Cardiovascular Research (2015) **106**, 180–181
doi:10.1093/cvr/cvv116

EDITORIAL

A step closer to cardiac optogenetics *in vivo*

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Online publish-ahead-of-print 25 March 2015

This editorial refers to 'Systemic gene transfer enables optogenetic pacing of mouse hearts' by C.C. Vogt *et al.*, pp. 338–343.

Imagine having the means to fully control excitation in the heart—to be able to trigger waves at precise locations and with desired properties and to be able to precisely manipulate such waves, including to selectively overwrite them (that is, to terminate an arrhythmia without brute force); imagine being able to do all this in the intact heart, *in vivo*, by light; imagine being able to 'see' all this in real time, optically. The idea of 'all-optical cardiac electrophysiology'[1,2] may seem like science fiction, but recent advances in optogenetics[3–6] make it more and more tangible.

New technologies for cell-specific fast optical sensing or optical actuation have profoundly impacted neuroscience over the last decade and have become instrumental in the mechanistic dissection of brain function, *in vivo*.[7] These techniques rely on genetically encoded proteins, i.e. require genetic modification in the cells and tissues of interest, and most often employ transgenic animals. The cardiovascular area is lagging behind, and such transgenic mice, expressing optogenetic sensors and/or actuators, are still not widely available. Notably, the new CHROMus resource,[8] sponsored by the National Institutes of Health in the USA, promises to supply a variety of relevant transgenic mouse models for the cardiovascular system, enabling optogenetic manipulation and imaging. Furthermore, new gene editing techniques open the door to affordable commercial generation of desired transgenic mice or even larger transgenic animals in a shorter time frame. Although the transgenic approach is highly valuable for mechanistic studies, translational relevance requires the pursuit of alternative (non-transgenic) ways for genetic manipulation. Quick and effective cell-mediated,[9] adenoviral, or lentiviral expression of optical actuators in neonatal and adult cardiomyocytes has been shown *in vitro*,[10–13] but these approaches have not been demonstrated *in vivo*. While optogenetic interrogation of the mammalian brain in freely moving animals has become common place, comparable *in vivo* optogenetic manipulation of cardiac function is yet to be realized; a robust way to inscribe light sensitivity in the intact heart or heart structures of interest is a requisite step towards this goal.

Sasse's laboratory first demonstrated optogenetic manipulation of the mammalian heart using transgenic mice.[14] Following on this pioneering work, in this issue, they[15] report a minimally invasive non-transgenic approach to cardiac optogenetics for *in vivo* applications. A relatively straightforward systemic viral delivery of a depolarizing opsin, channelrhodopsin2 (ChR2), in adult mouse hearts is demonstrated, and robust optical responsiveness is confirmed at different regions of the ventricles in open-chest experiments. Employing endovascular gene delivery of ChR2 by adeno-associated virus serotype 9 (AAV9), with known high cardiac tropism,[16] Vogt *et al.*[15] achieve an impressive, almost exclusively cardiac-specific, expression despite the use of a ubiquitous (CAG) promoter. While the wild-type AAVs feature unique site-specific integration in the genome,[17] the AAV vectors devoid of viral genes, as used in this study, do not integrate in the genome but, remarkably, retain coveted long-term expression (10 months demonstrated here, 10 years seen in a patient[18]), seemingly without disruption of other genes. The low immunogenicity of AAVs that makes them an attractive tool for gene therapy in humans[19] was also confirmed here, with no inflammation reported.

The advantages of the reported minimally invasive method, compared with transgenic approaches for cardiac optogenetics, include: (i) easy scalability to larger animals (including established cardiac disease models), where *in vivo* insertion of optical conduits for stimulation or imaging is feasible;[20] (ii) robust combination of genetic manipulations in the same animal, e.g. combining spectrally compatible actuators and sensors, or combining opsins with depolarizing and hyperpolarizing effects for bi-directional control by light; and, importantly, (iii) the approach provides a path to potential clinical translation. Considering the success of AAV-based clinical trials, including the CUPID trial[21] for heart failure patients, this is an important and relevant step, even if basic science applications are the main current focus of this technology.

The AAV-mediated optogenetic transformation is not without problems. Naturally occurring neutralizing antibodies make this approach somewhat subject-specific, though out of all 13 known serotypes, encountering antibodies against AAV9 is the least likely (found in $<$20% of humans).[22] This may have been a contributing factor in the lack of response in 26% of the studied animals by Vogt *et al.*[15] Furthermore, specificity of expression is dose-dependent, e.g. viral doses higher than the employed here (2×10^{11} viral particles per mouse) can yield non-myocardial expression for AAV9 in the diaphragm, liver, and skeletal muscle, and can even cross the blood–brain barrier to infect neurons and astrocytes,[23] which may or may not cause side effects. Cell- and tissue-specific promoters, combined with serotype tropism, can alleviate these problems but often at the cost of weaker expression. Interestingly, AAV9 has been reported to localize preferentially to ischaemic areas (border zone),[24] which can be leveraged for region targeting in cardiac applications.

The opinions expressed in this article are not necessarily those of the Editors of the *Cardiovascular Research* or of the European Society of Cardiology.

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A couple of findings by Vogt *et al.* are of particular interest. The demonstrated ability to optically pace with relatively low-level blue light in blood-perfused hearts (open chest)[14,15] holds promise for future *in vivo* use. Furthermore, the paradoxical inferior optical excitability of atrial tissue, reported here and in transgenic mice,[14,15] is at odds with the theoretically and experimentally found higher excitability in single atrial cells (compared with ventricular),[10,14] and can be indicative of the dominant role that cell–cell coupling plays in the response of cardiac tissue to light (less-coupled atria are less responsive). Finally, Vogt *et al.*[15] estimated that a minimum cell transduction rate of 40% was required for optical pacing in the ventricles. This number represents a relevant constraint for investigative purposes, i.e. to allow robust optogenetic perturbation of electrical activity at an arbitrary ventricular location, with a relatively small optical conduit. However, the creation of a space-localized optical biological pacemaker can be achieved by a much smaller number of optogenetically transformed cells (by gene or cell delivery), as long as the light-responsive region is consolidated, to provide enough charge for driving the myocardium.

Following the demonstration of this elegant approach for minimally invasive and stable optogenetic transformation of the heart, the next logical step is to take full advantage of the cell specificity of optogenetic targeting to strategic structures, e.g. sinoatrial node, atrioventricular node, or His bundle, or for dissection of neural–cardiac interactions,[25] as reported recently. Outstanding practical challenges to be addressed concern the light delivery to a desired cardiac location in the intact animal, for true *in vivo* optogenetic actuation, as commonly done in the brain. Two main approaches to optically stimulate and optically record appear feasible: endoscopically[20] using fibre optics and borrowing from microendoscopy applications in neuroscience, or through the implantation of miniaturized devices.[26] Additionally, spectral challenges also need to be resolved, i.e. red-shifted opsins are desirable for deeper penetration. However, the open-chest experiments by Vogt *et al.* suggest that for optical actuation, light absorption by haemoglobin may be an addressable problem, once the light is guided to the myocardial location.

In summary, Vogt *et al.* have demonstrated that making the heart sense light can be 'easy'; yet, bringing the light to the heart still faces further challenges *in vivo*. This work represents an important new development—the first experimental model for cardiac optogenetics that goes beyond *in vitro* and transgenic animal approaches. Such a model presents new opportunities to study the origin and control of cardiac arrhythmias by precise optical perturbations *in vivo*. Furthermore, the simplicity and the impressive reported efficiency of the method make it attractive for a much wider range of applications (beyond optogenetics) for gene delivery to the heart.

Funding

This work is supported by a grant from the National Institutes of Health—National Heart, Lung, Blood Institute R01HL111649 to E.E.

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