Neurosci. Bull. August, 2021, 37(8):1260–1263
https://doi.org/10.1007/s12264-021-00721-9

www.neurosci.cn
www.springer.com/12264

RESEARCH HIGHLIGHT

# A Temporal Precision Approach for Deep Transcranial Optogenetics with Non-invasive Surgery

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Received: 13 December 2020 / Accepted: 24 April 2021 / Published online: 6 June 2021


Optogenetics, an optical technique that uses light as a modality of biological control to manipulate neuronal activity, has revolutionized the understanding of individual neurons and the ability to decode neural circuit mechanisms in the nervous system [1]. Optogenetics technology has rapidly become a standard tool for understanding the mechanisms of cell types, neural circuits, and nervous systems under both normal and pathological conditions. For example, optogenetics has been developed to drive the expression of reporter proteins in specific cell types. These tools encompass gene delivery methods (e.g., biological, chemical, and viral transfection or chromosomal integration), as well as cell-type specific promoters and driver lines (e.g., GAL4/UAS or Cre-Lox systems) that have been identified or generated for various model organisms, such as worm, fly, fish, and mouse. Furthermore, optogenetics is a powerful technique to achieve precise regulation of specific cellular subtypes and circuits *in vivo*. Experiments have probed the function of brain circuits in awake and behaving animals with previously unimagined temporal and spatial resolution, revealing the complexity of behavioral control and new horizons of circuit function [2].

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The key to this new experimental technique is the development of new optogenetic probes, which are continuously advancing at an accelerating rate. In 2005, Boyden *et al.* first reported the successful *in vitro* transfection and expression of functional channelrhodopsin-2 (ChR2) in mammalian neurons *via* lentiviral gene delivery [3]. ChR2 is derived from *Chlamydomonas reinhardtii* and primarily expressed in cellular membranes. It permits temporal control of neuronal activity at the millisecond scale but at the risk of a high level of desensitization [4, 5]. Currently, the application of ChR2 provides greater flexibility in experimental design in addition to more powerful and refined operations. Therefore, ChR2 is widely applied in optogenetics and has been gradually applied in exploring neurological diseases.

Apart from optogenetics, there are several other methods for the regulation of neurons: ultrasound (US), electrical deep brain stimulation (DBS), and transcranial magnetic stimulation (TMS). But each has its shortcomings. For example, US stimulation is non-invasive, and suitable for stimulating neuronal populations but might generate ion fluxes by disrupting the integrity of otherwise ion-impermeant lipid bilayers [6]. Like all brain surgery, DBS is characterized by a small risk of infection, stroke, bleeding, or seizures. Compared to DBS, TMS is more readily accepted by patients because it does not require anesthesia and is minimally invasive with fewer side-effects [7]. The shortcoming of TMS is the inaccurate positioning. Another unavoidable defect of TMS is the challenge to the depth of stimulation-TMS can only stimulate the superficial cortex; it exerts no stimulating effect on subcortical areas [8]. Inaccurate positioning of TMS is also shared by US stimulation.

By comparison, optogenetics has unparalleled advantages; it enables the almost instantaneous response of

opsins to visible light along with the benefits of pinpoint optical targeting and high spatiotemporal resolution. This highly rapid and selective neuromodulation by visible light is unachievable by other modalities [1]. However, the transmission of visible light generally requires the invasive implantation of external objects and equipment into the brain, which may cause tissue damage and increase the risk of infection and ischemia. Moreover, scattering and absorption in skull and tissues can potentially cause light decay and limit the transmission of a sufficient photon density to stimulate the neural activity in deep brain structures (Fig.1A). At present, it is urgent to develop new tools to solve these problems.

Recently, Professor Karl Deisseroth's team reported the potent fast red-shifted opsin ChRmine, which exhibits significantly larger photocurrents with hundred-fold improvement in operational light sensitivity compared to existing fast red-shifted variants and rapid off-kinetics suitable for millisecond-scale control over neural activity, which may be proper for deep transcranial optogenetics [9]. Based on these characteristics, the authors attempted to elucidate whether ChRmine can achieve rapid transcranial deep brain photoactivation under the premise of ensuring safety (Table 1). The ventral tegmental area (VTA), 4.5 mm deep from the skull surface, was chosen to examine its effect. When a 400-μm optical fiber was positioned directly above the surface of the intact skull and short pulses of 635-nm light delivered at a certain pulse duration, irradiance, and frequency, the light-responsive neurons were activated. Also, they found that the neural responsivity

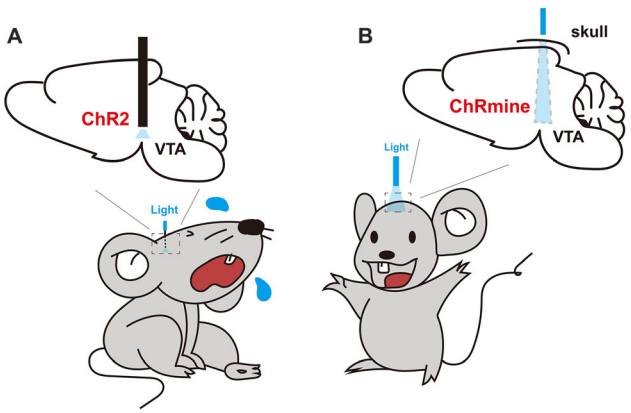


**Fig. 1** Optogenetics with microbial channelrhodopsin-2 (ChR2) *vs* the potent fast red-shifted opsin ChRmine. **A** Microbial channelrhodopsins (ChRs) enable cell type-specific excitation or light inhibition of neuronal activity; however, the delivery of visible light often requires invasive implantation of foreign materials and devices into the brain, which damages tissue and increases the risk of infection and ischemia. **B** Deep transcranial photoactivation with ChRmine raises the possibility of exploring interventions for brain disorders where the light source is well-separated from the target cells with non-invasive implantation of light stimulation devices.

improved with increased pulse duration. Further, they explored whether a lower irradiance could activate cells with an extension of pulse time. The results demonstrated that, with an extended pulse time up to 100 ms, the VTA neurons were activated, even when the light delivered to the surface of the skull was as low as 40 mW/mm$^2$. In addition, the spike latencies shortened upon strengthening the irradiance both *in vivo* and *in vitro*.

To explore the depth limit at which ChRmine-expressing neurons can be activated by transcranial light transmission, AAV8-CamKIIα::ChRmine-oScarlet-Kv2.1 was injected into the brains of rats at different depths. Notably, they found that at a 100-ms pulse width of 400 mW/mm$^2$, the reliable light stimulation dropped to 7 mm, and only approximately 0.02% of the initial irradiance (400 mW /mm$^2$) penetrated to this depth, and this is almost the sensitivity limit of ChRmine. Amazingly, they also found that none of these conditions applied to deep transcranial optogenetics caused tissue damage, which is highly critical for its human application in future.

Can deep transcranial activation of ChRmine regulate behaviors of mice *in vivo*? By injecting AAV8-EF1α::DIO-ChRmine-oScarlet into the VTA of DAT::Cre mice, ChRmine was specifically expressed in dopamine neurons in the marginal cortex. The authors proposed to elucidate whether transcranial optical stimulation could specifically regulate appetite. The results demonstrated that mice expressing ChRmine increased the proportion of time spent in the irradiated paired area in the real-time place preference assay test at irradiance ≥200 mW/mm$^2$ (20 Hz, 5 ms) with minimal toxicity, whereas the control mice did not. And the same behavior was evoked even after 6 months of chronic viral expression. Also, the place preference was not affected by the power of the light stimulation, but related to its frequency.

Further, the authors examined whether this method could be adopted to treat brain diseases. With the hippocampal alginate model of temporal lobe epilepsy, ChRmine optogenetic methods were applied to target hippocampal parvalbumin (PV$^+$) inhibitory (GABAergic) interneurons to provide feedforward inhibition and stop wild-type animal seizures. By subjecting the experimental group to light stimulation (40 mW/mm$^2$, 50 ms on, 100 ms off, lasting 10 s) during epileptic seizures, the seizure duration was truncated by 51% ± 2%; however, when ChRmine was used to target the wider GABAergic hippocampal septal neurons (not just PV$^+$ neurons), only 27% ± 4% of epileptic seizures were inhibited. These results suggested that deep brain optogenetics with ChRmine indeed can treat brain diseases, at least epilepsy.

Finally, the authors assessed whether systemic viral delivery using ChRmine could adjust neuromodulation without brain tissue damage. Here, they administered

**Table 1** Differences between ChRmine and ChRs for optogenetics.

| Characteristics | ChRmine | ChRs |
|---|---|---|
| Provides high temporal control enabled by fast opsins | Yes | No |
| Requires invasive implantation of foreign materials and device into the brain | No | Yes |
| Limits transmission of sufficient photon densities | No | Yes |
| Is suitable for deep transcranial optogenetics | Yes | No |

*ChRs* channelrhodopsins.

AAVPHPeB-Tph2::ChRmine-eYFP *via* retro-orbital injection to target serotonergic 5-HT neurons in the dorsal raphe nucleus of the brainstem in wild-type mice. After 6 weeks, they found that ChRmine-expressing mice, but not control mice, showed social preference in a three-chamber sociability task with no mood impairment after transcranial stimulation. In addition, systemic injection and intracranial injection did not differ in the photoactivation of neurons. Collectively, these results demonstrated that deep transcranial ChRmine photoactivation can promote specific adaptive behaviors without any intracranial surgery.

Notably, Karl Deisseroth and colleagues reported a new optogenetic approach, which is undoubtedly a major breakthrough in the field. Optogenetics has more unparalleled advantages than US, DBS, and TMS [10]: Optogenetics can provide opsins with the advantages of precise optical aiming, and high temporal and spatial resolution. This new discovery has improved the deficiencies of optogenetics, advancing the treatment of brain diseases such as epilepsy.

More deeply, the future applications of optogenetics may not be limited to the central nervous system, but may also be applied in non-neuronal systems, including glial, muscle, cardiac, and embryonic stem cells [11]. For instance, the heart and its associated diseases also poses a major challenge in human research. Optogenetics can bring the dawn and new options for its treatment.

However, one of the limitations is that this method can only reach 7 mm, which makes its application in humans and other non-human primates almost impossible. This warrants further in-depth research. Given that the human brain is much larger than the rodent brain, the other limitations of ChRmine is how to apply it in the clinic. Although it can be used in small brains to alter neuronal activity and behavior, it may have some difficulties in the application to large brains. In addition, the expression of exogenous genes is also a potential risk for application to human beings. In a nutshell, improving the deficiency of optogenetics will have great significance in the management of diseases. More importantly, these new breakthroughs provide new insights for the in-depth exploration of optogenetics and further advances the course of optogenetics.

In summary, Professor Karl Deisseroth and his colleagues applied the powerful rhodopsin ChRmine to achieve transcranial light activation of defined neural circuits (including midbrain and brainstem structures), reaching an unprecedented depth of 7 mm with millisecond precision. With ChRmine's systemic viral delivery, behavioral regulation can be demonstrated without surgery, enabling implant-free deep brain optogenetics (Table 1). Notably, this is a significant new achievement of optogenetics (Table 1). In summary, the researchers demonstrated a method of deep light activation of ChRmine-neurons, which can accurately control the peak time and provide an excellent in-depth manipulation of brain tissue in a completely non-invasive manner, with minimal optical power requirements (Fig. 1B, Table 1). This offers more advantages than luminous implants and intracranial surgery (Fig. 1A, Table 1). Of note, the deep-cranial optogenetics method has been applied in various behavioral environments.

**Acknowledgements** This Research Highlight was supported by the Natural Science Foundation of Zhejiang Province (LR18C090001), the National Natural Science Foundation of China (92049104, 81801102, and 82071230), and a Research Start-up Project by Hangzhou Normal University (4125C5021920453 and 4125C50220204109).

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