Cardiac optogenetics: a decade of enlightenment

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Abstract | The electromechanical function of the heart involves complex, coordinated activity over time and space. Life-threatening cardiac arrhythmias arise from asynchrony in these space–time events; therefore, therapies for prevention and treatment require fundamental understanding and the ability to visualize, perturb and control cardiac activity. Optogenetics combines optical and molecular biology (genetic) approaches for light-enabled sensing and actuation of electrical activity with unprecedented spatiotemporal resolution and parallelism. The year 2020 marks a decade of developments in cardiac optogenetics since this technology was adopted from neuroscience and applied to the heart. In this Review, we appraise a decade of advances that define near-term (immediate) translation based on all-optical electrophysiology, including high-throughput screening, cardiotoxicity testing and personalized medicine assays, and long-term (aspirational) prospects for clinical translation of cardiac optogenetics, including new optical therapies for rhythm control. The main translational opportunities and challenges for optogenetics to be fully embraced in cardiology are also discussed.

The year 2020 marks a decade of developments in cardiac optogenetics since the publication of the first studies that adopted this technology from neuroscience and applied it to the heart to optically control its function[1–4] (FIG. 1). Optogenetics uses light-sensitive proteins, which are genetically expressed in the cells or tissues of interest, and optical components for contactless control and monitoring of biological function in a highly precise, spatiotemporal manner. To date, no other approach (electrical, mechanical, chemical or non-optogenetic optical approaches) has matched the combination of space–time resolution, specificity and richness of control afforded by optogenetic tools (FIG. 2a).

Cardiac optogenetics emerged after 30 years of research using optical tools to track cardiac arrhythmias, starting with early optical mapping in the 1970s[5,6], and following in the footsteps of neuroscientists who first utilized this technique to control neuronal activity. Cardiologists have embraced the power of optical mapping, which enables improved mechanistic understanding of cardiac electrical disturbances with unprecedented space–time resolution, to test hypotheses about arrhythmia development and termination as well as to inform the development of new device technologies and treatments. Optogenetic sensing with genetically encoded calcium indicators (GECIs) was a natural progression for cardiac optical mapping, with early adoption (before 2010) for long-term monitoring of conduction abnormalities in transgenic mice and for tracking the integration of transplanted cells for cardiac repair in animal models[7–9]. The genetic aspect of these optical measurements empowered cell-specific probing in the heart and overcame the typically acute nature of traditional optical mapping experiments with small-molecule dyes. Around the time of publication of the studies using GECIs (2006), the term optogenetics was coined[10], after the first demonstrated utility of fast kinetics optogenetic actuators to perturb neural activity and brain function[11–13] on the basis of the newly discovered, light-sensitive ion channels from green algae[14,15].

The broadest utility of using light to perturb and to monitor biological processes is captured in a brief review[16] and early work by Miesenbock and colleagues[17,18]. These ideas (the combination of optogenetic actuators and sensors for control of function) are also pertinent to cardiac research, particularly for developing new strategies to control cardiac arrhythmias, which are complex and dynamic life-threatening, space–time events. FIGURE 1 and TABLE 1 summarize the timeline of experimental and computational studies that have contributed to various aspects of cardiac optogenetics, including reviews[19–51]. This Review reflects on a decade of important developments and applications of optogenetics to the heart, focusing on both near-term (immediate) translation and longer-term (aspirational) clinical translation of this technology in cardiology.

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Key points

- Cardiac optogenetics leverages genetically encoded optical sensors and actuators to empower basic and translational research, as evidenced by an impressive growth of published reports over the past decade.
- Immediate translational opportunities reside in all-optical platforms that are inherently high-throughput, offering rapid testing and discovery of new drugs and therapies with the use of patient-derived cells for personalized medicine and myocardial regeneration.
- Optogenetics offers technical innovations for cardiac rhythm control in patients and long-term opportunities for clinical translation for ultra-low-energy rhythm control, precise autonomic neuromodulation and painless defibrillation, which are based on the cell-specificity and space–time resolution of optogenetics.
- Challenges in translating cardiac optogenetics to the clinic are mainly linked to safe opsin delivery to the heart, cell-specific expression and engaging the opsins with light deep within the tissue.

Optogenetic tools

Optogenetic sensors: GECI and GEVI. Optical tracking of changes in membrane voltage and intracellular calcium allows a comprehensive assessment of cardiac activity with very high spatiotemporal resolution. Optogenetic sensors add both cell and organelle specificity as well as the ability to perform in vitro and in vivo longitudinal studies. The cameleon proteins were the first GECIs to be developed and use calmodulin as a sensing element fused to fluorescent proteins excitable with blue–green light[52]. Various kinetic properties were optimized in the GCaMP family of GECIs (consisting of green fluorescent protein, calmodulin and M13 protein), with GCaMP7 variants[53] being the latest member. Following the first cardiac applications of GCaMPs around 2006 (REFS[7–9]), these GECIs remain by far the most widely used optogenetic sensors for in vivo experiments, especially for cardiac applications. The red-shifted variants, including the R-CaMP[54,55] and the R-GECO[56] family of GECIs, were developed to be used in combination with blue-excitable optogenetic actuators. One study developed versions with improved sensitivity (jR-CaMP and jR-GECO) that were better suited for use with channelrhodopsin 2 (ChR2) actuation[57]. Finally, a near-infrared (NIR) fluorescent GECI, NIR-GECO[58], was developed in 2019, with improved spectral separation from optogenetic actuators and potential voltage sensors, while also providing deeper probing owing to NIR light penetration. Some of these GECIs, such as jR-GECO, come close in performance (in terms of kinetics and quantum efficiency) to the best synthetic calcium indicators when used in vitro. For long-term, repeated probing in vitro, as well as for in vivo measurements, GECIs are extremely valuable and reasonably well tolerated. Additional advantages of GECIs include organelle-specific targeting, such as nuclear, mitochondrial and sarcoplasmic reticulum targeting, to study compartment-specific changes in calcium levels[37,59]. In cardiac applications, red-shifted GECIs have been used in combination with optogenetic actuators[60–63], optogenetic voltage sensors that are blue-shifted or NIR[60,64,65], or NIR synthetic dyes with excellent performance, such as BeRST1 or di-4ANBDQBS[62].

Genetically encoded voltage indicators (GEVIs) have lagged behind the GECIs in development and deployment in mammalian cells, including cardiomyocytes, owing to difficulties in achieving fast kinetics of sensing as well as their potential to interfere with native electrophysiology (as many large membrane proteins would). However, great progress towards this goal has been achieved in the past decade, and some of these developments have been used in cardiac research. The first, more widely used GEVIs were VSFP2.3 (REFS[66,67]) and ArcLight[68]. VSFP2.3, ArcLight and the red-shifted variant FlicR1 (REF.[69]) (excitable at 570 nm) are all based on a phosphatase voltage-sensing domain (Ci-VSD). These GEVIs have been validated for use in cardiomyocytes in vitro[64,70–73], as well as to monitor cardiac electrical activity in transgenic mice in a cell-specific manner[74,75]. VSFP2.3 and ArcLight have relatively slow kinetics compared with synthetic voltage-sensitive dyes and cannot accurately capture the upstroke and high-frequency components of the action potential; however, these indicators are useful in reporting rate responses and relative changes in action potential duration. The faster ASAP family of GEVIs[76–78] now includes the latest variant, ASAP3, with high response to two-photon excitation in vivo (in the brain of mice)[76]. The GEVI Voltron, developed in 2019, uses a Halo-tag and a synthetic dye as a fluorophore instead of a fluorescent protein, thereby representing an optogenetic–chemogenetic hybrid GEVI for in vivo use[79]. Voltron525 shows the highest sensitivity to voltage, although a wide spectrum of other sensors, excitable between 525 nm and 635 nm, have been generated with the Voltron modular design, leveraging the spectral properties of fluorophore partners[79]. ASAP and Voltron have not yet been used to record cardiac action potentials. The motivation to obtain optical sensors designed for all-optical electrophysiology (considering the shorter-wavelength excitatory and silencing opsins) led to the development of a range of NIR-absorbing GEVIs, starting with the Quasars[80] and, more recently, Archon1 (REF.[81]), NIR-Butterfly[82] and Voltron635 (REF.[79]) (FIG. 2b). The drive to generate these NIR sensors is also motivated by their expected superior performance in vivo to shorter wavelength indicators for probing deep within the tissue by avoiding haemoglobin absorption.

CaViar[80], a combined GEVI–GECI construct, was designed and applied to study the zebrafish heart in vivo[83]. In later studies, CaViar was used in combination with the actuator CheRiff for all-optical electrophysiology studies in human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPSC-CMs)[60]. In addition to voltage and calcium, cytosol and mitochondria redox state have been recorded using an optogenetic sensor (the glutaredoxin 1 (Grx1)–roGFP2 fusion protein) during human cardiomyocyte differentiation[84].

Unresolved challenges exist for the routine deployment of GECIs and GEVIs and other optogenetic sensors in whole hearts, especially in vivo. Robust expression of the sensors without interference with native function is a desirable target; to date, the work has been primarily done in transgenic mice. Optical measurements are confounded by the mechanical motion of the heart, the dense muscle tissue and the high absorbance of haemoglobin[21,85]. For in vivo measurements, ratiometric sensors or other methods for motion correction

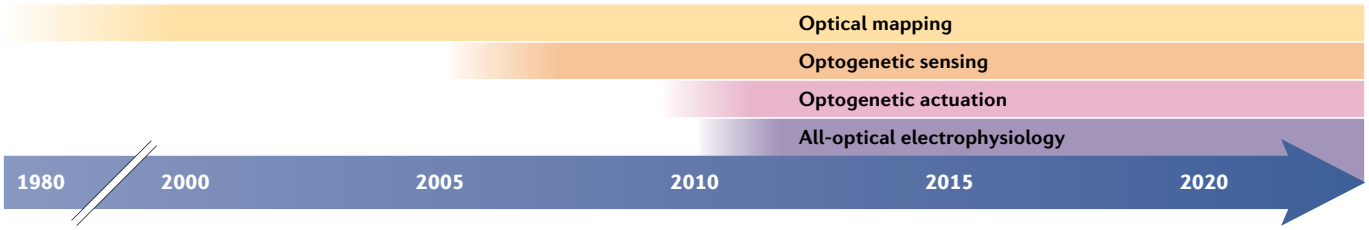


Fig. 1 | **Timeline of optical tools applied to the heart.** Beginning with optical mapping over 40 years ago, optical tools have been used extensively for cardiac research. In the past decade, optogenetics enabled the combination of optical sensing and optical actuation for the development of all-optical cardiac electrophysiology systems. See TABLE 1 for specific applications of cardiac optogenetics, including translational aspects, with the relevant publications since 2010, including Review articles.

are necessary. Continuous monitoring of in vitro cardiac function is more straightforward. The optogenetic toolbox (actuators and sensors) is ever-expanding and efforts are focused towards the development of high-throughput discovery systems with rational design of features, as exemplified by some new GEVIs[76,81].

Comprehensive reviews on optogenetic sensors (GECIs and GEVIs) offer details on the spectral and biophysical properties of these proteins and their use in generating transgenic animals for research[24,27,34,37,38,86–91]; those with specific relevance to the heart are shown in TABLE 1.

Excitatory and inhibitory optogenetic actuators. Optogenetic actuators of voltage are light-sensitive proteins that can be expressed in mammalian cells and can transform photon flux into transmembrane ion flux, thereby manipulating transmembrane potential on a millisecond scale. The discovery and cloning of ChR2, a cation-selective, light-sensitive ion channel, in 2003 (REF.[14]) led to the development of an extensive toolkit of optogenetic actuators (opsins). This toolkit includes depolarizing (excitatory) opsins (such as ChR2 (REF.[14]), CheRiff[80], Crimson[92] and ReaChR[93]) and hyperpolarizing (inhibitory) opsins (such as BLINK-1 (REF.[94]), PAC-K[95], GtACR1 (REF.[96]), archaerhodopsin T (ArchT)[97], halorhodopsin (Halo)[98] and Jaws[99]) that are activatable across a wide spectrum of wavelengths (FIG. 2b). All these optical actuators are microbial opsins that use all-*trans* retinal as a chromophore, with which the opsins form covalent bonds.

The most widely used optogenetic actuator is an H134R mutant of ChR2 with a larger photocurrent[13]. A vast number of other ChR2-based, depolarizing opsins have been developed to change their spectrum, kinetics or dynamic range[14,100,101]. FIGURE 2b shows the blue-shifted CheRiff[80], red-shifted Crimson[92] and red-shifted ReaChR[93]. These optogenetic actuators are both voltage-dependent and light-dependent, which means that they are nonlinear transducers of light to voltage. These actuators have voltage rectification, that is, the ChR2 current is able to limit itself after the membrane potential becomes depolarized, even if the light remains on during longer pulses. Understanding the nonlinearity of the function of ChR2-based opsins (through computer modelling[102]) is essential. These properties (the self-closing through voltage rectification) can be beneficial for optimizing the energy for stimulation[103] and can be leveraged for more intelligent control of actuator function. Opsins can also be modified through rational design[104] or metagenomic discovery[105] to tailor them to cardiac research.

Of note, depending on irradiance and pulse duration, excitatory opsins can act as complex modulators of cardiac function, beyond simple excitation and pacing. As discussed later, excitatory opsins can modulate the shape of the action potential[106–108], as well as cardiac rhythm even when constant light is applied[48,103,109], and the conduction properties and wave dynamics[48,110–112] of cardiac tissue. Excitatory opsins can also provide inhibition of activity during strong illumination and long-duration pulses[109]. Unlike electrical current input, optogenetic actuation can be long-lasting and can provide feedback-controlled, complex modulation over time[113,114] and over time and space[111,112].

Fast, bidirectional, optical modulation of voltage is particularly attractive, but the hyperpolarizing (inhibitory) opsins have been less robust than ChR2 and derivatives. Cardiac research has adopted the early tools ArchT[97] (a proton pump) and Halo[98] (a chloride pump). FIGURE 2b shows Jaws[99] (a red-shifted version of a chloride pump), GtARC1 (REF.[96]) (an anion-selective ion channel), BLINK-1 (REF.[94]) (a potassium channel-based inhibitory opsin) and PAC-K[95] (a newly developed, two-component potassium channel system). The search for improved hyperpolarizing opsins continues because the currently available pump-based tools lack sufficient photocurrent; in particular, the GtARC1-based tools are highly dependent on the cellular chloride reversal potential and the potassium channel opsin systems are difficult to express in cells or cannot be deployed on the same millisecond timescale as their depolarizing counterparts.

Unique applications of voltage optogenetic actuators or other optogenetic actuators (that modulate signalling pathways) include mitochondria-specific targeting of ChR2, which led to the development of optometabolic tools for cardiac research[115]. Early deployment of optogenetic tools provided insights into L-type calcium channel clustering and its effects on channel gating[116]. Optogenetic actuators for G protein-coupled receptor (GPCR) signalling (known as opto-XRs or opto-GPCRs) have been used for precise space–time manipulation of rhythm by very low light intensities via the G_q signalling pathway with the use of genetically

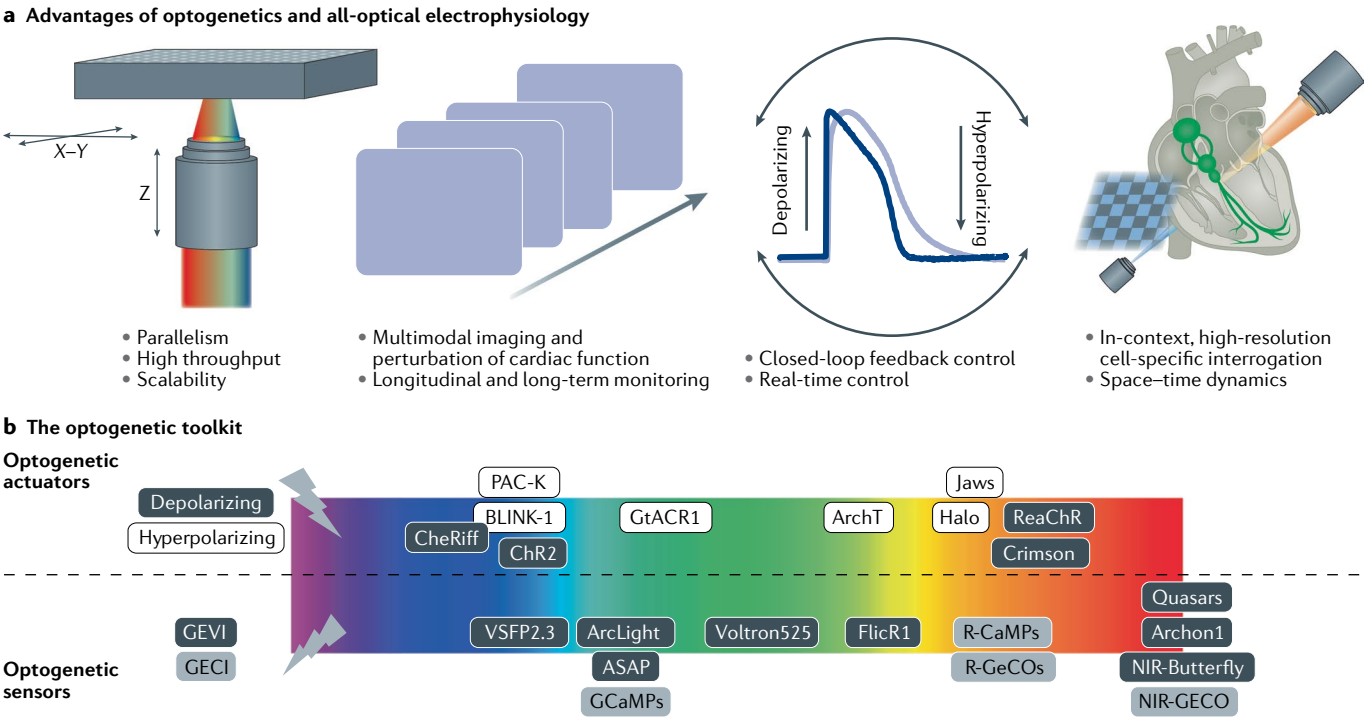


Fig. 2 | **Advantages of all-optical electrophysiology and the optogenetic toolkit. a** | Advantages of optogenetics and all-optical electrophysiology in enabling cardiac applications through spectral compatibility of optogenetic actuators and optogenetic sensors. Unique features include inherent parallelism, scalability, capacity for long-term monitoring of function, bidirectional multimodal imaging and perturbation of cardiac function, and closed-loop feedback control of electrical events and wave parameters, as well as cell-specific and organelle-specific targeting with high spatiotemporal resolution. Taken together, optogenetic technology is superior to electrical and chemical methods for actuation and sensing. **b** | All-optical electrophysiology draws upon an extensive toolkit of optogenetic actuators of voltage, including depolarizing (excitatory) opsins (such as channelrhodopsin 2 (ChR2), CheRiff, Crimson and ReaChR) and hyperpolarizing (inhibitory) opsins (such as BLINK-1, PAC-K, GtACR1, archaerhodopsin T (ArchT), halorhodopsin (Halo) and Jaws) that are activatable across a wide band of wavelengths. Relevant optogenetic sensors include genetically encoded voltage indicators (GEVIs), such as VSFP2.3, ArcLight, ASAP, Voltron525, FlicR1, Quasars, Archon1 and near-infrared (NIR)-Butterfly, and genetically encoded calcium indicators (GECIs), such as GCaMPs, R-CaMPs, R-GECOs and NIR-GECO, with peak excitation wavelengths ranging from 450 nm to 660 nm. These spectral properties and biophysical performance have enabled various combinations of actuators and sensors to be deployed in cardiac research.

expressed melanopsin[117] or via G_s signalling with the use of JellyOp (an opsin photopigment) expression[118]. Further discussion of the variety and properties of the opto-XR or opto-GPCR classes of optogenetic actuators can be found in previous reviews[38,119]. Comprehensive reviews on optogenetic actuators and their biophysical properties are listed in TABLE 1[20,38,41,100,101].

A large number of cardiac studies have used all-optical electrophysiology[1,4,60,62,63,106,110,111,120–144] by combining optogenetic actuators with optogenetic sensors, synthetic voltage-sensitive and calcium-sensitive dyes, or dye-free optical methods to image cardiac activity. Computational modelling of cardiac optogenetics has complemented experiments by providing insights into optimal design and interpretation of findings in cardiac cells and tissues (summarized in TABLE 1)[102,103,107,108,143,145–150].

Near-term translation of optogenetics

Clinical trials using optogenetics for correction of retinal disorders are underway and, so far, the approach has been safe. However, gene and light delivery to the human heart is much more challenging than delivery to the localized, immune-privileged setting of the eye. Therefore, the immediate translation of cardiac optogenetics is for outside-the-body deployment and is likely to draw upon some of the advantages of optogenetics (FIG. 2a), such as scalability, parallelism (the ability to observe and control a large number of locations simultaneously in an easily reconfigurable manner) and the ability to perform repeated measurements in long-term or longitudinal studies. At the time that optogenetics was first used to control neurons, another scalable technology emerged, that of human iPSCs. Human iPSC-CMs have been used in cardiology for disease modelling and cardiotoxicity testing[151,152]. Since 2013, the combination of optogenetics (optogenetic sensing, actuation or all-optical electrophysiology) and human iPSC-CMs has proved to be a fruitful union[60–64,70–73,113,129,132,133,138,140,144,153–164] (TABLE 1). Previous publications, illustrated in FIGS 3 and 4, present four areas of application that we consider an immediate and important translation of optogenetics for the cardiovascular field.

High-throughput drug discovery. The drug development process is lengthy and costly and relies on high-throughput screening for target identification, target validation, compound discovery and hit validation. All-optical electrophysiology[20,80,129] could augment

Tandem cell unit
A multicellular unit composed of light-sensitive, non-excitable cells that are electrically coupled to non-transduced excitable cells.

each of these steps in the drug development pipeline, as reviewed in detail previously[33]. FIGURE 3a illustrates how bidirectional, optogenetic voltage control (with the use of depolarizing ChR2 and hyperpolarizing ArchT opsins), combined with an optogenetic voltage readout (Quasar1) can produce similar results to the classic (electrical) voltage clamp when studying ion channel responses to drugs[65]. Considering the nonlinear light–opsin photocurrent relationships, this voltage control applies for a certain dynamic range, depending on the opsins. The main benefit of the optogenetic approach compared with the automated planar-patch systems, which have undergone rapid development in the past decade, is the non-contact nature of actuation and sensing by light, lending this technique to scalability and enabling work with a variety of cell types. Ultimately this motivates rigorous experimental design with a greater number of testing conditions.

High-throughput cardiotoxicity testing. Preclinical testing for all drugs involves a cardiotoxicity assay, which includes the detection of electrophysiological abnormalities. This assessment is currently based exclusively on compound testing in heterologous systems for potassium voltage-gated channel subfamily H member 2 (also known as hERG) channel blocking, a prime target for drugs. However, ongoing efforts are underway to consider more comprehensive cardiotoxicity assays with the use of human iPSC-CMs[152]. Given that drug actions are rate-dependent, it is important to capture cellular electromechanical responses under controlled, paced conditions. Electrical pacing is not easily scalable; therefore, contact-free optical methods offer an attractive alternative. Similarly, optical sensing has proved to be valuable in increasing throughput as well as enabling long-term observations (FIG. 3b). The utility and limitations of all-optical electrophysiology (optogenetic actuators and optogenetic sensors) to address the needs of drug testing was investigated computationally and experimentally by Klimas and colleagues[129]. An alternative implementation of optogenetic pacing without genetically modifying the cardiomyocytes is to use dedicated, light-sensitive, non-excitable cell constructs that can couple to cardiomyocytes, known as the tandem cell unit approach[4]. To date, experimental studies have not reported interference with native electrophysiology or notable limitations of optogenetic tools in drug testing.

After early reports on the use of optogenetic pacing of stem cell-derived cardiomyocytes[153,155], several groups focused on developing automated high-throughput all-optical platforms for cardiotoxicity testing. Optopatch, a fully optogenetic construct (GEVI–GECI

Table 1 | **References for key aspects of cardiac optogenetics**

Aspect of cardiac optogenetics	2010–2011	2012–2013	2014–2015	2016–2017	2018–2019	2020
All-optical cardiac electrophysiology	1,4	120	106,111,121–128	60,129–135	136–142	48,62,63,110,143,144
All-optical electrophysiology: high-throughput drug screening	–	–	–	60,61,129,133	65,70,140	62,63,164
Applications to advancement of induced pluripotent stem cell-derived cardiomyocytes	–	153	64,72,73,154,155	60,61,129,132,133,156–159	70,71,113,138,140,160–163	62,63,144,164
Cell-specific control: neuro-cardiology	–	212	127	210,211,213,215	142,203,204	51,110
Cell-specific control: other	1,4	146	124,214	39,74,132,180,182,216	191,217,218	143,181
Computational cardiac optogenetics	150	102,145,146	103,107,108	147,148	112,149	114,143
Optogenetic sensing of cardiac activity[a]	–	–	59,64,72,73,75,83,154,197,220	60,61,74,133,157	70,71,86,138	62,63
Optical cardiac pacing	1–4	102,153	91,103,106,124–126,128,170–173	60,61,129,132,133,135,156–159,174,175	141,161–163,176	62,63,110,144,178
Optical cardioversion–defibrillation, ablation	–	–	111,121,122,128,197,198	96,130,131,147,174,190,199	95,112,136,137,149,179,191,194–196	48,114,189
Optical control of cardiac ion channels, G protein-coupled receptor signalling, energetics	–	116	117	115,119	65,84,118	–
Optical control of excitation waves	–	–	111	131,190,199,200	112,137,138	48,110,143,181
Optical control of action potential shape	–	–	103,106,108	–	113	143
Optical closed-loop feedback control	–	–	111,126	29,190,199	112,113,137,139,191	48,114
Towards an optical voltage clamp	–	–	107	–	33,65,113	114
Towards low-energy optical pacing and defibrillation	4	102,146	103,124,173	131	112,136,149	48,114
Towards in vivo deployment: considerations	–	146	85,128,171	130,147	136,176,179,191	178,192,193
Review articles	–	19,20	21–25,27	28–39	40–47,50	49–51

[a]Papers from before 2010 used genetically-encoded calcium probes.

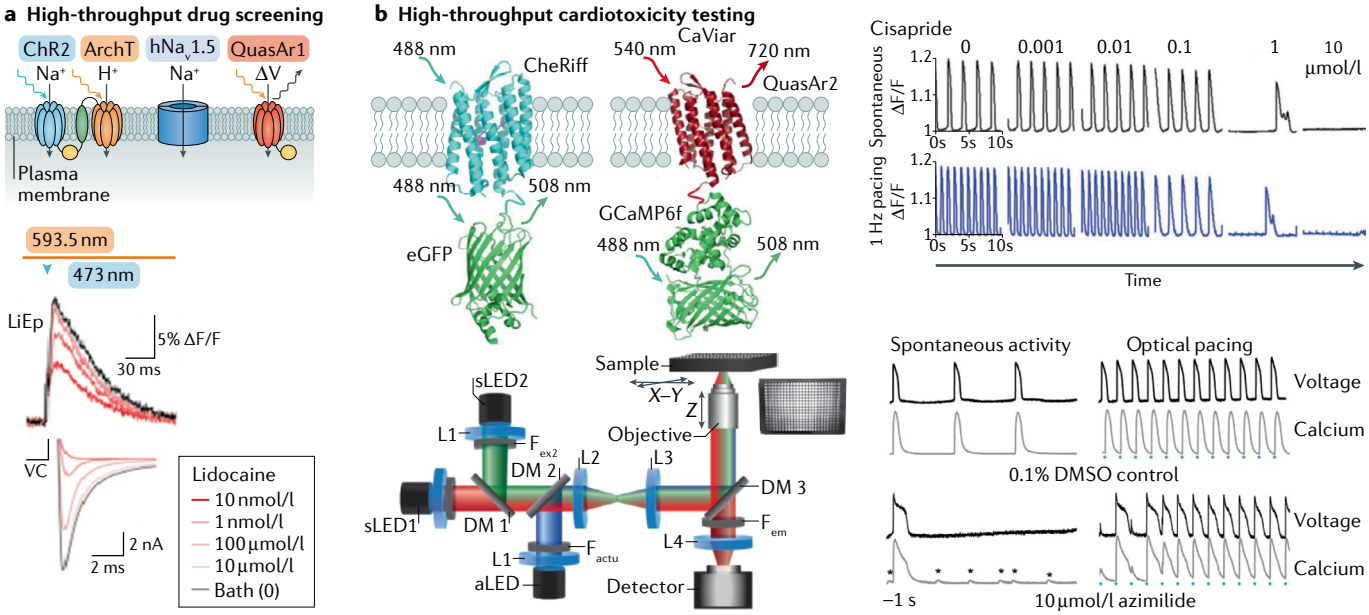


Fig. 3 | **Near-term translation for high-throughput drug screening and cardiotoxicity testing. a** | High-throughput drug screening. Optogenetic voltage sensors (QuasAr1) and bidirectional optogenetic actuation (depolarization via channelrhodopsin 2 (ChR2) and hyperpolarization via archaerhodopsin T (ArchT)) can be used in an all-optical electrophysiology setup to reveal drug effects on voltage-gated ion channels in heterologous systems. Sodium channel $hNa_v1.5$ ($hNa_v1.5$) and responses to lidocaine are shown. Light-induced electrophysiology (LiEp) and classic voltage clamp (VC) both capture functional cellular responses to lidocaine for light-induced voltages within the range of operation (before rectification) of the optogenetic actuators. **b** | High-throughput cardiotoxicity testing. CheRiff (an optogenetic actuator) and CaViar (an optogenetic construct for dual-voltage and calcium imaging) were expressed in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), and cell populations (expressing the actuator or expressing the sensor) were intermixed in a connected network (top-left panels). This approach was applied to perform dose–response testing with several cardiotoxic drugs. The top-right panel shows the voltage responses to cisapride for spontaneous activity and in response to 1 Hz optical pacing; early afterdepolarizations are induced at 1 µmol/l. Cisapride is a gastrokinetic drug that was removed from the US market in 2000 because of cardiotoxicity. The bottom-left panel shows OptoDyCE[62,129], an automated, high-throughput, all-optical electrophysiology setup for optogenetic actuation and optical–optogenetic sensing of voltage and calcium with the use of spectrally compatible proteins and/or small-molecule probes. The bottom-right panel shows the responses of human iPSC-CMs in 384-format plates to 0.1% dimethyl sulfoxide (DMSO) versus 10 µmol/l azimilide; the spontaneous and optically paced activity is shown (the blue dots are the optical pulses). Azimilide prolongs the action potential duration and can induce small, localized, spontaneous calcium-release events, seen in the calcium records (marked by asterisks). Although azimilide is a class III antiarrhythmic drug, it can be cardiotoxic and cause torsade de pointes. aLED, light-emitting diode used for actuation; DM, dichroic mirror; eGFP, enhanced green fluorescent protein; F, optical filter; L, lens; sLED, light-emitting diode used for excitation in sensing. Part **a** modified with permission from REF.[65]. Part **b** top panels modified with permission from REF.[60]. Part **b** bottom panels modified with permission from REF.[62].

CaViar sensor in tandem with an optogenetic actuator CheRiff) was developed by Cohen and colleagues[80] and combined with high-end macroscopic systems for cardiotoxicity testing with the use of human iPSC-CMs[60,133] (FIG. 3b). Variants of the system have been translated to an industrial setting for drug discovery[140]. Our group developed a simpler approach for automated, all-optical, dynamic cardiac electrophysiology (OptoDyCE)[62,129], which integrates an optogenetic actuator (ChR2) with spectrally compatible, high-performance, synthetic dyes for calcium and voltage or red-shifted, optogenetic calcium sensors (FIG. 3b). The system is low-cost, using LEDs and temporal multiplexing to simultaneously record voltage and calcium responses or cell contraction with a single camera. The high-content, drug-response measurements in human iPSC-CMs have been used to construct populations of human iPSC-CM computer models for personalized medicine[164]. Such work has been extended to include long-term, high-throughput microfluidic perfusion[63] and to allow long-term pacing and recording to enable chronic drug testing, while other research groups used some of these all-optical electrophysiology tools for iPSC-CM phenotyping and drug screening[61,157]. Some studies have specifically leveraged optogenetic pacing, pairing it with high-throughput microelectrode recordings to capture drug responses[158,159,161]. These approaches have sparked interest from industry and the FDA and are likely to influence drug development in the near-term.

Short-term and long-term actuation and sensing in human iPSC-CMs. Human iPSC-CMs have transformed cardiac research, but concerns about the immature state of the iPSC-CMs have prevented their rapid translation into the clinic[151]. Optogenetic tools can have a crucial role in phenotyping, long-term monitoring and maturation of these cells. The main advantage of optogenetic sensors compared with synthetic voltage or calcium dyes is the opportunity to acquire repeated measurements over long periods of time. Since 2014, multiple studies have used iPSC lines with constitutively expressed GEVI ArcLight to monitor cell changes during the differentiation and

maturation into cardiomyocytes and cellular responses to pharmacological agents[64,70–73,154]. Both control cells and cells from patients with inherited arrhythmogenic disorders have been characterized in detail with the use of ArcLight imaging[71,72], GCaMP calcium monitoring[72] and a combination of ArcLight and a GECI (R-GECO)[64]. Over the past 5 years, efforts have involved linking phenotypes to single-cell transcriptomic signatures[70] and leveraging optogenetics to monitor redox states in engineered human tissues[84].

Optogenetic actuation has been applied in creative ways to address iPSC-CM immaturity. FIGURE 4a illustrates work by Quach and colleagues to transform a classic (electrical) dynamic clamp assay into an optical dynamic clamp to yield a more hyperpolarized and mature resting membrane potential in human iPSC-CMs[113], to be closer to that of an adult cardiomyocyte. Viral expression of ArchT in human iPSC-CMs and computer-controlled, real-time manipulation by light (LED) was used to perform 'optical injection' of the inward-rectifier potassium current (I_{K1}) to make the resting membrane potential more negative, thereby shifting the electrophysiological phenotype of human iPSC-CMs to be more adult-like. Furthermore, a study from 2020 applied the same approach (optical dynamic clamp) to inject a synthetic, antiarrhythmic, frequency-dependent current in atrial cardiomyocytes[114]. Extending this idea to an all-optical clamp would enable multicellular and tissue-level applications. However, more work is needed to calibrate voltage responses and to implement real-time processing for multiple cells.

Dynamic stimulation (electrical or mechanical) of human iPSC-CMs has been identified as a potential approach to inducing a more mature cellular phenotype. Several studies in 2019 leveraged optogenetic actuators to explore chronic optical stimulation[162,163] (FIG. 4a). One study used closed-loop control for overdrive pacing of human iPSC-CMs during weeks in culture and quantified cell remodelling[162]. Another study used optogenetic pacing of engineered human tissues over 3 weeks and monitored their contractile performance[163]. The stimulated tissue constructs showed electromechanical remodelling, with faster contractions, shorter action potentials and lower L-type calcium current than unstimulated samples. The optically tachy-paced engineered human tissues were more susceptible to arrhythmias[163]. Overall, optogenetics enables scale up and accelerates the development of more mature and physiologically relevant human iPSC-CMs for drug screening and for applications in regenerative medicine.

Applications for disease modelling and personalized medicine. Optogenetic tools have been used in several studies on patient-specific disease modelling with human iPSC-CMs. For example, optogenetic sensors (GEVI and GECI) were used to monitor responses in Timothy syndrome, a calcium-channel genetic disorder[64]. Another study used control iPSC-CMs and iPSC-CMs derived from patients with catecholaminergic polymorphic ventricular tachycardia or long QT syndrome type 2 to phenotype and test the iPSC-CMs for pharmacological interventions[72]. A 2019 study leveraged optogenetic pacing to phenotype catecholaminergic polymorphic ventricular tachycardia subtypes with the use of engineered human tissues[160]. FIGURE 4b shows an example of an all-optical electrophysiology platform used to phenotype a rare genetic disorder with cardiac abnormalities[156]. Ogden syndrome[165] involves a point S37P mutation in *NAA10* (that encodes the catalytic subunit of *N*-α-acetyltransferase 10, responsible for the N-terminal acetylation of certain proteins). Patients with Ogden syndrome die young and some develop torsade de pointes, which is an extremely rare presentation of abnormal heart rhythm in children. Overall, the ability to phenotype patient-specific iPSC-CMs quickly and to quantify cellular responses to therapeutic interventions is at the core of personalized medicine. Optogenetic tools offer an enabling technology for such interventions and might help provide better stratification during preclinical testing of drugs, taking into account patient sex, race, age and other aspects through increased throughput and improved experimental design.

Long-term translational prospects

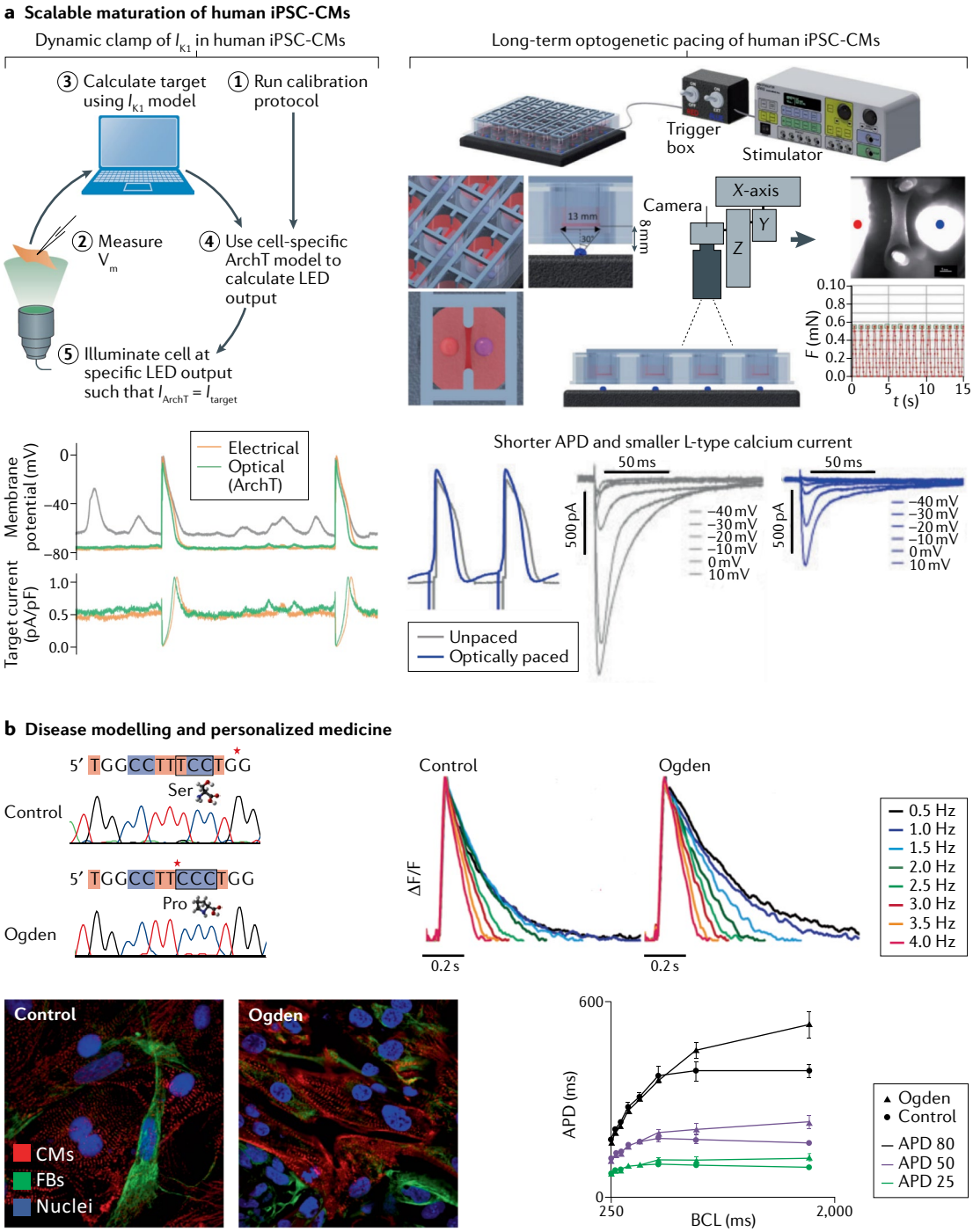
Arrhythmia management. Implantable electronic pacemakers and implantable cardioverter–defibrillators are the gold standard for arrhythmia management[166]. Despite their established value as safe devices, they still have substantial deficiencies. In particular, high-voltage shocks damage myocardial tissue, and device battery life is limited. Batteries are drained when shocks are frequent, such as in patients with ventricular electrical storm[167]. Shocks are also painful, causing patient anxiety and depression[168]. Optogenetics has the potential to address these deficiencies by offering routes for non-electrical, low-energy pacing and cardioversion that can be cell-specific and painless.

Optogenetic pacing for rhythm control. Optical pacing would be an attractive alternative to electronic pacemakers if, by leveraging the advantages of optogenetics (FIG. 2a), it could be achieved at lower energy, thereby extending battery life[20,22], while providing the additional advantages of cell specificity and distributed actuation. In 2010–2011, the first reports demonstrated the utility of optogenetic pacing of cardiomyocytes with the use of blue-light pulses[1–4]. Over the past decade, multiple other studies have used optogenetic pacing in cultured cell systems in vitro, in perfused hearts ex vivo and in anaesthetized animals[60–63,91,102,106,124–126,128,129,132,133,135,141,143,153,156–159,161–163,169–178] (TABLE 1). The hearts of transgenic species have been optically paced, including zebrafish, *Drosophila* and mice, as well as mouse and rat hearts virally transduced to express optogenetic actuators. Although long-term optogenetic pacing with an implantable device has not yet been demonstrated in an awake animal, in this section, we summarize crucial advances towards this goal.

A study in 2010 was the first to use optogenetics to pace the mammalian heart with the use of light[2] (FIG. 5a). Transgenic mice expressing ChR2 within the cell membrane of cardiomyocytes enabled optical pacing of the ventricles and atria at physiologically relevant rates with the use of short (<10 ms) pulses and well-tolerated

Optical dynamic clamp
A real-time feedback system to 'inject' a desired current into light-sensitized cells or tissue according to a computer model and voltage measurements.

Cardioversion
A procedure that restores normal sinus rhythm to a heart that has an arrhythmia.



light levels. Prolonged depolarization was observed during longer pulses, both in vitro and in mouse hearts in vivo, suggesting that optical termination of arrhythmias was possible, as demonstrated later by this group and others[130,131,179]. Among the pioneering studies of optogenetic pacing, Arrenberg and colleagues demonstrated cell-specific expression of opsins within the specialized conduction system of zebrafish hearts[1]. They used transgenically expressed halorhodopsin and ChR2 either to optically block intrinsic activation or to optically pace zebrafish hearts at specific rates. Using patterned illumination to switch rapidly between a normal activation sequence and an arrhythmia, they demonstrated for the first time the utility of optogenetic tools for spatiotemporal control of cardiac activity. Their optogenetic interrogation of zebrafish hearts provided new insights into endogenous sinus node electrophysiology.

Clinical deployment of optogenetic pacing will involve methods beyond transgenic approaches, such as gene therapy, because viral vectors (including lentiviruses, adenoviruses and the safer adeno-associated viruses (AAVs)) provide the most efficient way to inscribe light sensitivity to the heart and are suitable for clinical use[102,106,155,170,176]. Experiments in 2015 demonstrated how discrete regions of the adult rat heart could be enabled for optical pacing by local ChR2–AAV9

◀ Fig. 4 | **Near-term translation to enhance stem cell technology and personalized medicine. a** | Scalable maturation of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Left: an 'optical dynamic clamp' for computer-controlled, real-time manipulation of live cardiomyocytes (CMs) was used to 'inject' the desired ion current by light-emitting diode (LED) light when an optogenetic inhibitor ArchT was expressed in human iPSC-CMs. The dynamically clamped current was the inward-rectifier potassium current (I_{K1}) and its 'optical injection' yielded more hyperpolarized resting potential, consistent with mature CMs. The bottom-left panels show records without the dynamic clamp (depolarized resting membrane potential (grey) and wandering baseline), electrical dynamic clamp (orange) and optical dynamic clamp (green). This more mature CM 'phenotype' was used for studying drug responses. Right: the figure shows a system for long-term optogenetic stimulation of engineered human tissue constructs and tracking the induced force responses. The system was applied over 3 weeks and the optically paced constructs (3-Hz, 30-ms pulses at 0.3 mW/mm^2, 15 s on and 15 s off) showed electromechanical remodelling with faster contractions, shorter action potential durations (APDs) and lower L-type calcium current. In this study, the optically tachy-paced engineered human tissues were more susceptible to arrhythmias than unpaced engineered human tissues, but the arrhythmias could be prevented by pharmacological means. **b** | Disease modelling and personalized medicine. The example of disease modelling shown is of a rare X-linked genetic disorder, Ogden syndrome[165], with a point S37P mutation in *NAA10* (left panel), which encodes the catalytic subunit of *N*-α-acetyltransferase 10 (involved in N-terminal acetylation of proteins). Cells from patients with Ogden syndrome and cells from age-matched and sex-matched healthy individuals (control) were transformed into human iPSCs and then differentiated into CMs (the bottom-left panels show immunofluorescence images with labelling for CMs, fibroblasts (FBs) and nuclei). After 3 months, the iPSC-CMs were transduced with ChR2 adenovirus and their function was characterized with all-optical electrophysiology. The patient-derived cells showed APD prolongation selectively at lower pacing rates or longer basic cycle lengths (BCLs) compared with control cells, consistent with clinical findings that patients with Ogden syndrome have cardiac problems and arrhythmias, including bradycardia and torsades de pointes, and eventually die young. APD25, APD50 and APD80 are APDs at 25%, 50% and 80% repolarization. Part **a** left panels modified with permission from REF.[113]. Part **a** right panels modified with permission from REF.[163]. Panel **b** modified with permission from REF.[156].

viral injections[128] (FIG. 5b). Several weeks after the injections, hearts were responsive to optical pacing in both open-chest and ex vivo studies. Multisite optical pacing further demonstrated the application of optogenetics for ventricular resynchronization therapy[128]. Another study from 2015 demonstrated optogenetic transduction of the heart that did not require open-chest surgery and myocardial injections. Instead, ChR2–AAV9 was systemically delivered to mice[171], capitalizing on the cardiac tropism of this AAV serotype and achieving robust myocardial responsiveness to optical pacing a year after transduction.

Delivery of non-excitable, light-sensitized cells could be an alternative to viral transduction for optical cardiac pacing. In 2011, Jia and colleagues termed this approach the tandem cell unit strategy[4,124] and validated it in vitro using ChR2–HEK cells coupled to either adult dog cardiomyocytes or monolayers of neonatal rat cardiomyocytes. In a hypothetical tandem cell unit strategy, patient-derived cells would be induced to express an opsin to provide robust depolarizing current upon photoactivation. After delivery to a patient's heart, those cells would couple to cardiomyocytes to enable optical control of heart rhythm. Although exogenous delivery of light-sensitized cells has not yet been implemented, in vitro and in vivo proof-of-concept results of the tandem cell unit concept have been reported for non-excitable cells of the heart, including cardiac fibroblasts, myofibroblasts[132,143,169,180,181], cardiac progenitor cells[132] and macrophages[182], all of which can electrically couple to cardiomyocytes to entrain the myocardium after optical stimulation.

Low-energy optogenetic pacing could emerge as a disruptive technology if optogenetic transduction and light delivery are safe and minimally invasive[20,22,23]. Strategies for translating optogenetic pacing to the clinic include obtaining a better understanding of opsin operation and function in cardiomyocytes (atrial, ventricular and Purkinje cells)[102,103,146,175], optimizing optogenetic actuators for cardiac cells, obtaining a deeper understanding of cell-resident cofactors (for example, all-*trans* retinal at the right doses considerably lowers the threshold for stimulation)[173] and selectively engaging the specialized conduction system of the heart[1,126,146]. Computational modelling is an effective platform for testing these strategies and has provided new insights into the energetic benefits of longer stimulation pulses[102,103] and constant ultra-low illumination[103]. Such long-duration pulses are not feasible using electrical stimulation owing to the generation of cytotoxic electrochemical by-products[103,183]. Results from human heart computational models have revealed that the energy required to pace the heart is substantially reduced when ChR2 is exclusively expressed within the Purkinje system and light is delivered to the bundle of His[146]. Cell delivery of designated, non-excitable 'spark' cells, as in the tandem cell unit approach, could also lower the energy required to pace the heart if the cells are clustered[124]. Finally, low-energy solutions for rhythm control might also arise from optogenetic modulation of intracellular GPCR signalling, such as the G_q[117] and G_s[118] pathways.

Optogenetic termination of cardiac arrhythmias. The critical dependence on coordinated space–time actuation is a major challenge in arrhythmia termination. Focused actuation in either space or time alone is often inadequate for termination of complex arrhythmias. Conventional defibrillation is a high-energy termination strategy that depolarizes a large myocardial mass (>95%)[184] to block fibrillatory wavefronts, thereby preventing the formation of new re-entrant waves[185,186]. Low-energy, electrical strategies rely on the proper timing of low-amplitude depolarizing stimuli to extinguish arrhythmic wavefronts and include anti-tachycardia pacing[187] and low-energy anti-fibrillation pacing[188]. Anti-tachycardia pacing is a feature in current implantable cardioverter–defibrillators but is limited to actuation of specific locations and, therefore, is effective in terminating only non-complex, re-entrant arrhythmias. By contrast, optogenetics enables low-energy strategies that can be distributed over large areas or deliver long pulses, capturing multiple phases of the arrhythmic activity or having light that is patterned, possibly in real-time, for mechanistic termination of fibrillatory wavefronts. The light intensity could also be titrated to terminate rotors with the use of sub-threshold illumination[48]. By avoiding engagement of the cardiac nociceptors and preventing skeletal muscle hypercontractures, the cell-specificity of optogenetics ensures painless termination of an arrhythmia.

In an early study of optical cardioversion to terminate re-entrant arrhythmias in healthy mouse hearts,

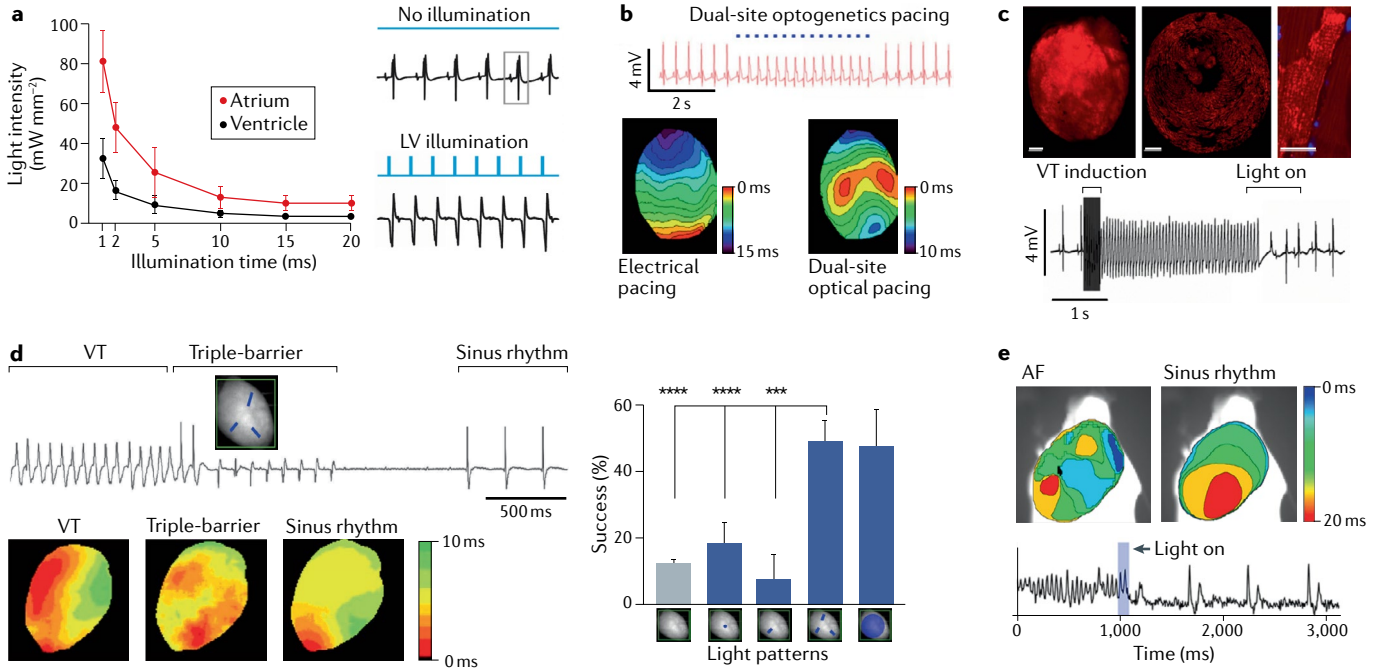


Fig. 5 | **Long-term translation of cardiac optogenetics for rhythm control.** The figure shows proof-of-concept results from multiple studies. **a** | Photostimulation intensity and duration required for in vivo pacing of transgenic channelrhodopsin 2 (ChR2) mouse hearts[2]. Electrocardiogram (ECG) signal before and after pulsed photostimulation indicating 1:1 capture during optical pacing of the left ventricle (LV). **b** | ECG indicating dual-site, optical pacing of the ventricles in a perfused rat heart at locations of myocardial injections of adeno-associated virus (AAV)-CAG-ChR2-green fluorescent protein (GFP), and activation sequences generated by electrical pacing at the apex (left panel) and by dual-site optical pacing of the ventricles (right panel)[128]. **c** | Optogenetic pacing and defibrillation in hearts after in vivo gene transfer[147]. Stable expression of ChR2 within a mouse heart 16 months after systemic injection of AAV9-ChR2-mCherry; epicardial surface (top-left panel), left ventricular cross-section (top-middle panel) and ventricular cardiomyocyte (top-right panel). The bottom panel shows an ECG with termination of ventricular tachycardia (VT) by epicardial illumination of the ventricles. **d** | ECG showing cardioversion after VT onset using a triple-barrier pattern (top-left panel)[131]. Activation sequences relative to the ECG; VT activation (bottom-left panel), photostimulation (bottom-middle panel) and restored sinus rhythm (bottom-right panel). The right panel shows the percentage of spontaneous cardioversion of VTs (grey bar) and VTs interrupted with each of the four light patterns shown on the *x*-axis (blue bars); *P* values (***$P < 0.001$, ****$P < 0.0001$) determined by one-way ANOVA. **e** | The top panels show activation maps with re-entrant conduction during atrial fibrillation (AF) (left panel) and subsequent restoration of sinus rhythm after optical cardioversion (right panel)[191]. The bottom panel is the optical voltage signal showing chaotic AF that converts to sinus rhythm after exposing the right atrium to a 100-ms light pulse. Panel **a** modified with permission from REF.[2]. Panel **b** modified with permission from REF.[128]. Panel **c** modified with permission from REF.[147]. Panel **d** modified with permission from REF.[131]. Panel **e** modified with permission from REF.[191].

illumination of the anteroseptal epicardium with one light pulse had an 85% success rate[147] (FIG. 5c), and the success during acute infarction was 88%. In computer simulations of an infarcted human heart, epicardial illumination successfully terminated infarct-related ventricular tachycardia by initiating transmural depolarization to block the re-entrant wavefront[147]. In a later study, ChR2–AAV9 constructs were used in combination with rapamycin (to suppress the immune response) in rats to demonstrate ventricular cardioversion[130]. Application of a single epicardial light pulse achieved 97% cardioversion for monomorphic ventricular tachycardia and 57% for polymorphic ventricular tachycardia. Another study also successfully terminated ventricular tachycardia by open-chest application of successive light pulses in rats after myocardial infarction[189].

An ex vivo study from 2018 found that ventricular tachycardia was terminated in transgenic ChR2-expressing mice after global illumination of the epicardial surface with a single light pulse[136]. Optical mapping revealed two primary cardioversion mechanisms that were motivated by optical depolarization of the entire epicardium: the termination of a re-entrant core and subsequent refractoriness, and an unpinning and drift of the re-entrant core until collision with refractory tissue[136]. Although global epicardial depolarization by light excels as a low-energy solution to cardioversion, it is not easy to implement in vivo where it is necessary to perform cardioversion with minimal area of illumination. A study in ChR2-transgenic mouse hearts addressed this issue using patterned light to annihilate arrhythmic wavefronts using mechanistic, multi-barrier illumination patterns[131] (FIG. 5d). Cardioversion of ventricular tachycardia required triple-barrier optical depolarization, where light was restricted to three lines oriented approximately 120° apart. This illumination pattern yielded an optical cardioversion success rate similar to that of illuminating the entire left ventricle, but used only 25% of the light energy.

Atrial cardioversion is a mid-future opportunity for translating cardiac optogenetics to the clinic. After early proof-of-principle in vitro work in monolayers and slices of rat atrial cardiomyocytes[122,175,190], followed by computational studies[149], two rodent studies demonstrated

optogenetic termination of atrial fibrillation[179,191]. One study used mice with cardiac expression of ChR2 as well as a loss-of-function mutation in *Cx40* that promoted atrial fibrillation[179]. In open-chest experiments, the success rate of terminating atrial fibrillation was >90% using focused, low-light illumination (<0.5 mW/mm^2). Optical atrial cardioversion was still possible 8 months after systemic viral delivery of ChR2, showing promise for the clinical translation of this approach. In other studies in rats, ReaChR–AAV9 with the atria-specific promoter from human *NPPA* was topically applied to the right atrium using the gene painting technique[191]. After 4 weeks, cholinergic-mediated atrial fibrillation was induced in the anaesthetized animals and a computer-controlled, implanted LED delivered optical pulses to the right atrium when PR intervals consistent with atrial fibrillation were detected, providing a cardioversion success rate of 96%[191] (FIG. 5e).

Light penetration into the myocardium is an important consideration for the clinical translation of optogenetic cardioversion or defibrillation, especially for the ventricles[85]. Illumination with longer wavelength NIR light, in combination with red-shifted opsins, would provide deeper myocardial penetration of optical depolarization, thereby increasing the probability of capturing a critical mass of tissue. Up-conversion nanoparticles administered deep within the myocardium could also be engaged by NIR light, enabling the activation of optogenetic actuators via nanoparticle-assisted light conversion[192]. These innovations could be optimized for the clinic using computational models[28,147–149]. Interestingly, results from a recent study suggest that simple photon transport models could underestimate the depth of light penetration into the myocardium and its effect on opsin-expressing cells[193]. Additional work is certainly needed to better understand light–matter interactions within the context of using distributed light for optogenetic cardioversion.

Cardioversion using well-controlled, regional excitation or inhibition is difficult to achieve using electric fields but is a unique feature of optogenetics. Indeed, several studies have used hyperpolarizing opsins to modulate cardiac activity[95,96,169,194–196]. However, some hyperpolarizing opsins, especially anion-conducting opsins, such as ACR, have had controversial results in cardiac muscle[194]. Furthermore, termination of complex arrhythmias with use of optical hyperpolarization would require global actuation (expression and illumination) to prevent enduring wavefronts from re-activating hyperpolarized regions. One study used transgenic mice expressing the hyperpolarizing opsin ArchT to optically terminate ventricular arrhythmias[195]. Although cardioversion success was higher than in control hearts, actuation of the inhibitory opsin was inferior to actuation of an excitatory opsin, such as ChR2. A comprehensive list of studies that used optogenetic tools for cardioversion or defibrillation[48,95,96,111,112,114,121,122,128,130,131,136,137,147,149,174,179,189–191,194–199] is shown in TABLE 1.

Excitation wave control and feedback for rhythm management. The optogenetic approaches described above for pacing and cardioversion or defibrillation are guided by concepts used in present-day cardiac devices. Optogenetics also enables new innovations for rhythm management, including the control of action potential morphology and control of the speed and direction of targeted wavefronts, a concept known as optical wave steering[29]. These innovations in rhythm management could neither be realized using present-day devices nor be rigorously tested in living tissue before the advent of cardiac optogenetics. Indeed, multiple experiments have demonstrated that wavefronts can be modulated by light[48,110–112,131,137–139,143,181,190,199,200] (FIG. 6a,b), among which some have implemented quasi-real-time, closed-loop control whereby the trajectory of a wavefront is sensed and then altered using patterned illumination (FIG. 6c). This approach is particularly promising for ultra-low-energy cardioversion. However, the implementation could be challenging due to the substantial spatiotemporal data that would need to be processed in quasi-real-time by an implanted device. Even so, closed-loop sensing and actuation of cellular function is a powerful feature of optogenetics that is useful not only in cardiology but also in neuroscience[201].

An early experiment demonstrated optical wave steering in cardiomyocyte monolayers by combining dye-free optical imaging for wave sensing with patterned light for localized optogenetic actuation[111] (FIG. 6a). A digital micromirror device was driven by sequences of programmed images to project light for the modulation of conduction velocity, to induce unidirectional block, to initiate rotors and to instantaneously reverse the chirality of rotors. This study demonstrated how optical wave steering could provide insights into a range of excitable tissue disorders, including functional and anatomical re-entry[29,111]. In other experiments, opsins were expressed in non-cardiomyocytes, including 3T3 fibroblasts[181], cardiac myofibroblasts[143] and stellate ganglion neurons[110], to modulate conduction speed. Excitation wave dynamics have also been studied and manipulated within geometrically patterned cultures of human iPSC-CMs and engineered excitable cells using all-optical sensing and actuation[138].

Optical wave steering using patterned light is particularly useful for investigating and implementing mechanism-based termination of re-entry. For example, mechanisms of atrial rotor termination have been revealed by illuminating ChR2-expressing rat atrial myocyte layers or slices with specific patterns of light[190]. Stable rotors were extinguished when an optically positioned, temporary conduction block intersected both the rotor core and an unexcitable boundary. A 2018 study used optogenetics to guide spiral waves towards termination along well-defined trajectories by optically positioning functional conduction blocks with high spatiotemporal precision[112] (FIG. 6b). Results from these mechanistic studies and other studies using optogenetics[111,136,190] have provided new insights into guided rotor termination. Extending this approach to perfused, ChR2-expressing mouse hearts, Scardigli and colleagues designed and tested a novel system engineered for real-time optical sensing and stimulation to control excitation waves[137] (FIG. 6c). A region-averaged fluorescence signal of electrical activity was monitored, and

Gene painting
Transduction of tissue with a gene by 'painting' it with adenovirus, using a solution containing the vector, an adhering polymer and a weak protease to promote vector penetration.

Up-conversion nanoparticles
Optical nanomaterials, typically doped with lanthanide ions, that up-convert two or more lower energy photons (longer wavelengths) into one high-energy photon (shorter wavelength).

Optical wave steering
A procedure in which specific space–time dynamic light patterns are used to control the speed and direction of an electrical wavefront within excitable tissue that expresses an opsin.

patterned light pulses were applied to the epicardium when a threshold was exceeded. The system reacted to non-stationary wave dynamics within 2 ms. This system was able to accelerate bundle-branch-mediated ventricular depolarization, restore atrioventricular conduction after atrioventricular node inhibition, and generate stable, apex-to-base re-entrant circuits, whereby sensing at the base and stimulation at the apex provided a virtual excitation pathway[137,139].

Although these innovations for rhythm control have been informed by cardiac optogenetics, their clinical implementation does not necessarily require optogenetics. Some control strategies could be deployed by distributed arrays of electrodes, but optical methods remain

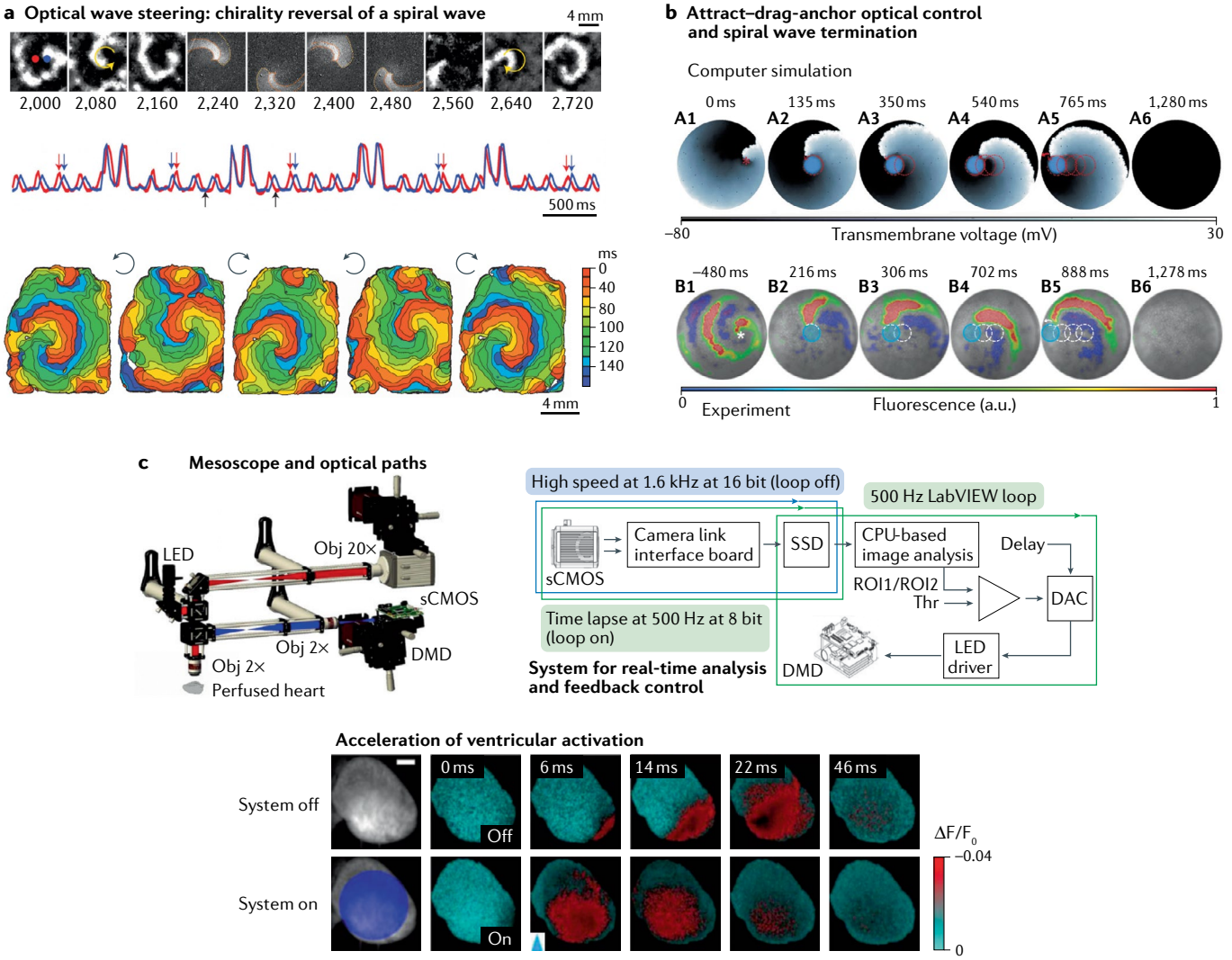


Fig. 6 | **Long-term translation of cardiac optogenetics for wave control and feedback control.** The figure shows proof-of-concept results from multiple studies. **a** | The top images show an anticlockwise spiral wave (frames 2,000–2,160), an optically applied, computer-generated clockwise spiral wave (frames 2,240–2,480) and the persisting spiral wave after chirality reversal (frames 2,560–2,720)[111]. The activity signals from the red and blue pixels indicated in the top panel show four light-controlled chirality reversals. Computer-generated, blue-light spirals were imposed at random phases for just over a cycle, as seen in the four higher-intensity transients (middle panel). Black arrows indicate the time period shown in the top panel. Red and blue arrows indicate the switch in order of excitation at the chosen locations due to chirality reversal. The bottom panel shows activation maps for the initial spiral wave and the four resultant spirals after each of the chirality reversals. **b** | Attract–drag–anchor control of a spiral wave core towards termination[112]. The top panel shows the successful termination of a spiral wave in silico by capturing the core by using circular depolarizing light pulses and 'dragging' the core to the left boundary in a stepwise fashion. The bottom panel shows the successful termination of a spiral wave in vitro in a manner similar to that shown above. For each light spot, the current location of the applied light is indicated with a filled blue circle. The movement of the tip of the spiral wave, as it is anchored to the location of the light spot at previous time points, is indicated in each frame as a dashed red (in silico) or white (in vitro) line. **c** | The top-left panel shows a fluorescence mesoscope used for optical mapping and photostimulation. Voltage-sensitive dye fluorescence was imaged using a scientific complementary metal oxide semiconductor (sCMOS) camera, and a digital micromirror device provided patterned light for photostimulation. The top-right panel shows the hardware used for real-time analysis of fluorescence and feedback control. The bottom panel shows optical membrane potential images acquired before and after photostimulation (blue arrowhead), revealing acceleration of ventricular activation after large-field illumination of the epicardium of a perfused mouse heart[137]. CPU, central processing unit; DAC, digital-to-analogue conversion; DMD, digital micromirror device; LED, light-emitting diode; Obj, objective; ROI, region of interest; SSD, solid state drive; Thr, threshold. Panel **a** modified with permission from REF.[111]. Panel **b** modified with permission from REF.[112]. Panel **c** modified with permission from REF.[137].

superior in their spatiotemporal capacity for precise wave control. Furthermore, optogenetic tools developed for research, such as the optical dynamic clamp[113], have enabled experimental testing of novel in silico strategies to design synthetic anti-arrhythmic ion channels that have gating properties that are modulated by the rate of electrical excitation, thereby enabling the potential for fully autonomous in situ termination of arrhythmias[114].

Leveraging cell-specific control of cardiac function. Cardiac function cannot be controlled in a cell-specific manner using electrical current. Pharmacological agents provide cellular specificity but have inferior resolution of spatiotemporal actuation. Optogenetics uniquely provides both the spatiotemporal resolution of cellular activation, or inhibition, and the cell and organelle specificity necessary to bring about new cardiac therapy paradigms (FIG. 7).

Cre–*lox* recombination and cell-specific promoters are used to target opsin expression within specific cell types[202] (for example, macrophages) (FIG. 7a). In a Cre–*loxP* transgenic approach, a Cre-responder animal would have a *loxP*-flanked STOP cassette upstream of the opsin gene, preventing its expression. This animal would be crossed with a Cre driver animal expressing Cre recombinase under the control of a cell-specific promoter, such as the *Myh6* promoter for cardiomyocytes[126,131], the *Th* promoter for sympathetic neurons[127,203] or the *Chat* promoter for parasympathetic neurons[142,204]. In the mice progeny, the opsin would then be expressed only within cells in which the promoter is active. Alternatively, with viral transduction, cell specificity is determined by the site of delivery (if localized), by tissue tropism of the viral vector and by the promoter used to drive expression. For example, for both localized and systemic vector delivery, the use of ubiquitous promoters, such as the cytomegalovirus or the CAG promoters, in combination with the cardiotropic AAV9 vector result in preferential ventricular myocyte transduction, with much lower expression in atrial myocytes and non-myocytes. To drive more robust atrial myocyte expression, one study used local gene painting and the *NPPA* promoter[191] (FIG. 5e).

A 2015 study used transgenic mice and Cre–*lox* recombination to express ChR2 in either cardiomyocytes (*Myh6* promoter) or cells of the specialized conduction system (*Cx40* promoter)[126] (FIG. 7b). This cell-specific expression provided insight into the arrhythmogenic role of the myocardium and the specialized conduction system during local ischaemia. In open-chest experiments, optical stimulation (photostimulation) of the cardiomyocytes triggered sustained arrhythmias after coronary artery ligation, but photostimulation of the specialized conduction system did not. Similar viral vector-based transduction of the Purkinje network could yield low-energy optogenetic pacing[146] (FIG. 7c), as discussed above. In addition, specific cell populations or myocardial regions prone to arrhythmogenic activity could be optically targeted to suppress arrhythmias.

Neurocardiology is a classic area that has benefited from the cell specificity of optogenetics. The complexity of the intrinsic cardiac autonomic network has been revealed using Cre–*lox* recombination in transgenic mice or viral delivery of fluorescent protein vectors, with impressive resolution[142], particularly after tissue clearing[204]. Neuron-specific expression of opsins enables precise functional mapping of cardioneural circuits, similar to what has been done for the brain[205]. Original studies in this area used transgenic mice with ChR2 expression targeted to either catecholaminergic neurons (using a *Th* promoter)[127] or cholinergic neurons (using a *Chat* promoter)[142]. Selective photostimulation of intrinsic catecholaminergic neurons in perfused mouse hearts initiated sudden and dramatic increases in heart rate and contractility[127] (FIG. 7d), consistent with downstream activation of β-adrenergic pathways and increases in cytosolic cAMP levels[203].

Another study used a silicon-encapsulated micro LED placed on the right atrium of perfused mouse hearts to photostimulate cholinergic neurons[142] (FIG. 7d). The RR interval quickly increased after illuminating the right atrium, consistent with activation of the $G_{\alpha i/o}$-coupled cholinergic M_2 muscarinic receptors of sinus node cells[206]. LED positioning altered the responses between heart rate slowing without atrioventricular delay and atrioventricular delay without heart rate slowing, confirming a cholinergic neural network whereby the sinoatrial node and the atrioventricular node are innervated by distinct axons[207,208]. Photostimulation also maintained substantially low heart rates for up to 30 min of continuously pulsed illumination[142], indicating that continuous photostimulation of the cholinergic network could be an approach for in vivo heart rate control. In other studies, photostimulation of autonomic neurons in the stellate ganglion and the vagus nerve increased and decreased heart rate, respectively[204] (FIG. 7e), supporting efficacious optical neuromodulation for the treatment of rhythm disorders[209]. Beyond rhythm control, in vitro studies with co-cultured cardiomyocytes and optogenetically enabled sympathetic neurons have revealed the effects of neuron–cardiomyocyte interactions on electrical wave dynamics[110] as well as the effect of cardiomyocytes on neuronal maturation[210].

Optogenetic suppression of sympatho-mediated ventricular arrhythmias has been reported in canine studies of acute ischaemia[211]. AAV9–ArchT was injected locally in the left stellate ganglion neurons, which were subsequently illuminated by an LED for an hour after coronary artery ligation. The resulting neuronal hyperpolarization greatly reduced the incidence of ventricular tachycardia and fibrillation[211], providing proof-of-concept results of optogenetic arrhythmia therapy in a large-animal model. In earlier studies, excitatory opsins were expressed within specific neural populations of the cardiac centres of the brainstem. These neural populations were then photostimulated to provide myocardial protection during ischaemia–reperfusion injury in rats[212] and to increase exercise capacity in healthy rats[213]. Optogenetic neuromodulation to improve cardiac function and the associated devices that would deliver light to the cardiac ganglia and peripheral nerves, including the vagus nerve, are important mid-term future translational opportunities. Cell-specific control provided by optogenetics currently

does not exist for electrical neuromodulation therapies, such as electrical stimulation of the vagus nerve.

Any cardiovascular cell type could probably be inscribed with light-sensitivity, especially as new Cre-expressing animal models become available and viral vectors are optimized. For example, one study used Cre–*lox* recombination in mice to target the expression of ChR2 to resident cardiac macrophages with the use

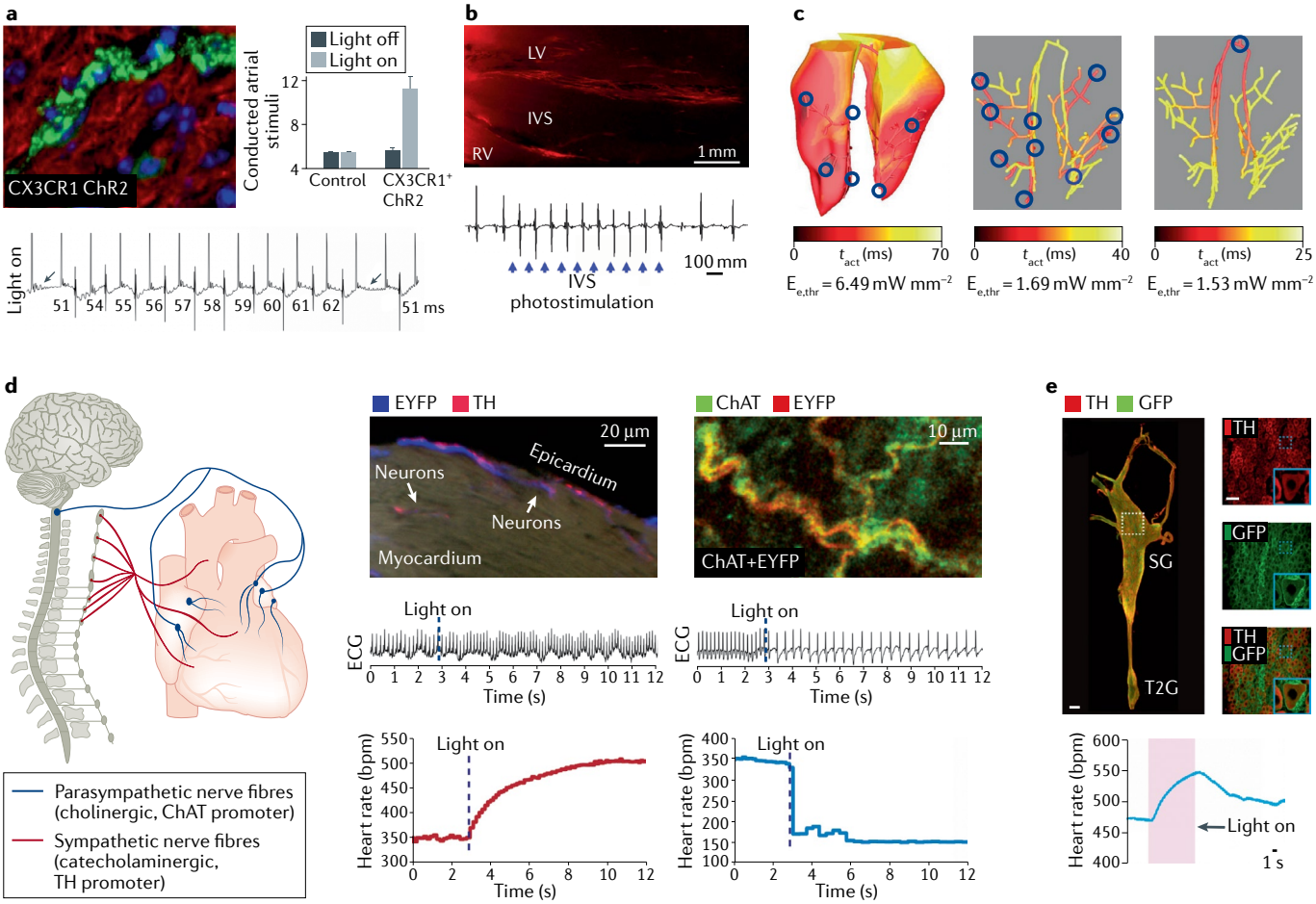


Fig. 7 | **Long-term translation of cardiac optogenetics for cell-specific control.** The figure shows proof-of-concept results from multiple studies. **a** | The top-left panel shows an atrioventricular node section from a CX3CR1^{+} channelrhodopsin 2 (ChR2) mouse, showing endogenous ChR2–yellow fluorescent protein (YFP) signal (green) expressed in macrophages and additional staining for HCN4^{+} cardiomyocytes (red) and nuclei (blue)[182]. Bar graphs of control and CX3CR1^{+}ChR2 hearts showing the number of conducted atrial stimuli between two non-conducted impulses of a Wenckebach period during light-off and light-on cycles (top-right panel). The bottom panel is an electrocardiogram (ECG) from a CX3CR1^{+}ChR2 mouse heart, illustrating an increased number of conducted atrial stimuli during a light-on cycle. Arrows indicate failure of conduction leading to a missing QRS complex. Numbers indicate atrioventricular delay in milliseconds. **b** | Evidence of selective expression of ChR2 in the conduction system of a mouse heart by the presence of ChR2-expressing bundles in the interventricular septum (IVS), as well as in the right ventricle (RV) subendocardium[126] (top panel). ECG signal showing ectopic beats originated by epicardial photostimulation of the IVS, where light penetration through the myocardium activated Purkinje fibres (bottom panel). **c** | Energy requirements of optogenetic pacing measured using a computer model of human ventricles[146]. The left panel shows the response to illumination of ChR2 gene delivery sites (blue circles) in regions of dense Purkinje system arborization. The middle panel shows the response to the same illumination pattern as in the left panel, but with gene delivery specific to the Purkinje system only. The right panel shows the response to His bundle illumination for the Purkinje system-specific gene delivery with light delivered at a single strategic site, requiring approximately four times lower energy (threshold for excitation, $E_{e,thr}$) for ventricular pacing than the approach shown in the left panel. **d** | Cell-specific expression of ChR2 within the cardiac autonomic nervous system. Choline acetyltransferase (ChAT) is an opsin expression promoter for parasympathetic neurons whereas tyrosine hydroxylase (TH) is a promoter for sympathetic neurons (top-left panel). The top-middle panel shows expression of enhanced YFP (EYFP)–ChR2 (blue) in TH-expressing sympathetic neurons (red) of the left ventricle (LV) of a mouse heart[127]. The middle-bottom panel shows the heart rate response during photostimulation of intrinsic cardiac TH-expressing neurons[51]. The top-right panel shows the colocalization of ChAT (green) with EYFP–ChR2 (red) within the nerve bundles of the right atrium[142]. The bottom-right panel shows the heart rate response during photostimulation of intrinsic cardiac ChAT-expressing neurons[142]. **e** | The top panel shows an image of the right paravertebral chain of a mouse stained with TH (red) and green fluorescent protein (GFP; green), with expression of ChR2 in the sympathetic neurons of the stellate ganglion (SG)[204]. The insets show single-plane images of the SG. Blue dashed boxes indicate the location of higher magnification images shown in the blue boxes. The bottom panel is a representative heart rate response during photostimulation of the craniomedial right SG. T2G, second thoracic ganglion. Panel **a** modified with permission from REF.[182]. Panel **b** modified with permission from REF.[126]. Panel **c** modified with permission from REF.[146]. Part **d** top-middle panel modified with permission from REF.[127]. Part **d** bottom panels modified with permission from REF.[51]. Part **d** top-right panel modified with permission from REF.[142]. Part **e** top panel modified with permission from REF.[204].

of the *Cx3cr1* promoter[182] (FIG. 7a). Atrioventricular conduction was improved after macrophage photostimulation, underscoring the importance of connexin 43 coupling between macrophages and cardiomyocytes for normal atrioventricular node conduction. Other studies used targeted optogenetic sensor expression to assess the electrotonic coupling of excitable and non-excitable cells[74] and to modulate coronary artery vasomotor tone[214]. A comprehensive list of studies that have applied optogenetic tools for cell-specific control of neurocardiac function[110,127,142,203,204,210–213,215] and control of myocardial function via non-myocyte cells[1,4,39,74,124,126,132,143,146,169,180–182,191,214,216–218] is provided in TABLE 1.

Conclusions

Over the past 10 years, the potential of cardiac optogenetics to empower basic and translational research has been demonstrated by an impressive growth in the number of published reports (TABLE 1). These include fundamental developments in translating optogenetics from the brain to the heart, the development of optogenetically enabled, high-throughput screening technologies for cardiotoxicity and successful proof-of-concept applications of optogenetics for cardiac rhythm control and defibrillation. Immediate translational opportunities reside in all-optical platforms that use iPSC-CMs for pharmaceutical discovery, development and toxicity screening because this strategy does not require incorporation of optogenetic tools in patients (BOX 1). All-optical platforms are inherently high-throughput, offering rapid testing, revision and re-testing of new drugs and therapies, particularly those involving patient-derived cells and tissues for personalized medicine and myocardial regeneration.

Challenges in translating cardiac optogenetics to the clinic are substantial and confounded by the obvious need for new therapies to be better than the current standard of care (BOX 1). Present-day cardiac devices have reliable efficacy and are fairly safe, setting a high barrier for optogenetics-inspired therapies. Nevertheless, the desire to improve patient quality of life is a strong motivation for further device innovations. Technical challenges for translation reside in the methods used to deliver opsins and light to the heart. To achieve opsin expression in patients would require gene therapy via AAV-based vectors or similar, and a minimally invasive procedure would be required for cardiac site-specific transduction of these vectors. Efficient expression of opsins in the presence of neutralizing antibodies in the host against the viral vectors is a challenge as is the risk of cardiotoxicity associated with viral capsids and opsins, as covered in a previous review on the current state of human gene therapy[219]. The possibility for the algae-derived opsins to trigger an immune response when expressed in the human heart deserves serious investigation before clinical deployment. Engaging resident opsins with light is further confounded by the vigorous contraction of the heart and the lack of a stabilizing surface to anchor an optical device. Visible light absorption by the blood remains a challenge that has motivated recent work to develop long-wavelength opsins, up-conversion of nanoparticle mediators for blue-light opsins, and other performance-boosting strategies. Finally, to leverage fully the cell-specific optogenetic control of cardiac function, the palette of well-characterized, cell-specific promoters would need to be expanded, at least to the level of what is currently available for neuroscience.

Even with these challenges, the envisioned clinical opportunities for cardiac optogenetics are plentiful (BOX 1). Exquisite spatiotemporal control provided by light and the cell-specific expression provides new fundamental methods for painless rhythm management. These methods include 'wave steering', ultra-low-energy cardioversion or defibrillation, the shaping of action potentials with the use of both depolarizing and hyperpolarizing opsins, and long-term hyperpolarization to inhibit cell excitability. Optical therapy for atrial arrhythmias is likely to be on the horizon in the mid-future. As the general population ages, the market potential for optogenetic treatment of atrial arrhythmias grows. Patients with arrhythmias could receive cardioversion repeatedly with the use of light without the pain and tissue damage that occurs with current ablation therapy. Upon eventual translation into humans, optogenetics could be the specific therapy, whereby 'optoceuticals', through cell-specific expression, would modulate the

Box 1 | **Translational opportunities and challenges for cardiac optogenetics**

Translational opportunities

- Optical platforms are inherently high-throughput, offering immediate near-term translation in cardiotoxicity testing, drug discovery and optimization of patient-derived cells and tissues for personalized medicine and cardiac regeneration.
- Long-term clinical translation can include fundamentally new methods for painless and ultra-low-energy (ventricular) cardioversion or defibrillation and rhythm control, enabled by the cell-specificity of optogenetics and its exquisite level of spatiotemporal feedback control, such as 'wave steering' rather than a brute-force shock.
- Targeted optogenetic disruption of re-entrant circuits can offer non-destructive, tissue-sparing methods for diagnosis and control of high-prevalence atrial arrhythmias compared with current ablation methods.
- Optogenetic methods can yield a range of 'optoceuticals' for cardiac-specific neuromodulation, control of inflammation, signalling and gene expression to improve heart function.
- Cardiac applications can be informed and empowered by fundamental discoveries enabled by optogenetics, including a version of an 'optical clamp'.

Translational challenges

- Gene therapy is part of optogenetic approaches, and all the technical and regulatory challenges that come with gene therapy affect the clinical translation of optogenetics. Cardiac optogenetics-inspired disruptive technology solutions have to out-compete the well-established and fairly safe electrical therapy devices for the heart.
- There are challenges in using adeno-associated virus-based viral vectors, the most common gene-therapy tools, to inscribe light sensitivity to the heart, including their lack of specificity, the physical inaccessibility to obtain adequate site-specific expression of the target gene, the need to overcome neutralizing antibodies against the viral vectors, and the potential cardiotoxicity of the viral capsid or the opsins.
- There are major challenges for implantable light-delivering and/or light-sensing devices due to lack of easy access and/or stabilizing surface, limited light penetration in the thick, dense cardiac muscle, and the mechanical motion of the heart.
- The limited palette of well-characterized promoters does not allow as extensive cell selectivity of optogenetic targeting in the cardiovascular system as in the brain.

activity of cardiac neurons, control inflammation and activate or suppress cell signalling and gene expression. Continued research and development in cardiac optogenetic technologies will undoubtedly inform and guide the development of a new generation of cardiac therapies.

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