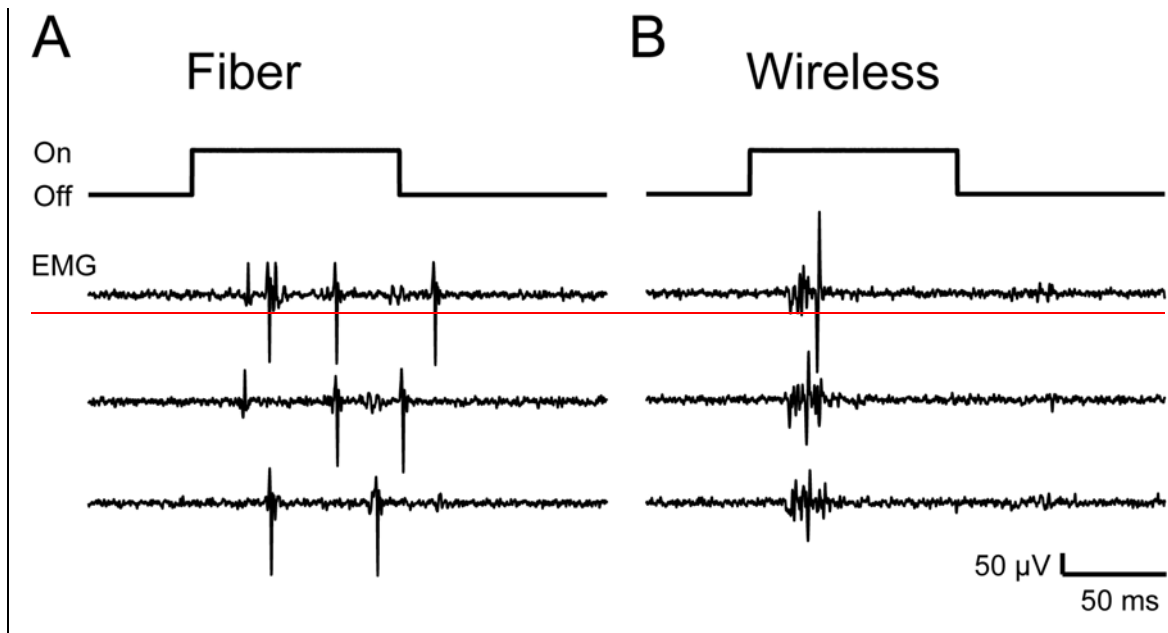
Figure 3. Optogenetic stimulation of M1 by the wireless LED receiver unit leads to hindlimb muscle twitches. (A) Three consecutive EMGs from the right hindlimb with light stimulations delivered by an optic fiber on the left M1 are plotted. (B) Three consecutive EMGs from the right hindlimb with light stimulations delivered by the wireless LED receiver unit are plotted. Top traces show the timing of optical stimulation.

Supplementary Video 1.

Video of a Thy1-ChR2-YFP mouse on a raised platform. The mouse is implanted with the wireless LED receiver on its head. Whole body muscle twitches are seen at each occasion of LED activation (at about 7th, 28th, and 44th second).

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