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# Encode the "STOP" Command by Photo-Stimulation for Precise Control of Rat-robot *

Sicong Chen, Yi Qu, Songchao Guo, Zhaoyue Shi, Kedi Xu* and Xiaoxiang Zheng*

*Abstract*—**Studies on behavior control are important for bio-robots designation. For auto or manual navigation of the bio-robots, the accuracy of the command execution is especially critical. In this paper, we reported a precise "STOP" command for the rat-robots by optical stimulation of the central nervous system (CNS). We labeled dorsolateral periaqueductal gray (dlPAG) neurons with light sensitive channelrhodopsin-2 (ChR2) and directly probed the optical fiber to reactivate these neurons. The rats showed freezing behavior only upon the optical stimulation with an appropriate range of laser intensity and stimulation frequency. Neuron spikes and local field potential (LFP) were simultaneously recorded with optical stimulation by optrodes on free moving rat-robots. Together, our findings demonstrated the utility of deep brain optical stimulation for the stopping behavior of rat-robot control and indicated a potential application of optogenetics for precise control of bio-robots in further work.**

## I. Introduction

Brain-computer interface technology has been widely applied on bio-robots to accomplish the interaction between brain and outer devices [1-5]. In our previous work [6-9], like most of the traditional bio-robots, micro electrodes were implanted into the rat brain to perform rat-robot navigation. Electrical stimulations of medial forebrain bundle (MFB) acted as reward systems that could indirectly drive the rat walk forward. Combined with stimulations through electrodes in primary somatosensory cortex, barrel field (S1BF), left or right turn could be performed under a typical 2-weeks training. Electrical activations of periaqueductal gray (PAG) nucleus were implemented to "STOP" the rat as the rat would freeze after stimulation. Though we have succeeded in rat-robot navigation, optimizing the voltage of electrical stimulation became one of the key factors in fabricating rat-robots. Low voltage stimulation might be invalid while high voltage might lead obvious side effects. For example, serious fear-like responses, such as the decrease of activity and heightened nervousness, were observed for a long time after PAG electrical stimulation. These unstable results of the behavior controlling under the electrical stimulation showed poor maneuverability owning to the individual difference, which either might result from the general activation of all excitable cells in the target area or from the unintended stimulation of adjacent non-targeted brain regions [10]. Thus, it is crucial to develop a novel strategy for rat-robot navigation which is more target specific for the sake of improvement on precise behavior controlling.

Recently, optogenetic technology was boomingly applied in the studies of nucleus function [11, 12], neural circuits [13, 14], and central nervous system (CNS) diseases [15, 16]. The advantages of optogenetics contain high temporal resolution, limited spatial activation and neuron type specific activation [17]. Thus, optogenetics provide an alternative for neuromodulation by photo-stimulation. An algal light-gated ion channel protein channelrhodopsin-2 (ChR2) for photo-stimulation was frequently employed to evoke the action potential of excitable cells. With the help of optical neural interface, it allowed precise quantitative coupling between optical excitation and neuronal activation [18]. These characteristics made optogenetics a challenge to traditional electrical stimulation on bio-robots design and application.

In this paper, we report an engineering application of optogenetics in encoding a "STOP" command in rat-robot control with optimal selected laser intensity and frequency. Compared with traditional electrical stimulation, this method gave us more stable freezing results with precise millisecond time scale, which was important for the direct, real-time control of rat-robots, and especially for further study of high-accurate navigation

## II. Materials and Methods

### A. Animals

All experiments were performed on male Sprague-Dawley rats (180g) purchased from Zhejiang Academy of Medical Sciences (Hangzhou, China). The rats were individually housed in a specific room with temperature and humidity controlled between 23~25°C and 30±5%. The light/dark cycle in this room was 12h/12h.

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Sicong Chen is with Biomedical Engineering and Qiushi Academy for Advanced Studies (QAAS), Zhejiang University, Hangzhou 310027, P.R.China.

Yi Qu is with Qiushi Academy for Advanced Studies (QAAS), Zhejiang University, Hangzhou 310027, P.R.China.

Songchao Guo is with Qiushi Academy for Advanced Studies (QAAS), Zhejiang University, Hangzhou 310027, P.R.China.

Zhaoyue Shi is with Qiushi Academy for Advanced Studies (QAAS), Zhejiang University, Hangzhou 310027, P.R.China.

*Kedi Xu is with Qiushi Academy for Advanced Studies (QAAS), Zhejiang University, Hangzhou 310027, P.R.China. (email:xukd@zju.edu.cn; phone/fax: +86 571 87952865).

*Xiaoxiang Zheng is with Qiushi Academy for Advanced Studies (QAAS), Zhejiang University, Hangzhou 310027, P.R.China. (email:zxx@mail.bme.zju.edu.cn; phone/fax: +86 571 87952865).

### B. Viral infection

Adeno-associated virus was applied to deliver target gene to adult rats by stereotactic injection. Adeno-associated virus serotype 2 (AAV2) carrying ChR2 and red fluorescent protein (mCherry) genes under the control of h-synapsin promoter (AAV-Syn-ChR2-mCherry, ~$5*10^{12}$ titer, Neuron Biotech, Shanghai) were used. Typical coordinates from bregma for dorsolateral periaqueductal gray (dlPAG) injections were lateral, 2.3 mm; posterior, 7.2 mm; and 5 mm ventral at 16° to the surface of skull. For the virus injection process, 200nl virus was pumped at the speed of 100nl/min each time. The injection was then paused for 3 minutes to ensure the virus spreading and tissue absorption. Pumping-pausing process was repeated for 5 times to reach a final virus volume of 1μl. The syringe needle was then kept still for extra 7min before lifting up for 0.5mm, followed with another 10min before complete removal. Antibiotics were applied on the surface of the skull before suturing the skin. After 3 weeks for gene expression, rats were ready for further experiments.

### C. Optics setup

The implantation of optical fiber was conducted two weeks after the virus injection. The rats were anesthetized, stereotaxically fixed. A hole was carefully drilled in the skull by craniotomy above the PAG area. After debridement , the 50/125 (50μm core and 125μm cladding) multimode glass optical fiber with FC interface was then stereotaxically implanted through the hole right into the targeted area, with the fiber tip 0.5mm above the location of previous virus injection. The rats were allowed for recovery for one week.

The optical path consisted of a laser device (DFSSL DRIVER, China) providing 500mW max output at the wavelength of 473nm, a "inter fiber" (3 meters, 50/125 multimode glass optical fiber) coupled with the laser device, and a FC interfaced fiber implanted into the brain, which was coupled with the inter fiber through a ceramic connector. The laser device was triggered by a dual-channel digital stimulator (PG4000A, Cygnus Technology) under the "Train Mode" with a "manual trigger" key. The laser power at the end of the inter fiber ceramic connector and the laser attenuation of the FC interfaced fiber tail were measured by an optical power meter (LTE-1A, Chinese Academy of Sciences, Beijing). For avoiding the heat injury to the brain, the final power of the laser from the ceramic connector was controlled at 3-10mW as input intensity (corresponding intensity directly illuminated the brain was about 1.5-5mW, resulting from ~50% energy loss owing to the interface).

### D. Behavioral Recording

Rat behaviors were rated by direct observation. A binary way of recording was adopted, as yes or no. And the echograms of the rats were defined as described:

*Freezing*: The rat stops moving, keeps a statue-like posture, with increased muscle tonus as suggested by the extension of neck and/or limbs and rising of head, trunk and/or tail.

*Walking*: Typical locomotion of the rat with lowered trunk and tail.

*Escaping*: Running furiously fast with unpredictable direction.

*Jumping*: Upward leaps directly to the border of the open-field.

### E. Neural Signal Recording

For recording neural signals during optical stimulation, a multi-electrode array bundled with optical fiber (optrode) was implanted into dlPAG. Neuron spikes and Local Field Potential (LFP) were simultaneously recorded and processed by a Plexon Multichannel Acquisition Processor (1-40 kHz rate, Plexon Inc., Dallas, TX). The trigger signals transmitted to laser device were also recorded synchronously through "Data 1 input port" of Plexon. To analyse the data, the Offline Sorter Software Ver.3 (Plexon, Inc., Dallas, TX, USA) and Matlab Ver.10b (Mathworks, USA) were employed.

### F. Brain Slice Preparation

Rats were anesthetized with 1% pentobarbital sodium, perfused transcardially first with 0.9% saline for 200ml, then PBS-buffered 4% paraformaldehyde (pH 7.0) for 300ml. The brains were removed and submerged into PBS-buffered 4% paraformaldehyde (pH 7.0) overnight, then placed into 20% and 30% sucrose solution for each day respectively. The brains were cut into coronal sections at the thickness of 50 μm each with a freezing microtome.

### G. Statistics

Data were separated into two groups upon whether the 473nm laser stimulated ChR2-positive dlPAG or not. T-test was employed to compare PAG Stimulation and Control groups on various measures. Here we applied $p<0.05$ indicating static difference, while $p<0.01$ showed significant difference.

## III. Result and Discussion

### A. Experiment 1: Brain Slices

Brain slices were obtained 4 weeks after virus injection as described in "materials and methods, F". Initial experiments in brain slices indicated the precise region of virus injection and ChR2 expression. Also, the images showed structurally intact neuron cells expressing ChR2-mCherry in dlPAG and partial dorsomedial periaqueductal gray (dmPAG) (Fig. 1a and Fig. 1b).

### B. Experiment 2: Optical Stimulation in Anesthetic Rats

For testing whether the dlPAG neurons could be activated by optical stimulation, the optrode implanted anesthetic ChR2-positive rats were used. In Fig. 1c, the raw records of LFP indicated that the spike fire rate increased obviously when the light was on and could recovery to normal scale when the light was off. It proved that optical stimulation could induce dlPAG neurons response under the anesthetic state.

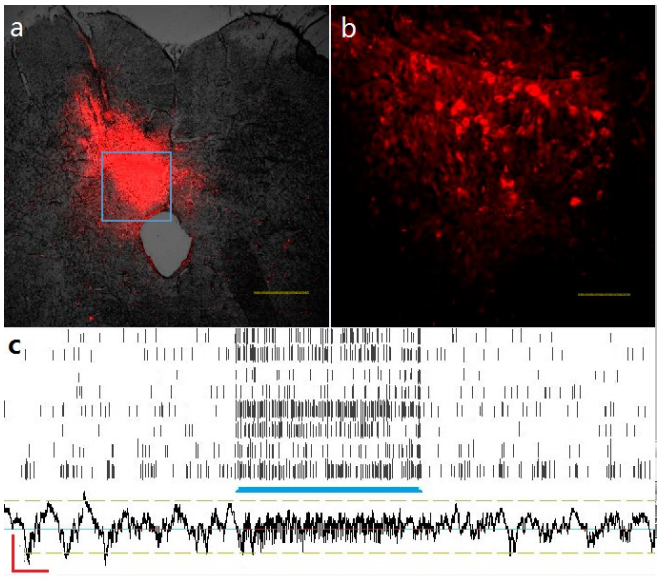


Figure 1. a) The brain slice captured by laser scanning confocal microscope (Olympus FV1000) under 4X objective, bar=0.5mm. The area in blue rectangle was enlarged under b) 20X objective, bar=100 μ m. Red fluorescence showed structurally intact neuron cells expressing ChR2-mCherry. c) The raw data of neuron spikes (upper panel) and LFP (lower panel) recorded by Plexon in an anaesthetic rat. Blue line indicates a 10 seconds optical stimulation (pulse width=15ms, frequency=20Hz). Red bar=500mV, 2s. It proved that optical stimulation could induce dlPAG neurons response.

### *C. Experiment 3: "Command" Optimization in Awaken Rats*

In order to record the field potential, neuron spikes and animal behaviors simultaneously under the optical stimulation, in a bowl-shaped transparent cage (50cm in diameter, 50cm in height), awaken ChR2-positive rats with optrode implanted were connected to Plexon and monitored under a video camera. Under the increasing intensity of the optical stimulation (5.9mW, 6.3mW, 6.8mW and 7.6mW, measured from the ceramic connector of the inter fiber), different frequencies (5Hz, 20Hz and 50Hz with constant pulse number, see Table 1) were tested for optimizing the "STOP" command. Combined with field potential recordings, the behavior result (Table 2) indicated that the intensity of the input laser should be above ~6.3mW but beneath ~6.8mW (measured from the ceramic connector of the inter fiber). Meanwhile, the laser frequency also has significant influence on the rat behavior. When stimulated with low frequency (5Hz, 200 pulses for 40s), no obvious behavior change was observed. However, the spike firing rate increased ($p=0.016$, vs. no stimulation, Fig. 2) under high power (7.6mW) stimulation. Fear-like reactions were observed when using 20Hz (200 pulses for 10 seconds) stimulation. The rats displayed a statue-like state when the laser power reaches 6.3-6.8mW while acted more violently with higher stimulation laser power. The highest frequency

Table 1. Parameters of different optical stimulation patterns.

| | Pulse width | Number | Duration |
|---|---|---|---|
| 5Hz | 15 ms | 200 | 40 s |
| 20Hz | 15 ms | 200 | 10 s |
| 50Hz | 15 ms | 200 | 4 s |

Table 2. The behavior results under different stimulation protocols.

| | 5.9mW | 6.3mW | 6.8mW | 7.6mW |
|---|---|---|---|---|
| 5Hz | No response | No response | No response | No response |
| 20Hz | No response | Freezing | Freezing[a] escaping | Escaping[b] jumping |
| 50Hz | No response | Escaping | Escaping[b] jumping | Escaping[b] jumping |

a. Due to the individual differences, some rats exhibited freezing behaviors while others showed escaping behavior; b. Some rats exhibited only escaping behavior while some others showed escaping accompanied jumping.

(50Hz, 200 pulses for 4 seconds) stimulation could activate robust flight reactions but not freezing behavior. The neuron spike firing rate exhibited similar trends with rat behavior. Higher firing rates were recorded when using higher optical stimulation frequency and intensity (Fig. 2).

Our Results emphasized that frequency and intensity of optical stimulation were critical factors for coding "STOP" command. As Tsai reported, the release of the neural transmitter by optical stimulation is highly related to the stimulation frequency [19]. They found that the ChR2 transduced dopaminergic neurons in ventral tegmental area could be activated and elicited dopamine transients by phasic optical stimulation but not by longer, lower-frequency optical stimulation. The robust release of the dopamine could further induce the animal behavior [19]. Similar to Tsai's result, freezing and flight reactions were also induced by optical stimulation with higher-frequency in our experiment. These violent fear reactions might related to the robust release of GABA generated by higher frequency optical stimulation on GABA-ergic neurons, which were known had exert tonic control over the neural substrates of aversion in dlPAG [20]. Besides the stimulation frequency, the laser intensity also played an important role in rat-robot behavior controlling. The low intensity optical stimulation failed in causing fear-related reactions, which may either due to the insufficient illuminate region or due to the lower laser power failed to initiate the action potential of surrounding neurons. Although violent fear reactions were observed with higher laser intensity, a decrease of neuron firing rate was recorded when stimulating with 7.6mW laser intensity (20Hz and 50Hz), indicating the loss of neuron activity (Fig. 2). This might be

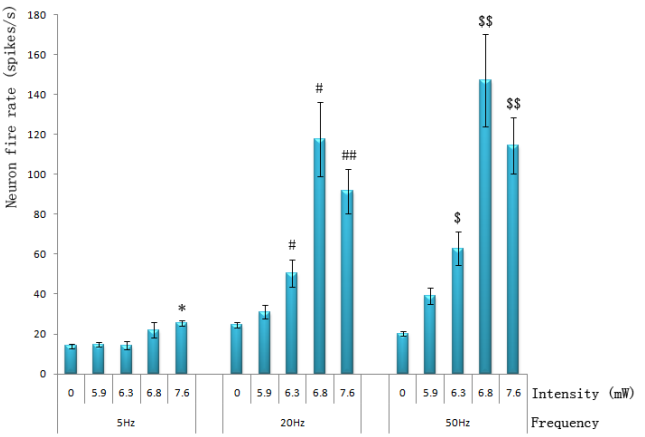


Figure 2. Neuron fire rate under different stimulation protocols. Intensity=0 means no stimulation. *, # and $ means $p<0.05$ vs. no stimulation state of respective frequency group (* for 5Hz, # for 20Hz and $ for 50Hz); ## and $$ means $p<0.01$ vs. no stimulation state of respective frequency group.

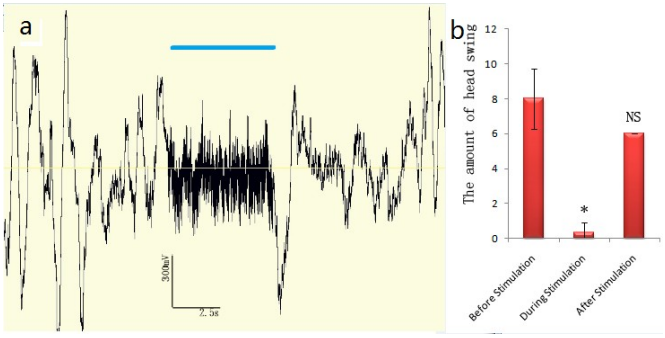


Figure 3. a) The LFP recorded before, during and after optical stimulation in free moving ChR2-positive rats. The blue line indicates a 5 seconds optical stimulation with the parameters of 20Hz, ~6.3W laser intensity. Bar=300mV, 2.5s. b) The amount of head swing in 10 seconds before, during and after optical stimulation. * means $p<0.01$ vs. no stimulation state (both before and after stimulation states), NS means no static significance vs. before stimulation state ($p=0.18$).

explained by the inactivation of the ChR2 channels under robust optical stimulation with higher frequency and intensity [19], indicating no necessity of applying higher laser intensity.

### *D. Experiment 4: "Command" Testing in Free Moving Rats*

A navigation track of 20cm in width, 300cm in length with 20cm in height transparent walls was established to test the effect of optical stimulation encoded "STOP" command on free moving rat-robots. The ChR2 transduced rats were gently placed on one side of the track. After free moving for 5 minutes, the rats were optically stimulated with 20Hz (6.5mW) for 10s. The rat-robots immediately exhibited a statue-like posture upon optical stimulation and kept freezing throughout the stimulation procedure. Typical LFP signals before, during and after 5s optical stimulation were recorded (Fig. 3a). Unlike the electrical stimulation, the rats could quickly recover from the freezing state once upon the termination of optical stimulation (illustrated by head swing number in Fig. 3b). This result suggested that the optogenetics encoded "STOP" commands could be applied in precise control of the rat-robots.

## IV. Conclusion and Further Work

In this paper, a novel "STOP" command encoded by optigenetic stimulation was applied on ChR2 transduced rat-robots. The laser intensity and stimulation frequency were optimized to achieve the accurate control of rat behavior. Our results prospected the possibility of using optogenetics in controlling animal behaviors. On account of the advantages of optogenetics, further research will focus on real-time optical navigation of rat-robots with other commands (e.g. FORWARD, LEFT, and RIGHT commands). Furthermore, wireless LED based remote stimulation modules will be devised, with the final aim for the precise navigation of rat-robots, even for precise behavioral control of other bio-robots.

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