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REVIEW

Optogenetic Pacing: Current Insights and Future Potential

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Abstract: Optogenetics combines the biological techniques of optics and genetics and uses light to control the activities of living tissues such as neurons and heart. Optogenetic actuators like channelrhodopsin (ChR), halorhodopsin (NpHR), and archaerhodopsin (bacterio-opsin) provide specificity for neuronal or cardiac controls, and the field has made much progress in heart research since its introduction almost a decade ago. This review will provide information about the history, research highlights and clinical applications of optical coherence tomography (OCT) technology. The clinical translation of cardiac optogenetics will be towards human and larger mammalian animal model applications and ultimately optogenetics may have the power to restore normal heart rhythm and greatly improve quality of life.

Keywords: optical coherence tomography, channelrhodopsin, halorhodopsin, archaerhodopsin, *Drosophila*, heart

Introduction of Optogenetics

Optogenetics is a combined biological technique from both the optics and genetic fields that uses light to control the activities of living tissues such as neurons and heart.

Optical coherence tomography (OCT) provides novel three-dimensional (3D) imaging.[1–3] Combined with OCT, optical coherence microscopy (OCM) provides high-resolution imaging.[4–10] The image resolution of OCT (~5–10 μm in tissue) and OCM (~1–3 μm) is 50x–100x greater than conventional ultrasound, MRI, or CT. OCT and OCM have been used for medical imaging. Commercially, OCT systems provide a series of applications, such as in interventional cardiology for diagnosis,[11] in ophthalmology and optometry for the retina[12] and in dermatology to improve diagnosis.[13]

Specific neuronal or cardiac control are provided by optogenetic actuators such as channelrhodopsin (ChR),[14] halorhodopsin (NpHR),[15] and archaerhodopsin (bacterio-opsin). ChRs are retinylidene proteins (rhodopsins) that are sensory photoreceptors responded to light.[14] NpHR is a chloride ion-specific light-gated ion that responds to green/yellow light,[15–18] that senses light in vertebrate retina rhodopsins.[18] Bacterio-opsins are a family of receptor proteins in archaea that have light inhibiting action potential.[19–22]

The History of Optogenetics

In 2002 the American scientist Boris Zemelman and the British scientist Gero Miesenböck used fly rhodopsin photoreceptors to control neural activity[23] and in

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2005 Peter Hegemann expressed ChR2 in mammalian cells and oocytes, Zhuo-Hua Pan transfected neurons in a manner that allowed them to be electrically active responsive to light[24] and Kramer and Isacoff developed organic photoswitches interacting with ion channels.[25,26]

Also in 2005, Lima and Miesenböck were the first to demonstrate the use of optogenetics control the animal behavior,[27] Karl Deisseroth and Feng Zhang were the first to use channelrhodopsin[28] and Georg Nagel first reported a single-component light-activated cation channel,[28] while Lynn Landmesser and Stefan Herlitze controlled neuronal activity using ChR2 and were also the first to use vertebrate rhodopsin in neurons.[29]

Alexander Gottschalk and Georg Nagel first used Channelrhodopsin-2 (CHR2) for controlling neuronal activity in 2005[30] and in the same year they were also the first to make a ChR2 mutant (H134R) for modifying neuronal activity.[31]

In 2006 Atsushi Miyawaki et al and Roger Tsien et al developed optogenetic recordings called calcium indicators (GECIs)[32,33] in flies and zebrafish,[34,35] while Nakai et al developed the first GECI to be used in mammals,[36] and in 2007 Feng Zhang and Karl Deisseroth with Georg Nagel, Alexander Gottschalkn, Peter Hegemann and Ernst Bamberg were the first to publish optogenetic inhibition research in mammals.[37]

Awards in OCT field include the inaugural HFSP Nakasone Award to Karl Deisseroth in 2010, the InBev-Baillet Latour International Health Prize to Gero Miesenböck in 2012, the Brain Prize to Ernst Bamberg et al in 2013, and the Else Kröner Fresenius Research Prize to Karl Deisseroth for in 2017.

Optogenetics was chosen as the Method of the Year 2010 and the Breakthroughs of the Decade by the prominent research journals Nature Methods and Science, respectively (https://www.medinc.co.uk/optogenetics-breakthrough-of-the-decade-by-dr-zulfiquar/). The application of the first Drosophila heart study using OCT in 2015 was featured on the Discovery channel and in the Boston Globe (Light - powered hearts? https://www.bostonglobe.com/lifestyle/2015/10/25/light-powered-hearts/ETWV7DZU6pwMNm1P59TLGL/story.html).

Drosophila Heart Has Alterations in Development

The *Drosophila* heart has marked morphological and functional changes during development as seen through a longitudinal study of various development stages. The heart beat is reduced dramatically when the fly is in the pupal stage and stops beating during pupae 2 (Figure 1). These data show that a circadian clock gene *dCry* affected heart[38] in heart development and functioning.[38]

Blue Light Optogenetic Pacing in Drosophila Melanogaster

An integrated ultrahigh resolution OCM imaging and optogenetic pacing system used to non-invasively monitor *Drosophila* heart in response to optical stimulations have clearly shown mCherry fluorescence signal in the heart of a ChR2-mCherry transgenic fly compared to a wild-type control fly (Figure 2) [39] and using these transgenic *Drosophila* models, the fly heart showed successfully pacing.[39]

Red Light Optogenetic Pacing in Drosophila Melanogaster

Optogenetic fly models are sensitive to the fact that red light is absorbed less strongly than blue light to increase the excitability of the heart tissue and flies expressing ReaChR were able to be tachypaced under red light stimulation (Figure 3).[40,41]

Optogenetic Control of Cardiac Arrhythmia

Optogenetics has the capability to treat the cardiac conduction system, restore pacemaking ability and terminate cardiac arrhythmias.[42] Cardiac tissue exposed to optogenetic tools can provide mechanistic insights into arrhythmia.[43] Shift light tuned behavior through photosensitive ion channels and pumps (opsins) by optogenetic methods and pacing of cardiac preparations have now been successful in several experimental models.[44] The opsins induce reliable, precise stimulation or silencing of electrophysiological activity in the cardiac cells.[45] ChR2 expressed in cardiomyocytes can sensitively activate Ca2+ signaling properties[45] and ChR2 expression in transgenic mice controlled heart muscles in vivo.[42] Stimulation of G_s-signaling in cardiomyocytes and the whole heart by optogenetics was documented in the light-sensitive G_s-protein coupled receptor in mice cardiac tissue.[46] Self-sustained spiral waves in heart can be manipulated precisely to influence cardiac function and overall dynamics in cardiac excitable media.[47] Studies have demonstrated that near-infrared (NIR) light has the ability for tissue-penetration and NIR had the potential to manipulate cardiovascular diseases non-invasively.[48] The red-shifted opsins achieved greater tissue

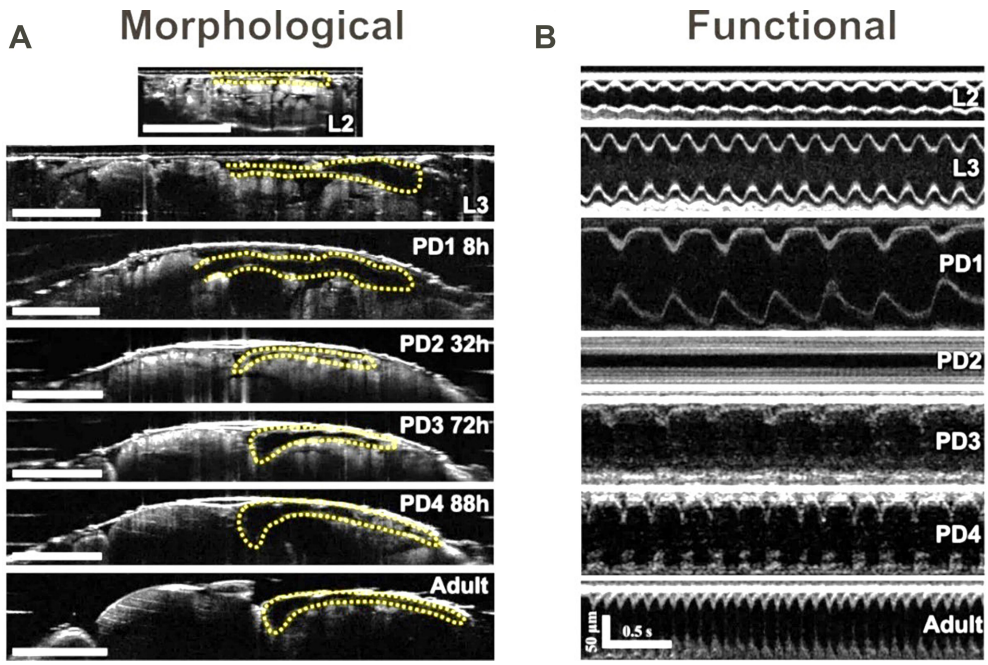

Figure 1 (**A**) Heart remodeling during *Drosophila* lifecycle were by OCM sections. (**B**) Heart variations at different developmental stages were by M-mode images. Reproduced from Alex A, Li AR, Zeng XX, et al. A circadian clock gene, cry, affects heart morphogenesis and function in drosophila as revealed by optical coherence microscopy. *PLoS One*. 2015;10(9):e013723. Creative Commons license and disclaimer available from: http://creativecommons.org/licenses/by/4.0/legalcode.[38] Scale bars represent 500 μm.

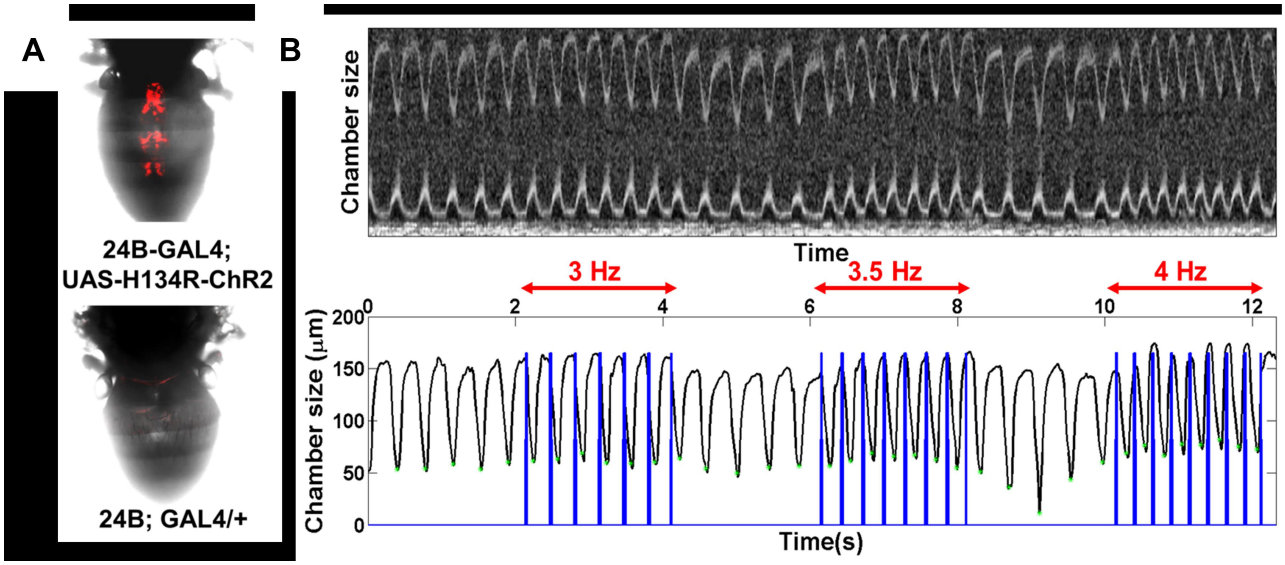

Figure 2 Optogenetic pacing in *Drosophila*. (**A**) mCherry fluorescence signal was clearly observed in the heart of an adult 24B-GAL4; UAS-H134R-ChR2 fly specimen. No fluorescence signal was observed from the wild-type control fly (24B-GAL4/+). (**B**) M-mode OCM image and measurements of heart chamber size showing successful pacing of a pupa heart using blue light pulses. Alex A, Li A, Tanzi RE, Zhou C. Optogenetic pacing in Drosophila melanogaster. *Sci Advan*. 2015;1:e1500639. Reprinted with permission from Alex et al. Optogenetic pacing in Drosophila melanogaster.*Sci. Adv.* 2015;1:e1500639. Distributed under CC BY-NC.[39]

depths than conventional blue-sensitive channel-rhodopsins.[45] These studies have increased our understanding of cardiac physiology.

Rapid antitachycardia pacing produced by an electric shock can resynchronize the heart and terminate arrhythmias such as atrial fibrillation (AF).[49] Electric shock functions in electrical defibrillation in mice cardiomyocytes[50–52] and atrial neonatal rat cardiomyocytes.[53] Aging may significantly reduce heart rate via electrical pacing in *Drosophila*, similar to that seen in elderly humans. Age was also associated with

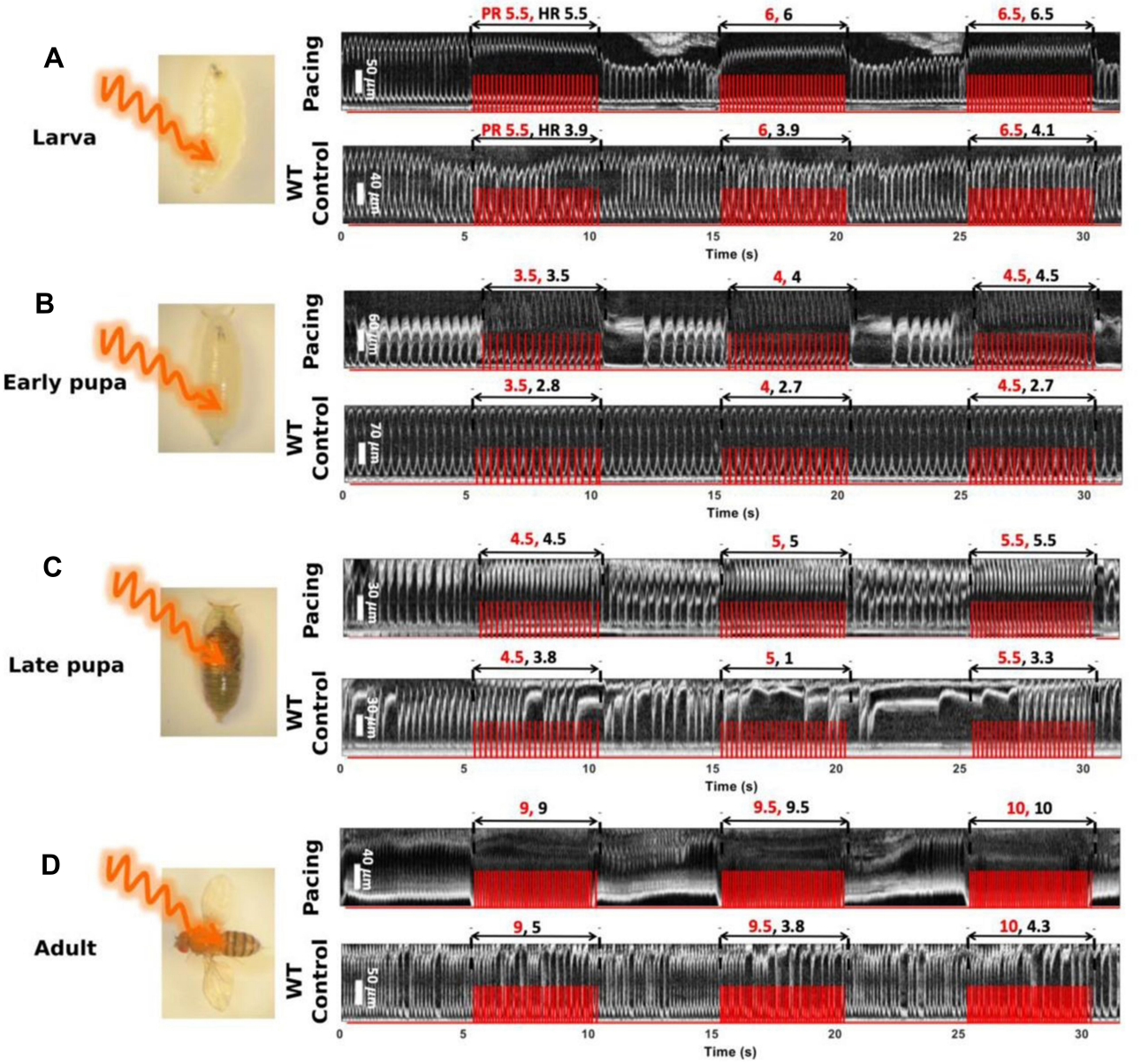

Figure 3 Flies are optogenetically cardiac paced by the ReaChR-expressing Drosophila using red light. ReaChR and WT flies of M-mode images were acquired during pacing at the larval (**A**), early pupal (**B**), late pupal (**C**), and adult (**D**) stages. Reproduced from Men J, Li A, Jerwick J, Li Z, Tanzi RE, Zhou C. Non-invasive red-light optogenetic control of Drosophila cardiac function. *Comm Biol*. 2020;3:336. Creative Commons license and disclaimer available from: http://creativecommons.org/licenses/by/4.0/legalcode. [40] Methods was used from reference[41] to create this image.

an increase in rhythm disturbances.[54] In *Drosophila*, NpHR stops the heart rate from beating in relation to light intensity.[55]

During *Drosophila* metamorphosis glutamatergic neurons provide extensive innervation to the adult heart. Muscles of the first abdominal cardiac chamber showed pacemaker action potentials.[56]

Optogenetic pacing of adult hearts may characterize the effects in flies.[57] *KCNQ1* in humans is related to myocardial repolarization and *KCNQ1* mutant *Drosophila* showed abnormal contractions and fibrillations.[57]

Of the genes identified in *Drosophila* genetic screens, mutants in a fly orthologue of epidermal growth factor (EGF) rhomboid 3 enlarged cardiac chambers.[58] Proper EGFR signaling maintains adult cardiac function.[58] A mutation in the Notch ortholog weary (*wry*) results in dilated cardiomyopathy.[59] Insulin-IGF receptor signaling regulates the age-dependent changes in cardiac function[60].

Multimodal and multisite pacing studies showed chronic stability and excellent biocompatibility in small animals.[61]

Clinical Applications of OCT in Heart

Coronary vasculature and coronary graft assessment are primary applications of OCT in cardiovascular medicine. Clinically, several systems have become commercially available.

OCT can assist patients who have stable coronary artery disease for a more detailed lumen segmentation. On the other hand, in patients with acute coronary syndrome, intraluminal thrombus can be detected at 100% with OCT in comparison to coronary angioscopy[62–65] which detected plaques in 79% and stenosis in 24% of patients.[66]

The Future of Optogenetics

Optogenetics can control and monitor the biological function of cells, tissues or organs. The field has made significant progress in heart research from its inception almost a decade ago. This review has provided information on the introduction, history, research highlights and clinical applications of OCT technology.

The direction of clinical translation of cardiac optogenetics in human application appears to be towards larger mammalian animal models and tools such as safe and stable opsin expression in heart.[43] Because optogenetics may restore normal heart rhythm to increase the overall quality of life[67–69] and action potential duration of ChR2- or NpHR can be modulated in opsin-expressing rat cardiomyocytes,[70] optogenetics may potentially play an important therapeutic role in treating heart diseases.

OCT will likely be of great assistance in *Drosophila* genetic screens that can be designed to identify additional cardiovascular-related genes and may also be valuable in assessing pre-clinical drug development cardiotoxicity, which account for approximately 20% of withdrawal of drug development.[71,72] Electrophysiology measures used to detect cardiotoxicity are often low throughput[67,73] and efficient high throughput screening tools that significantly reduce cost are needed.[71,72] Overall, it is clear that optogenetics has the potential for use in evaluating cardiotoxicity through high throughput and automation.

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Disclosure

The authors report no conflicts of interest for this work.

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