

## atrial fibrillation

# An automated hybrid bioelectronic system for autogenous restoration of sinus rhythm in atrial fibrillation

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Because of suboptimal therapeutic strategies, restoration of sinus rhythm in symptomatic atrial fibrillation (AF) often requires in-hospital delivery of high-voltage shocks, thereby precluding ambulatory AF termination. Continuous, rapid restoration of sinus rhythm is desired given the recurring and progressive nature of AF. Here, we present an automated hybrid bioelectronic system for shock-free termination of AF that enables the heart to act as an electric current generator for autogenous restoration of sinus rhythm. We show that local, right atrial delivery of adenoassociated virus vectors encoding a light-gated depolarizing ion channel results in efficient and spatially confined transgene expression. Activation of an implanted intrathoracic light-emitting diode device allows for termination of AF by illuminating part of the atria. Combining this newly obtained antiarrhythmic effector function of the heart with the arrhythmia detector function of a machine-based cardiac rhythm monitor in the closed chest of adult rats allowed automated and rapid arrhythmia detection and termination in a safe, effective, repetitive, yet shock-free manner. These findings hold translational potential for the development of shock-free antiarrhythmic device therapy for ambulatory treatment of AF.

## INTRODUCTION

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia and is known to have a negative impact on mortality, morbidity, and quality of life (1). Despite current treatment options, a large number of patients with AF still suffer from drug-resistant and symptomatic AF recurrences, even after multiple ablation procedures (2–4). For these symptomatic patients, electrical cardioversion remains the only proven and effective means for acute restoration of sinus rhythm (5, 6) and should therefore be applied each time AF recurs. Such restoration of sinus rhythm involves manual transthoracic delivery of electroshocks upon sedation to prevent pain and discomfort. AF duration is, however, inversely related to the success of maintaining sinus rhythm, meaning that continuous, rapid detection and termination are important for effective AF treatment (7, 8).

To meet this need for urgency, the detector (cardiac rhythm monitor) and effector (electric current generator) were incorporated in one device and implanted within the human body to allow continuous rhythm monitoring and therefore rapid detection and termination of AF. The use of such automated implantable atrial defibrillators was studied in the mid-1990s and proved to be safe and effective in the ambulatory treatment of AF (9, 10). Despite these advantages, most of the recipients of these devices discontinued therapy because of their intolerance to repeated cardioversion shocks (11). Non-selective excitation of nerves and skeletal muscles by these electroshocks causes severe pain, and electrical cardioversion may also result in myocardial tissue damage (12). Consequently, there is currently no therapeutic strategy for continuous, rapid, and shock-free

termination of AF. One can, however, hypothesize that the negative adverse effects of electroshock therapy can be overcome by allowing the heart, a bioelectric organ containing excitable cells, to produce the electric current required for arrhythmia termination and subsequent restoration of sinus rhythm. Thus, the effector function of an electrical defibrillator would be provided by the heart itself and would no longer rely on electronics but rather on bioelectricity.

This concept of autogenous arrhythmia termination can theoretically be realized by properly timed generation of endogenous cardiac currents after forced expression and activation of light-sensitive ion channels through optogenetics (13–15). Such an antiarrhythmic approach would make the delivery of painful electroshocks obsolete, thereby creating the possibility of continuous and rapid termination of AF. The anticipated benefit for patients would range from minimizing the effective duration of AF to avoiding AF-related symptoms, complications, morbidity, repetitive hospitalization, and subsequent application of electroshocks. This could potentially not only slow the natural progression of AF and improve prognosis but also increase the patients' quality of life (16, 17). Here, we combined an optogenetically established biological effector with a machine-based cardiac rhythm monitor to create a bioelectronic hybrid system for AF treatment. Our study shows that, once established in the closed-chest adult rat, this system allows continuous and rapid cardiac arrhythmia detection and termination in an automated, specific, and shock-free manner.

## RESULTS

### Local viral vector delivery by atrial gene painting

To equip the heart with the effector function of the envisioned AF termination system, we genetically modified the right atrium (RA) of adult rats with adenoassociated virus vectors (AAVs) encoding red-activatable channelrhodopsin (ReaChR; fused to the green fluorescent protein variant citrine) under control of the atrial myocyte-specific human natriuretic peptide precursor A (NPPA) gene promoter

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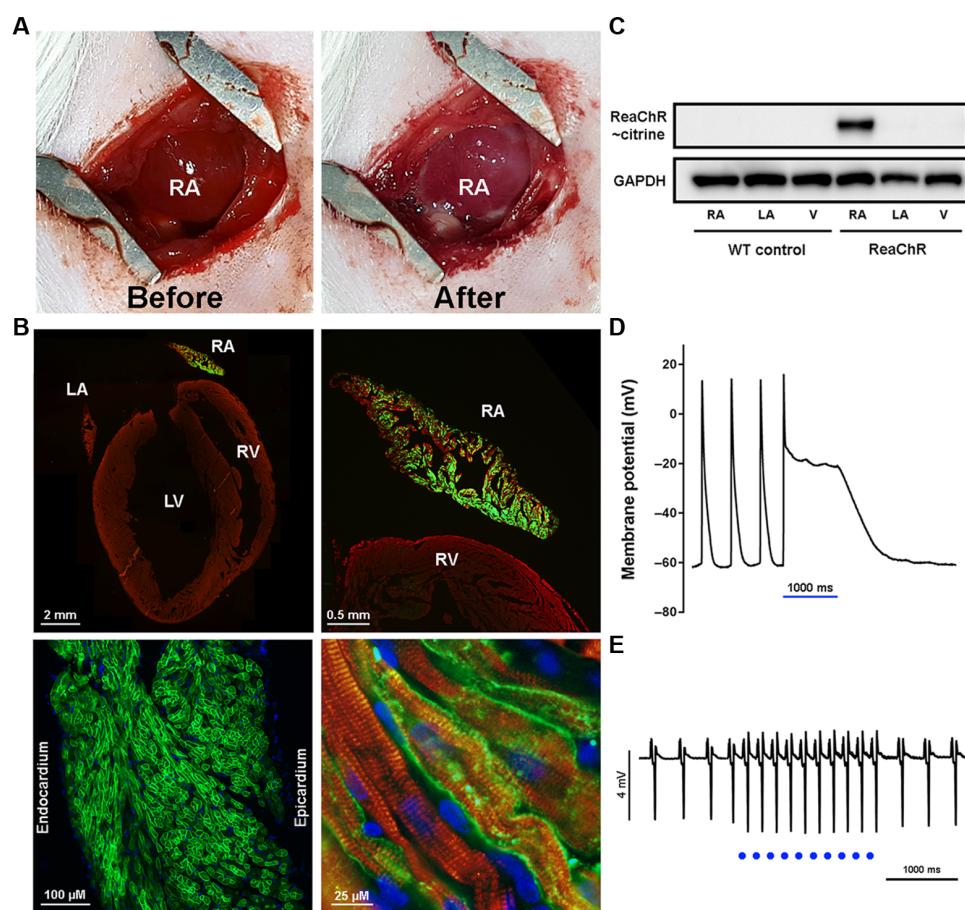
(AAV2/9.45.HsNPPA.ReaChR~citrine.SV40pA) or encoding citrine as control (AAV2/9.45.HsNPPA.citrine.SV40pA) (fig. S1). AAVs were delivered locally to the right atrial epicardium by gene painting (Fig. 1A) (18). Immunohistological analysis 4 weeks after gene painting with AAV2/9.45.HsNPPA.ReaChR~citrine.SV40pA showed transmural transduction of right atrial myocytes (average transduction rate,  $78 \pm 6\%$ ) and little transgene expression in the left atrium (LA;  $6 \pm 2\%$ ), right ventricular wall ( $0.3 \pm 0.3\%$ ), and left ventricular wall ( $0.1 \pm 0.1\%$ ) ( $n = 6$ ) (Fig. 1B, fig. S2, and data file S1), which was confirmed by Western blot analysis (Fig. 1C). Similar transduction rates were observed in citrine control animals (fig. S3). The average thickness of the right atrial wall, measured histologically, was  $0.31 \pm 0.11$  mm ( $n = 5$ ) (data file S1). Masson's trichrome staining revealed some thickening of the collagen-rich anterior epicardial layer of the RA suggestive of fibrosis, but there were no signs of inflammatory infiltrations (fig. S4). The functionality of ReaChR was verified by sharp electrode measurements of the RA, showing strong and sustained depolarization of the membrane during 470-nm light-emitting diode (LED) stimulation (Fig. 1D). The average time to maximum depolarization of the membrane upon 470-nm light stimulation was  $14 \pm 1$  ms, whereas the time to maximum repolarization after a 1000-ms light pulse was  $921 \pm 40$  ms.

### Ex vivo optogenetic pacing and atrial arrhythmia termination

The possibility for optical atrial pacing and atrial tachyarrhythmia termination was first studied ex vivo in isolated rat hearts using a Langendorff apparatus. Brief 470-nm light pulses targeting about  $20 \text{ mm}^2$  of the ReaChR-expressing RA allowed optical atrial pacing up to 5 Hz with 1:1 ventricular conduction (Fig. 1E), whereas optical pacing of the LA and ventricles was not possible, consistent with the observed minimal transgene expression in these areas. Light sensitivity thresholds for stable optical atrial pacing (100% atrial capture) were quantified by determining minimal light pulse duration for various light pulse intensities during 5-Hz pacing (fig. S5). Light pulse intensities of  $0.02 \text{ mW/mm}^2$  (with a corresponding light pulse duration of  $7 \pm 1$  ms) resulted in stable optical pacing of the RA for all hearts tested ( $n = 12$ ), whereas the RA could also be optically paced with 1-ms light pulses using a minimum light pulse intensity of  $0.8 \text{ mW/mm}^2$ . As expected, the RA of citrine control rats was not reactive to 470-nm illumination.

Next, we induced atrial tachyarrhythmias by epicardial electrical burst pacing of the RA or LA (2000 mV; pulse duration, 10 ms; cycle length, 20 to 40 ms; total time, 0.5 to 2 s). Before arrhythmia induction, the oxygenated Tyrode's solution of

the Langendorff apparatus was supplemented with the  $I_{KACH}$ -increasing muscarinic receptor agonist carbachol (a final concentration of  $4 \mu\text{M}$ ) because atrial tachyarrhythmias could not be induced reliably without an action potential duration (APD)-shortening drug. Optical voltage mapping confirmed that carbachol perfusion significantly reduced the average atrial APD at 80% repolarization ( $\text{APD}_{80}$ ) during 5-Hz pacing ( $43 \pm 1$  ms versus  $31 \pm 4$  ms,  $P = 0.004$ ; data file S1) with subsequent shortening of the atrial effective refractory period, thereby mimicking baseline conditions of atria that are prone to develop AF (19). After induction of sustained atrial tachyarrhythmias ( $>10$  s), a single epicardial 470-nm light pulse ( $3.5 \text{ mW/mm}^2$ ) targeting  $20 \text{ mm}^2$  of the center of the ReaChR-expressing RA (fig. S6A) resulted in 100% arrhythmia termination in all hearts tested ( $n = 12$ ) (Fig. 2, A and B), whereas in citrine control rats, 8% (SEM, 5%) of the arrhythmias were terminated ( $n = 4$ ,



**Fig. 1. Functional right atrial ReaChR expression after gene painting.** (A) Representative photographs of the RA before and directly after gene painting. AAV2/9.45.HsNPPA.ReaChR~citrine.SV40pA particles were applied to the surface of the RA in a fibrinogen solution after which thrombin was added to induce clot formation (pale covering). (B) Representative immunohistological staining of longitudinal sections of the heart for green fluorescent protein (green) and cardiac troponin I (red) (upper left and right panels) showing transgene expression in the RA. Cell nuclei are stained in blue. ReaChR expression was transmural (lower left panel), and the protein was mainly plasma membrane bound (lower right panel). (C) Western blot analysis of ReaChR~citrine expression in the RA, LA, and ventricle (V) in a wild-type (WT) control rat and in a ReaChR-expressing rat 4 weeks after gene painting. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. (D) Sharp electrode measurement of the ReaChR-expressing RA showing spontaneous action potentials silenced by a 1000-ms light pulse ( $\lambda = 470 \text{ nm}$ ; irradiance,  $2.55 \text{ mW/mm}^2$ ) leading to sustained depolarization during illumination. (E) Optical atrial pacing by 470-nm light pulses (blue dots) of the ReaChR-expressing RA resulting in 1:1 atrial capture and ventricular conduction. RV, right ventricle; LV, left ventricle.

$P = 0.001$ ). In addition, none of the arrhythmias in ReaChR-expressing hearts were terminated during no-light control experiments ( $n = 8$ ,  $P < 0.001$ ) or when stimulated with light outside the excitation spectrum of ReaChR (730 nm, 1000 ms, 3.5 mW/mm $^2$ ) ( $n = 4$ ,  $P = 0.001$ ). The efficacy of autogenous arrhythmia termination was independent of the atrial burst pacing location.

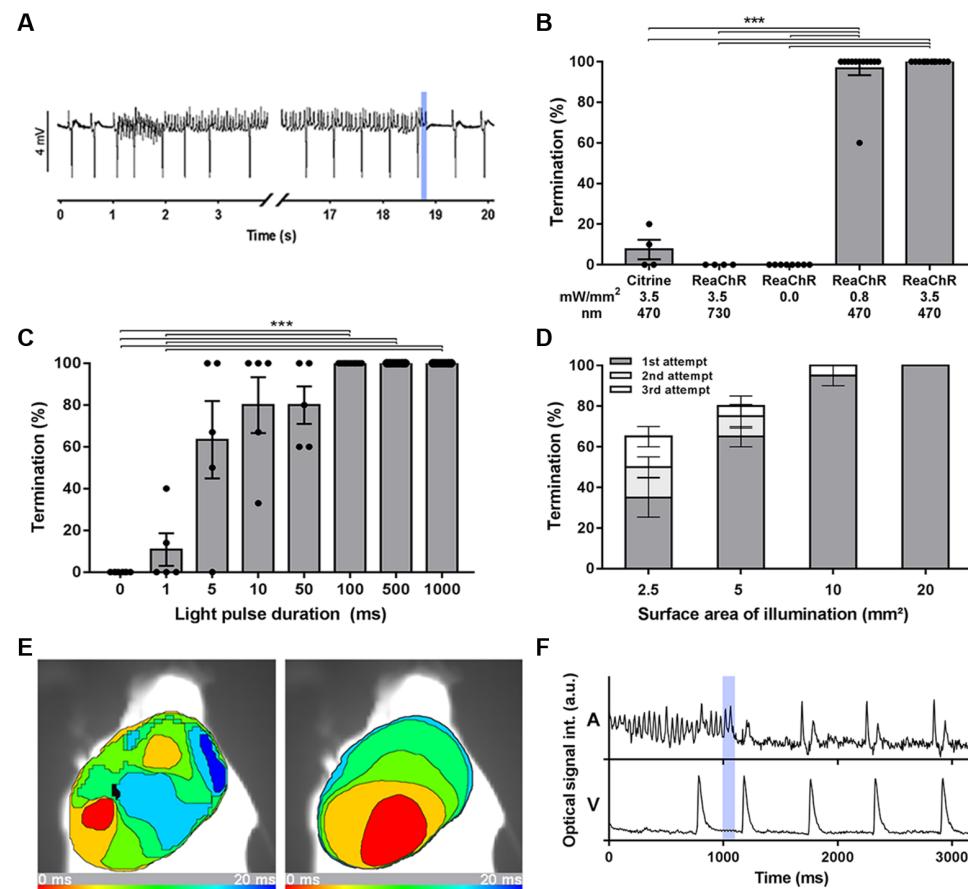
Termination was also tested with various light pulse intensities and durations and with different right atrial areas and locations of light exposure (Fig. 2, B to D, and fig. S6). We found that light pulses (3.5 mW/mm $^2$ ) of 100-ms duration illuminating 20 mm $^2$  of the RA allowed termination of all atrial tachyarrhythmias. When light pulse duration was increased to 1000 ms, a light pulse intensity of 0.8 mW/mm $^2$  sufficed to terminate 97% (SEM, 3%) of the atrial tachyarrhythmias ( $n = 12$ ). Reducing the right atrial surface area of illumination to 10 mm $^2$  (light pulse intensity, 2.0 mW/mm $^2$ ; single pulse of 500 ms) still resulted in 95% (SEM, 5%) termination efficacy ( $n = 4$ ). However, arrhythmia termination efficacy decreased when the illuminated area was further reduced to 5 mm $^2$  (65%; SEM, 5%) or 2.5 mm $^2$  (35%; SEM, 10%) ( $n = 4$ ). The impaired optogenetic arrhythmia termination efficacy by smaller surface areas of illumination was partly compensated by allowing three subsequent attempts [increase to 80% (SEM, 8%) and 65% (SEM, 17%) for illuminated areas of 5 and 2.5 mm $^2$ , respectively]. Illumination of the anterior region of the RA near the auricle was less effective compared to central RA illumination for the light pulses of 5 and 2.5 mm $^2$  (fig. S6C). Activation maps during AF confirmed global high-frequency activation of the atria resembling both non-organized and organized re-entry circuits, which were terminated after a single 470-nm light pulse leading to restoration of sinus rhythm (Fig. 2, E and F, and movie S1).

### Efficient autogenous AF termination *in vivo*

Next, we performed *in vivo* experiments in adult rats with gene-painted RAs to evaluate the feasibility of autogenous atrial tachyarrhythmia termination by optical stimulation of the blood-perfused heart. The RA of anesthetized and mechanically ventilated rats was visualized by a minithoracotomy of the fourth right intercostal space, followed by gentle rib spreading. Optical stimulation of the RA was performed with an external 470-nm LED illuminating about 20 mm $^2$  of the tissue (Fig. 3A). Optical pacing of the RA was feasible up to 10 Hz by brief 470-nm light pulses (1 ms, 3.5 mW/mm $^2$ ) in all ReaChR-expressing hearts tested ( $n = 12$ ) (Fig. 3B). Light sensitivity thresholds for

*in vivo* optical pacing were somewhat higher than for optical pacing in the Langendorff setup (fig. S5), which can be explained by the larger photon scattering and light absorption of light *in vivo* (20) due to the presence of blood instead of colorless Tyrode's solution.

Because of the differences in pathological entities, clinical outcome, and treatment options between AF and atrial flutter, atrial tachyarrhythmias were categorized in AF or atrial flutter based on three-lead body surface electrocardiographic (ECG) recordings and were considered to be sustained if lasting for >10 s. Electrical burst pacing of the RA, after a single intraperitoneal carbachol injection (50  $\mu$ g/kg), allowed reliable AF induction in which typically one of three episodes was sustained. After induction of sustained AF, a single brief 470-nm



**Fig. 2. Autogenous termination of atrial tachyarrhythmias ex vivo.** (A) Intracardiac ECG demonstrating termination of an atrial tachyarrhythmia with a single 470-nm light pulse (100 ms, 0.8 mW/mm $^2$ , light blue box) illuminating 20 mm $^2$  of the RA. Bipolar ECG needles were attached to the LA and ventricle to visualize both atrial and ventricular electrical activity on a single lead. (B and C) Quantification of light-induced termination of atrial tachyarrhythmias expressed as a percentage of successful attempts averaged for ReaChR-expressing and control hearts for various light pulse intensities and wavelengths (light pulse duration, 1000 ms) (B) or averaged for light pulse duration in ReaChR-expressing hearts only ( $\lambda = 470$  nm; irradiance, 3.5 mW/mm $^2$ ) (C). (D) Quantification of optical arrhythmia termination efficacy averaged per ReaChR-expressing heart ( $n = 4$ ) for various surface areas of illumination ( $\lambda = 470$  nm; irradiance, 2.0 mW/mm $^2$ ; light pulse duration, 500 ms). Illumination was repeated up to three times in cases of prior unsuccessful arrhythmia termination. (E) Electrical activation map of a ReaChR-expressing RA, derived from optical voltage recordings, showing a re-entrant conduction pattern during AF and subsequent restoration of sinus rhythm after optogenetic arrhythmia termination. (F) Optical voltage trace showing rapid and chaotic atrial electrical activation during AF, which converts to sinus rhythm with 1:1 ventricular conduction after exposure of the RA to a 100-ms light pulse ( $\lambda = 470$  nm; irradiance, 3.5 mW/mm $^2$ ; light blue box). int., intensity; a.u., arbitrary units. Data are means  $\pm$  1 SEM. Each dot represents a minimum of three independent measurements in one heart. \*\*\* $P \leq 0.001$  by (B) Mann-Whitney  $U$  test ( $n = 4$  to 12 hearts per group) or (C) Kruskal-Wallis test with Dunn-Bonferroni post hoc test ( $n = 5$  to 12 hearts per group).

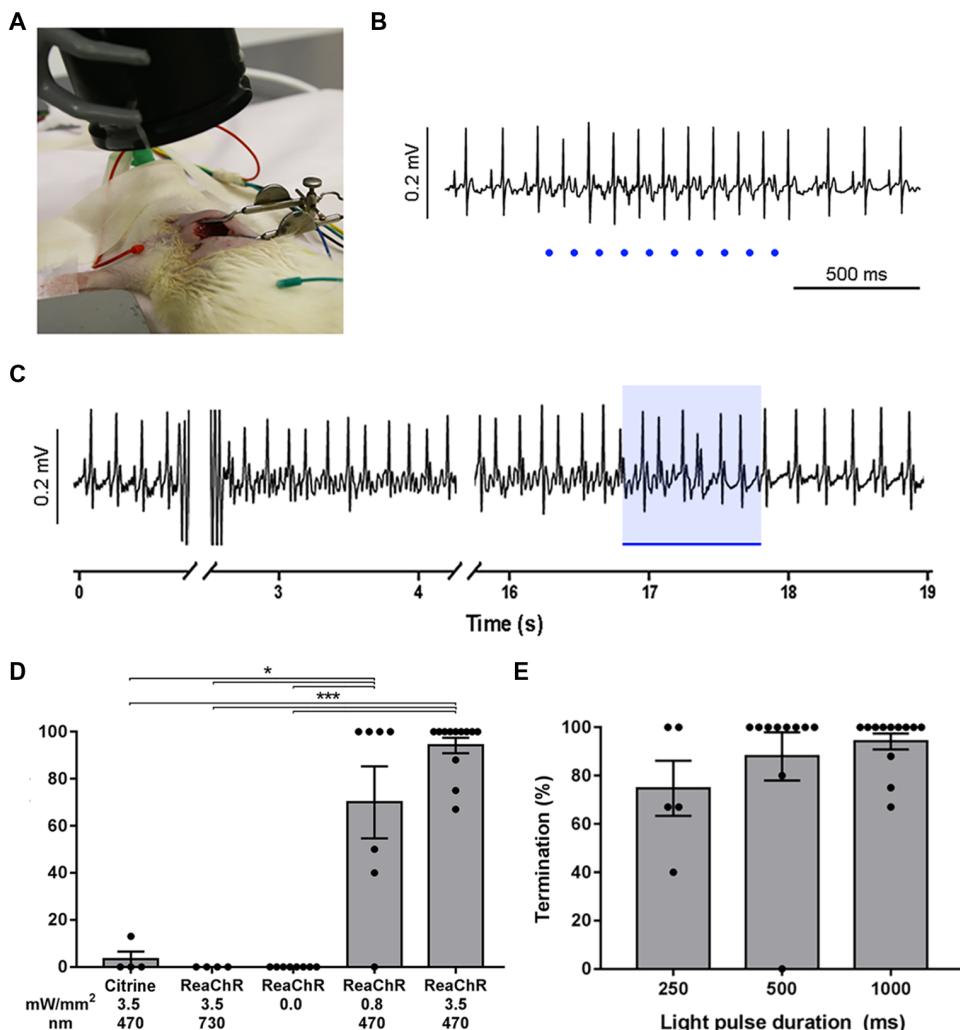
light pulse targeting 20 mm<sup>2</sup> of the RA terminated AF in all ReaChR-expressing rats tested ( $n = 12$ ) (Fig. 3, C and D), with an average termination efficiency of 94% (SEM, 3%) when a light pulse (3.5 mW/mm<sup>2</sup>) of 1000 ms was applied ( $n = 12$ ), compared to an average AF termination rate of 3% (SEM, 3%) in citrine control rats ( $n = 4$ ,  $P = 0.001$ ). None of the sustained AF episodes in ReaChR-expressing rats was terminated during no-light control experiments with the LED inactive ( $n = 8$ ,  $P < 0.001$ ) or when the RA was exposed to light outside the excitation spectrum of ReaChR (730 nm, 1000 ms, 3.5 mW/mm<sup>2</sup>) ( $n = 4$ ,  $P = 0.001$ ) (Fig. 3D). All atrial flutter episodes in ReaChR-expressing rats ( $n = 7$ ) were terminated after RA illumination with a single

1000-ms 470-nm light pulse (3.5 mW/mm<sup>2</sup>; fig. S7). Autogenous restoration of sinus rhythm remained efficient throughout the experiments, even after repetitive cardioversion attempts. There was a nonsignificant trend toward the decrease in light-induced AF termination efficacy ( $P = 0.178$ ) when light pulse duration and intensity were reduced (Fig. 3E).

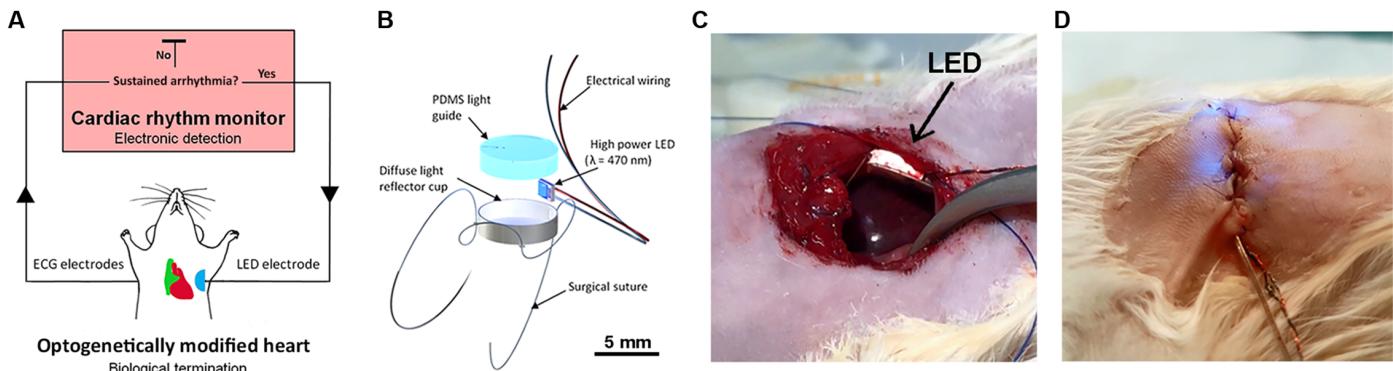
### Development of a hybrid system for automated detection and subsequent autogenous AF termination

Next, we combined this newly obtained antiarrhythmic function of the optogenetically modified heart (light-controlled electric current generator) with the arrhythmia detector function of a machine-based cardiac rhythm monitor (Fig. 4A) to establish an automated hybrid bioelectronic system in adult rats with closed chests. We developed an LED device small enough for implantation in the thoracic cavity (Fig. 4B and fig. S8). This LED device ( $\lambda = 470$  nm; diameter  $\times$  height, 6 mm  $\times$  1.5 mm; light-emitting surface area, 28 mm<sup>2</sup>) was sutured to the inside of the adult rat thoracic wall facing the anterior side of the RA (Fig. 4C). The LED implant was controlled by a custom-made AF detection algorithm based on ECG-based PR and RR interval irregularities (fig. S9). Human input was solely required for arrhythmia induction by electrical burst pacing. After intrathoracic implantation of the LED device and epicardial pacing electrode, the incision was completely closed in layers (Fig. 4D). Symmetrical respiration was present in all rats after LED implantation.

After the successful induction of atrial tachyarrhythmias, the algorithm detected all induced AF episodes without any false positives during sinus rhythm. Atrial flutters were not automatically detected by the algorithm because of their inherent regularity. Detection of AF was followed by a programmed delay of 10 s to ensure that AF was sustained before the LED was activated. Upon detection of AF and expiration of the programmed delay, automated LED activation (3.5 mW/mm<sup>2</sup>, 500 ms) resulted in 96% (SEM, 4%) termination of AF (corresponding to 29 AF episodes in four rats) (Fig. 5, A and B, and movie S2). Time to automated LED activation averaged  $23 \pm 11$  s (range, 10 to 48 s) after induction of AF (Fig. 5C). The spontaneous termination rate of sustained AF was, on average, 4% (SEM, 4%) (corresponding to 1 of 30 AF episodes in four rats), thereby confirming that the sustained AF episodes were stable for the time period needed for automated AF detection.



**Fig. 3. Autogenous termination of AF in vivo.** (A) Overview of the in vivo open-chest experimental setup showing a mounted 470-nm LED collimation lens directed at the RA after a minithoracotomy and subsequent rib spreading of the fourth right intercostal space. (B) Typical body surface ECG trace showing optical pacing of the RA with 470-nm light pulses (1 ms, 3.5 mW/mm<sup>2</sup>, blue dots) during 10-Hz pacing resulting in 1:1 ventricular conduction. (C) Typical body surface ECG trace demonstrating successful in vivo light-induced termination of AF and subsequent restoration of sinus rhythm by a single 470-nm light pulse (1000 ms, 3.5 mW/mm<sup>2</sup>, blue shaded region). (D and E) Quantification of light-induced termination of AF for all ReaChR-expressing hearts and citrine controls, expressed as a percentage of successful attempts averaged for all rats, for various light pulse intensities and wavelengths (light pulse duration, 1000 ms) (D) and various light pulse durations for ReaChR-expressing rats (light intensity, 3.5 mW/mm<sup>2</sup>) (E). Data are means  $\pm$  1 SEM. Each dot represents a minimum of three independent measurements in one rat. \* $P < 0.05$  and \*\*\* $P \leq 0.001$  by the Mann-Whitney U test ( $n = 4$  to 12 rats per group). For (E), the Kruskal-Wallis test found no significant differences ( $n = 5$  to 12 rats per group;  $P = 0.178$ ).



**Fig. 4. Overview of the hybrid bioelectronic system with implantable LED device.** (A) Schematic diagram of the automated hybrid bioelectronic system consisting of the optogenetically modified RA (green) and a cardiac rhythm monitor. The input of the cardiac rhythm monitor consists of analog body surface ECG signals that are automatically analyzed for rhythm anomalies. Upon detection of sustained AF, an output signal is generated, switching on the implantable LED device for 500 ms and leading to autogenous termination of AF and restoration of sinus rhythm by activation of the light-gated depolarizing ion channels present in the RA. Note that both the input and output of this hybrid bioelectronic system do not depend on physical contact with the heart. (B) Exploded schematic view of the implantable LED assembly. The LED is placed inside the reflector cup at a 90° angle to enable total internal reflection. The surgical suture is shown to illustrate the fixation method. (C) Demonstration of the LED implantation process, before closure of the chest, showing firm fixation of the LED implant to the inside of the thoracic wall of the rat heart at the height of the RA (without making physical contact with the heart). (D) Demonstration of the LED implant during illumination after surgical closure of the thoracic wall, muscle layers, and skin. The electrical wires entering the wound drive the pacing electrode and LED.

Automated AF detection and subsequent LED activation failed to terminate AF episodes in citrine control rats ( $n = 28$  in four rats; Fig. 5B). In addition, covering the LED with nontranslucent tape prevented AF termination in ReaChR-expressing rats ( $n = 17$  in 3 rats; Fig. 5B), thereby excluding that heat or electromagnetic fields produced by the implanted LED caused AF termination. Because this approach for atrial tachyarrhythmia termination relied on autogenous atrial depolarization rather than on extraneous high-voltage electroshocks, no electrical artifacts were observed on the body surface ECG. No bradycardias were observed after autogenous termination of AF: The average RR intervals before AF induction ( $0.216 \pm 0.041$  s) and 5 to 15 s after AF termination ( $0.216 \pm 0.042$  s) were similar ( $P = 0.867$ ) (Fig. 5D). The longest measured RR interval immediately after optical cardioversion was 0.332 s.

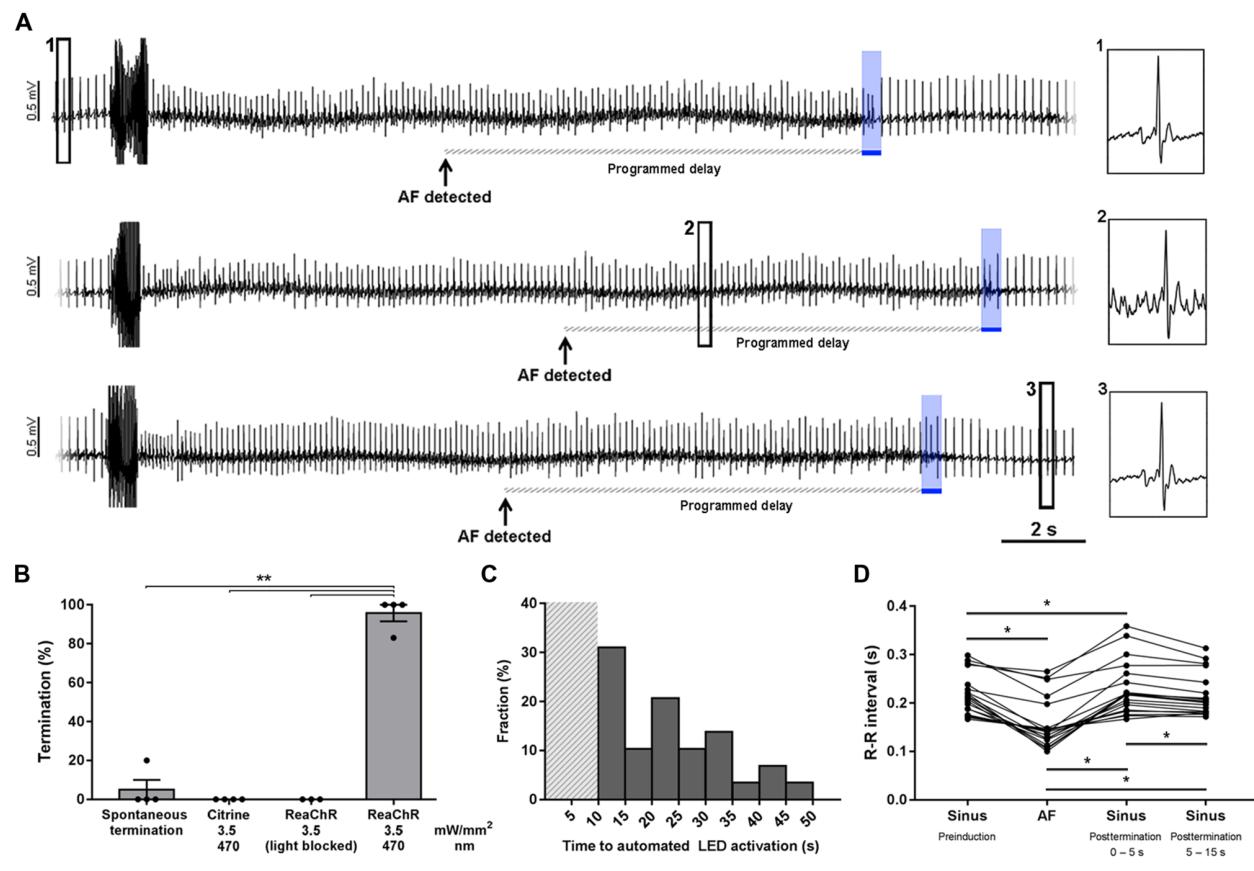
As a final experiment, the penetrance of 470-nm LED light in the blood-containing right atrial wall was investigated. We observed that  $35 \pm 5\%$  ( $n = 5$ ) of the emitted light passed the atrial wall, indicating that illumination was transmural in our experimental setup (data file S1).

### Evaluation of safety parameters

Various experiments and analysis were performed to assess the safety of atrial gene painting and autogenous AF termination by LED activation. Transgene expression after local gene delivery was almost completely limited to the targeted RA (Fig. 1B and fig. S2). ECG recordings of ReaChR-expressing rats showed that the heart rate ( $393 \pm 40$  beats per min versus  $384 \pm 37$  beats per min,  $P = 0.448$ ), P-wave duration ( $17.5 \pm 0.9$  ms versus  $17.7 \pm 1.3$  ms,  $P = 0.635$ ), and PR interval ( $47.6 \pm 3.1$  ms versus  $46.3 \pm 2.7$  ms,  $P = 0.158$ ) were not significantly different 4 to 8 weeks after the gene painting procedure when compared to baseline (before gene painting) or to citrine control animals (heart rate,  $P = 0.719$ ; P-wave duration,  $P = 0.972$ ; PR interval,  $P = 0.163$ ) (data file S1). Electrophysiological characterization of the optogenetically modified atria by optical voltage mapping revealed no abnormalities: The average APD<sub>80</sub> of the RA and LA during 5-Hz pacing was  $41 \pm 4$  and  $38 \pm 5$  ms, respectively ( $P = 0.347$ ),

whereas conduction velocity (CV) was  $60 \pm 9$  cm/s for the RA and  $61 \pm 16$  cm/s for the LA ( $P = 0.814$ ,  $n = 7$ ). No differences in APD<sub>80</sub> and CV were noted between hearts subjected to 5-Hz electrical or optical pacing. In addition, the average APD<sub>80</sub> and CV of the ReaChR-expressing RAs did not significantly differ from those of the citrine controls [ $41 \pm 4$  ms versus  $46 \pm 8$  ms,  $P = 0.178$  (APD<sub>80</sub>);  $60 \pm 9$  cm/s versus  $60 \pm 17$  cm/s,  $P = 0.912$  (CV)] (data file S1).

As the observed atrial APDs and CVs of ReaChR- or citrine-expressing hearts were similar to those reported in previous electrophysiological studies of unmodified Langendorff-perfused rat hearts (21, 22), we concluded that optogenetic modification by RA gene painting did not functionally impair excitability or gap junctional coupling. We did not observe any ventricular arrhythmias or ectopy after optogenetic AF termination or after targeted and prolonged illumination (470 nm;  $3.5 \text{ mW/mm}^2$  for 500, 1000, and 2000 ms and for five times 2000 ms with 1-s intervals) of the right ventricle during sinus rhythm. In addition, RA illumination during sinus rhythm never induced atrial tachyarrhythmias, as ectopic atrial excitation with light was immediately followed by sinus rhythm when illumination was stopped (fig. S10). Last, temperature measurements of the RA were performed to evaluate the thermal effects of the LED when switched on four times for 500 ms with 5-s intervals or once for 1000 ms. Measurements were performed ex vivo in an incubator set at 37°C. The temperature of the RA before illumination was  $36.8^\circ\text{C}$  (range,  $36.6^\circ$  to  $36.9^\circ\text{C}$ ) and increased to an average maximum temperature of  $37^\circ\text{C}$  (range,  $36.8^\circ$  to  $37.2^\circ\text{C}$ ) after the fourth consecutive 500-ms light pulse ( $n = 3$ ; fig. S11A). During the 1000-ms LED activation, the temperature increased from  $36.8^\circ\text{C}$  (range,  $36.7^\circ$  to  $37.0^\circ\text{C}$ ) to an average maximum temperature of  $36.9^\circ\text{C}$  (range,  $36.8^\circ$  to  $37.1^\circ\text{C}$ ;  $n = 3$ ; fig. S11B). A similar temperature response was noted in numerical simulations of a custom-made *in vivo* rat model (fig. S12), which, in addition, showed temperature decay (dissipation of the heat produced by the LED assembly) across the atrial wall. The thermal response of the LED light guide and the RA during LED illumination ( $3.5 \text{ mW/mm}^2$ ) thus remained well within the physiological temperature range.



**Fig. 5. Autogenous restoration of sinus rhythm in AF by an automated hybrid bioelectronic system.** (A) Representative body surface ECG traces showing three consecutive events of successful automated in vivo closed-chest detection and termination of AF by the hybrid bioelectronic system. Upon detection of AF by the custom-made algorithm and expiration of the programmed 10-s delay, the 470-nm LED implant is activated for 500 ms at 3.5 mW/mm<sup>2</sup> (blue boxes) resulting in autogenous termination of AF and subsequent restoration of sinus rhythm. Insets highlight (1) regular sinus rhythm before optical termination of AF, (2) rapid and irregular atrial activity during AF, and (3) regular sinus rhythm after the third of three consecutive and successful attempts of optical AF termination. Note that sinus rhythm is immediately restored after autogenous termination of AF without the occurrence of sinus bradycardia or other arrhythmias. (B) Quantification of optical and spontaneous termination of AF expressed as a percentage averaged for all rats. Also included are the spontaneous termination rate and control experiments. Each dot represents a minimum of five measurements in one rat. The error bar represents 1 SEM. (C) Histogram (bin size, 5 s) of the time to automated LED activation directly after AF onset of all autogenous termination attempts (29 AF episodes in four rats). Because of the programmed delay of 10 s, the LED could not be active in the first 10 s of AF (shaded area). (D) Graph showing the average RR intervals of the last 10 s before AF induction, the first 10 s of AF, and immediately (0 to 5 s) and later (5 to 15 s) after autogenous AF termination. Each line represents one measurement. Data are means. \*P < 0.05 and \*\*P < 0.01 by (B) Mann-Whitney U tests (n = 3 to 4 rats per group) or (D) two-sided paired t test (n = 20 AF episodes from four rats per group).

## DISCUSSION

Collectively, our results show that a hybrid bioelectronic system for autogenous restoration of sinus rhythm in AF can be achieved by combining a newly obtained antiarrhythmic effector function of the optogenetically modified atrium with an arrhythmia detector function of a machine-based cardiac rhythm monitor. In this scenario, the heart itself became an electric current generator for arrhythmia termination. This system allowed safe, effective, continuous, yet shock-free AF termination after activation of an implanted miniature LED in the adult rat with closed chest. Although optogenetics has been previously applied in whole-heart arrhythmia research (23–26), this study shows how electronic arrhythmia detection and biological arrhythmia termination can be incorporated into one automated hybrid system for effective AF treatment in the adult intact mammal. The present study could thereby contribute to a paradigm shift in the biomedical field, where diseased organs are no longer only considered as targets for therapy but now also as an integral part of the therapy itself by acquir-

ing specific machine-inspired effector functions, like that of a bioelectricity generator for restoration of sinus rhythm. Such creation of new biology for therapeutic purposes (synthetic biomedicine), combined with bioelectronic engineering, may provide the foundation for the development of distinctively innovative treatment options, including pain-free, ambulatory antiarrhythmic device therapy for AF.

Many patients with AF experience drug-resistant recurrences of symptomatic AF, even after multiple ablation procedures (2–4). These patients suffer from AF-related symptoms and show increased morbidity and mortality and impaired quality of life (27–29) while coping with lifelong adverse effects of medication and risk developing long-standing persistent AF (29, 30). At present, the only proven effective treatment option for these symptomatic patients that leads to acute restoration of sinus rhythm is repeated electrical cardioversion through application of high-voltage shocks administered in a hospital setting (5, 6). Our bioelectronic hybrid system was developed to fulfill the unmet need for therapies that deliver shock-free termination of AF

as a prerequisite for long-term continuous ambulatory treatment. The anticipated benefit of our system for the aforementioned patient group would range from minimizing the effective duration of AF episodes to avoiding AF-related symptoms, complications, repetitive hospitalization, and subsequent application of electroshocks. This may potentially slow the progression of AF and improve the prognosis while also increasing the quality of life for these patients (16, 17).

Considering these anticipated benefits, two subsets of patients seem poised to profit from shock-free defibrillation. The first subset of patients represents the relatively young and active individuals with frequent and highly symptomatic paroxysmal AF episodes; AF-related symptoms, morbidity, and adverse effects of medication have a considerable impact on these younger individuals (31–33). The second subset consists of patients with heart failure (HF) as comorbidity (34), as indicated in the Catheter Ablation versus Standard Conventional Therapy in Patients with Left Ventricular Dysfunction and Atrial Fibrillation (CASTLE-AF) trial (35). This multicenter randomized controlled trial revealed that HF patients with symptomatic AF who were assigned to rhythm control by ablation had fewer AF recurrences and showed significantly lower rates of death or hospitalization for worsening of HF than patients who were given pharmacological rate/rhythm control. However, multiple ablation procedures still resulted in a 50% AF recurrence rate within 60 months, whereas 30% of patients that underwent catheter ablation experienced an AF burden of  $\geq 25\%$ . Reduction of AF recurrence and burden by continuous and rapid AF termination provided by a bioelectronic hybrid system could potentially improve the outcome for patients with HF in whom ablation therapy is not successful.

Although several hurdles need to be overcome before clinical translation can be considered, findings of the present study may facilitate the clinical application of our approach to shock-free defibrillation. First, local transgene delivery through epicardial gene painting as performed in this study has the advantage that less vector particles are required compared to systemic vector administration. Epicardial painting resulted in efficient and targeted transgene expression in the RA with an about 100-fold lower vector dose than that required to obtain similar RA transduction rates after tail vein injection (36). Gene painting may therefore facilitate therapeutic applications because low transduction rates are considered one of the major limitations in cardiac gene therapy trials (37). In addition, local transgene delivery may reduce the chance of producing an adverse immune response against the vector capsid (38). Second, the present study also showed how to overcome the limitation of light delivery to the heart in a closed-chest animal model by demonstrating efficient optogenetic AF termination with a single intrathoracically implanted LED illuminating only a small part of the atria. The finding that light pulses of 2.5 to 5 mm<sup>2</sup> still allowed optogenetic arrhythmia termination may indicate that an implantable light source for human applications does not have to cover a large surface area, which may facilitate clinical translation. Clinical translation could be further facilitated by the expanding fields of optogenetics, microelectronics, and optics, which have made tremendous progress in the last few years: consider, for example, the development of ultra-sensitive light-gated ion channels (39) and biocompatible, stretchable, (multi-)LED arrays with customized specifications (40, 41). Using these thin ( $<1$  mm) and flexible materials, an LED sheet for human application could potentially be placed epicardially through minimally invasive thoracosopic or subxiphoidal approaches. In addition, because AAVVs can be immobilized on ultrathin biocompatible coatings (42), which could be

applied on the light-emitting surface of the LED sheet, a single, minimally invasive procedure may suffice for both targeted gene delivery and LED placement. Although additional research would be required for such translation, it is important to note that epicardial access and interventions by minimally invasive thoracoscopic surgery or a subxiphoidal approach are already feasible and safe, and device implantation for cardiac rhythm management is currently one of the mainstays of treatment. In addition, recent clinical trials have demonstrated that human cardiac gene therapy is feasible and safe (43–45), and new human cardiac gene therapeutic studies are being planned (46).

Although ReaChR was initially reported to be optimally excited by orange to red light wavelengths ( $\lambda = \sim 590$  to 630 nm) (47), subsequent studies showed that ReaChR is more sensitive to blue light stimulation (48, 49). Red light penetrates tissue more deeply than blue light, however the small thickness of the rat atrial wall did not necessitate the use of long wavelengths to produce a photocurrent strong enough for efficient AF termination. Whether the same holds true for human hearts remains to be tested. Although future studies assessing minimal thresholds values for optogenetic arrhythmia termination are needed, in our experiments, 470-nm light pulses of 500 ms at 3.5 mW/mm<sup>2</sup> [the approximate light intensity needed for maximum cell membrane depolarization (47)] led to effective AF termination *in vivo*. This amount of irradiation is well below the previously determined safety limit of 100 mW/mm<sup>2</sup> (50), allowing the possibility to increase the light intensity if necessary in future studies. The high optogenetic arrhythmia termination efficiency with moderate light intensities could have been facilitated by high transgene expression in the RA, as Bruegmann *et al.* (26) demonstrated that optogenetic AF termination efficiency and channelrhodopsin expression are strongly related.

### Limitations

AF is caused by a complex interaction between an initiating trigger and the underlying substrate, which can be structural, electrical, or both (7). In our study, the underlying substrate for AF maintenance was electrical (created by carbachol administration), whereas the initiating trigger was provided by electrical burst pacing. Although this AF model provided a solid starting point for this research, clinical translation requires future studies in larger animal models with clinically relevant AF. However, even if optogenetic arrhythmia termination would be hindered by structural remodeled atria, the system described in the present study allows for multiple shock-free interventions to compensate for such an effect. Another limitation of our AF model relates to the relatively small thickness of the atrial myocardial walls of rats compared to those of humans. The thicker human atrial walls may hinder optogenetic modification and activation of deeper atrial myocardium that may affect optogenetic arrhythmia termination efficacy. However, it is not yet known whether transmural illumination is an absolute requirement for efficient optogenetic arrhythmia termination. Previous ex vivo studies showed successful optogenetic termination of ventricular tachyarrhythmias in mice (24, 25) and rats (23), suggesting that optogenetic AF termination may be feasible in humans because rodent ventricles have a similar wall thickness as human atria (51, 52). Furthermore, in patient-specific computational models of fibrotic human atria expressing light-gated ion channels, Boyle *et al.* (53) demonstrated transmural optogenetic depolarization using 470-nm light pulses at 1.5 mW/mm<sup>2</sup>, suggesting that transmural optogenetic depolarization in human-sized atria may already be possible with the light intensities used in the present study.

### Path to clinical translation

Several essential steps need to be taken before a bioelectronic hybrid system for AF can be evaluated in a clinical setting. First, the feasibility and efficacy of optogenetic termination of atrial arrhythmias need to be validated in animals with human-sized atria, such as adult swine. During these experiments, both the genetic modification (choice of light-gated ion channel, gene delivery vehicle and procedure, and expression characteristics of transgene) and light delivery (location, timing, duration, surface area, and intensity of light) should be investigated to determine the optimal conditions for optogenetic AF termination. An improved understanding of the mechanism(s) and minimum requirements for optogenetic AF termination is required to guide the design and specifications of the light source implants for human applications. In addition, the influence of structural and/or electrical remodeling on optical termination efficiency needs to be assessed, considering that patients with drug-resistant AF may have advanced proarrhythmic atrial substrates. The insights gained from these exploratory studies would allow conclusive research toward clinical translation to be conducted in a constructive and responsible manner, with a focus on the long-term safety and efficacy of the bioelectronic system in freely moving animals with spontaneous episodes of AF. These studies would elucidate whether rapid and continuous AF termination could manage AF and whether reverse atrial remodeling and improved cardiac function are observed as a result of ambulatory AF termination. Such secondary effects could benefit patients with HF as comorbidity (35). Complementary future studies, evaluating minimally invasive atrial gene delivery and LED device implantation strategies, may widen the clinical applicability of the bioelectronic antiarrhythmic approach once proven to be safe and effective. Consistent positive results would ultimately provide an evidence-supported basis to initiate a clinical trial. Our study may, therefore, create the foundation for the design and development of pain-free antiarrhythmic device therapy through bioelectronic hybrid engineering with the potential to improve patients' prognosis and quality of life.

## MATERIALS AND METHODS

### Study design

The objective of this study was to assess the feasibility, efficacy, and safety of automated and biological shock-free termination of atrial tachyarrhythmias in a closed-chest animal model. Adult rats were randomly assigned to undergo local right atrial gene delivery of AAVVs encoding citrine-tagged ReaChR or, as negative control, citrine. Four to eight weeks after treatment, the efficacy of optogenetic termination of atrial tachyarrhythmias was studied ex vivo and in vivo using ECG as the primary readout. Atrial tachyarrhythmias that lasted for more than 10 s were considered sustained, and the RA was subsequently exposed to light pulses of different intensities, durations, and sizes. Optogenetic termination was considered successful when the arrhythmia stopped within 2 s after the start of the light pulse. Control experiments were performed in an identical fashion. Closed-chest in vivo experiments were carried out after implantation of a custom-made LED device, the activation of which was strictly controlled by a customized AF detection algorithm. The safety of the optogenetic intervention was evaluated by various assessments including electrophysiological measurements. Experimental blinding was not possible due to the nature of optogenetics (acutely detectable response upon illumination). The number of rats per experimental group varied between experiments and is specified in the figure legends. Each illumination

protocol per rat was repeated at least three times for the ex vivo and open-chest in vivo experiments and five times for the closed-chest in vivo experiments. All animal experiments were approved by the Animal Experiments Committee of Leiden University Medical Center and conformed to the Guide for the Care and Use of Laboratory Animals as stated by the U.S. National Institutes of Health. Raw data are reported in data file S1.

### Epicardial gene painting of the RA

Seven-week-old female Wistar rats (Charles River Laboratories) were anesthetized, intubated, and mechanically ventilated by inhaling 2 to 3% isoflurane in O<sub>2</sub> at 0.8 liters/min. Baseline ECG data were collected using an 8-channel PowerLab data acquisition device and recorded and analyzed using LabChart Pro software version 8 (both from ADInstruments). Adequate anesthesia was confirmed by the absence of reflexes. A minithoractomy was performed by incision of the fourth right intercostal space and subsequent rib spreading. The right lung and thymus were carefully moved to expose the RA. The pericardium at the height of the RA was incised, and the visible parts of the RA were washed with phosphate-buffered saline by wet swabs and subsequently dried with absorbent swabs. Twenty-five microliters of 6 mM EGTA (Sigma-Aldrich) was pipetted on the dried epicardial surface of the RA, resulting in the formation of a thin liquid film covering the RA, which was repeated three times every 5 min. Subsequently, the Sealer Protein solution (Baxter) mixed with AAVV was applied onto the dried epicardial surface of the RA in a dropwise fashion using an automatic pipet with a 20-μl filtertip. In addition, 50 μl of TISSEEL-Thrombin solution containing thrombin and CaCl<sub>2</sub> (Baxter) was pipetted onto the RA to induce formation of a fibrin clot thereby trapping the AAVV particles ( $4 \times 10^9$  genome copies). After a brief drying period of 5 min, the wound was closed in layers using polypropylene sutures. Animals received appropriate analgesia by subcutaneous injections of buprenorphine hydrochloride (0.05 mg/kg). In addition, rapamycin (3 mg/kg; LC Laboratories) was administered every other day by intraperitoneal injections for 3 weeks.

### Construction of the LED implant

To provide in vivo closed-chest illumination of the rat RA, a miniaturized light guide with an integrated LED was designed. This system is composed of a high-power blue ( $\lambda = 470$  nm) LED (LUXEON LXZ1-PB01, Lumileds), a diffuse light reflector cup and a transparent polydimethylsiloxane (PDMS; Dow Corning SYLGARD 184, Sigma-Aldrich) light guide plate (fig. S8). The high-power blue LED was operated at a typical forward voltage of 2.81 V and 180 mA (0.5 W), which resulted in a radiant flux of 3.5 mW/mm<sup>2</sup>. Insulated electrical wires with a diameter of 150 μm were soldered to the LED anode and cathode. Because the LED chip area is no more than about 1 mm<sup>2</sup>, the emanating light requires redistribution over a larger surface area. To spread the concentrated LED light uniformly over a larger surface area, the PDMS light guide and a diffuse light reflector cup (thickness, ±1.5 mm) were used. The diameter of the reflector cup is 6 mm resulting in an effective exposure area of ±28 mm<sup>2</sup>. The cup is filled with PDMS silicone elastomer in its liquid state. The PDMS was prepared with a curing agent in a 10:1 ratio and degassed inside a vacuum oven to remove bubbles. Curing was performed at room temperature for 48 hours to form a soft and flexible elastomer. The PDMS has a refractive index of about 1.4 and is transparent in the visible range with minimal light loss because of absorption. The LED light guide design uses a combination of total internal reflection (TIR) and a method for light extraction (diffuse reflectance)

to produce a uniform light-emitting surface. The LED is therefore placed tilted ( $90^\circ$  with respect to the surface to be illuminated) inside the diffuse reflector cup to achieve TIR. Rays of light can only exit the light guide when its critical angle is not exceeded. Light rays are emitted inside the PDMS by the tilted LED and reflected internally until the angle of incidence is below the critical angle, allowing the light ray to exit. The reflector cup improves the extraction efficiency due to the diffuse backscatter reflectance of the light.

### Optical cardiac pacing and optical termination of AF and atrial flutter in vivo

Rats were prepared for surgery and thoracotomized as described above. A single intraperitoneal injection of carbachol (50  $\mu\text{g}/\text{kg}$ ) (54) was administered 15 min before the induction of atrial tachyarrhythmias by electrical burst pacing of the RA. Carbachol injections were repeated every 30 min. Atrial tachyarrhythmias were subdivided in AF (defined as irregular atrial activity and RR intervals on the body surface ECG) and atrial flutter (defined as regular rapid atrial activity and RR intervals on the body surface ECG). Atrial tachyarrhythmia induction and optical arrhythmia termination protocols were identical to those applied ex vivo. For closed-chest experiments, a custom-made LED assembly was implanted at the inside of the thoracic wall by two sutures in such a way that it was facing the RA without directly contacting the heart. A custom-made, rigid bipolar pacing electrode was placed at the RA, and the wound was closed in layers as described above. The optical termination protocol (500-ms light pulse of 3.5 mW/mm $^2$ ; illuminating circa, 28 mm $^2$ ) was automatically activated upon detection of AF by a tailored algorithm implemented in LabChart. Real-time ECG analysis continuously monitored heart rate, and PR interval was averaged per three beats. Integrals for these parameters will accumulate when the heart rate suddenly changes (>50 beats within 1 s) or the PR interval cannot be calculated. A constant time decay allows the integral to return to zero when sinus rhythm is restored. Both integrals form the command leading to activation of the termination protocol when a threshold is reached. A programmed delay of 10 s was used upon automated detection of AF to make sure that the atrial arrhythmia was sustained. Because of the constant time decay, the optical termination protocol was automatically aborted in case AF terminated spontaneously during the programmed delay period. The closed-chest experiments were performed in four rats with a minimum of five sustained AF episodes per rat. RR intervals of the last 10 s before AF induction, the first 10 s during AF, and immediately (0 to 5 s) and later (5 to 15 s) after autogenous AF termination were calculated and averaged using LabChart. Data were derived from five consecutive AF episodes in each of the four rats used for the closed-chest rat experiments ( $n = 20$ ).

### Statistical analysis

Statistical analyses were performed using SPSS Statistics v23.0 (IBM Corporation). Because the successful arrhythmia termination rates were unequally distributed, the Mann-Whitney  $U$  test was performed for the comparison of illumination and control groups. The Kruskal-Wallis test with Dunn-Bonferroni post hoc test was performed for the comparison of different light pulse duration groups. Optical mapping data were normally distributed, and comparisons were made by using the two-sided Student's  $t$  test for unpaired data and the two-sided paired Student's  $t$  test for paired data. ECG parameters were compared with the two-sided paired Student's  $t$  test. Data were expressed as means  $\pm$  SD or, for arrhythmia termi-

nation efficacies, as SEM. Differences were considered statistically significant at  $P < 0.05$ .

### SUPPLEMENTARY MATERIALS

- [www.scientifictranslationalmedicine.org/cgi/content/full/11/481/eaau6447/DC1](http://www.scientifictranslationalmedicine.org/cgi/content/full/11/481/eaau6447/DC1)
- Materials and Methods
- Fig. S1. Maps of the AAVV genomes.
- Fig. S2. Transgene expression in different cardiac compartments after gene painting of the RA of adult rat hearts with AAV2/9.45.HsNPPA.ReaChR~citrine.SV40pA.
- Fig. S3. Transgene expression after gene painting of the RA of adult rat hearts with AAV2/9.45.HsNPPA.citrine.SV40pA.
- Fig. S4. Masson's trichrome staining of the RA and LA after gene painting of the RA with AAV2/9.45.HsNPPA.ReaChR~citrine.SV40pA.
- Fig. S5. Light intensity-duration curve for optical atrial pacing of adult rat hearts after gene painting of the RA with AAV2/9.45.HsNPPA.ReaChR~citrine.SV40pA.
- Fig. S6. Correlation between surface area and location of right atrial illumination on optogenetic termination of atrial tachyarrhythmias ex vivo.
- Fig. S7. Efficient autogenous termination of atrial flutter in vivo after gene painting of the RA of adult rat hearts with AAV2/9.45.HsNPPA.ReaChR~citrine.SV40pA.
- Fig. S8. Implantable LED device.
- Fig. S9. Schematic overview of the AF detection algorithm and experimental setup.
- Fig. S10. Prolonged illumination of the RA and ventricle of adult rat hearts after gene painting of the RA with AAV2/9.45.HsNPPA.ReaChR~citrine.SV40pA.
- Fig. S11. Ex vivo temperature measurements of the RA during LED activation.
- Fig. S12. Computational simulation of thermal heating after activation of the implantable LED device based on in vivo conditions.
- Data file S1. Raw data.
- Movie S1. Optical voltage mapping of the ReaChR-expressing RA during AF and subsequent optogenetic restoration of sinus rhythm.
- Movie S2. Summary of all essential experimental steps leading to the development of the automated hybrid bioelectronic system for autogenous restoration of sinus rhythm in AF.

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**Acknowledgments:** We thank R. Tsien (University of California, San Diego, CA), D. Duan (University of Missouri, Columbia, MO), R. J. Samulski, and A. Asokan (both from the University of North Carolina School of Medicine, Chapel Hill, NC) for providing DNA constructs; N. Harlaar for movie production; M. de Jong for performing the Western blot experiments; and A. Teplinen for technical assistance regarding patterned illumination experiments (all from the Department of Cardiology, LUMC). **Funding:** This work was supported by personal funding from the Netherlands Organization for Scientific Research (NWO, Vidi grant 1714336 to D.A.P.). D.A.P. is also a recipient of the European Research Council (ERC) Starting grant (716509). Additional support was provided by the Netherlands Heart Institute (ICIN grant 230.148-04 to A.A.F.d.V.). **Author contributions:** E.C.A.N., A.A.F.d.V., and D.A.P. conceived the study, interpreted results, and wrote the manuscript; R.H.P. and G.Q.Z. designed,

fabricated, and tested the implantable LED device; E.C.A.N. performed the ex vivo and in vivo experiments and statistical analysis and developed the AF detection algorithm; J.J.P. and L.V. performed the sharp electrode measurements and data analyses; T.J.v.B., K.Z., and M.J.S. helped in designing the hybrid system and interpreted the data; A.M.K., C.I.B., and A.A.F.d.V. designed and produced the AAVVs and assisted with surgical procedures. All authors refined the manuscript. **Competing interests:** The authors declare that they have no competing interests. **Data availability:** All data associated with this study are present in the paper or Supplementary Materials.

Submitted 2 July 2018  
Resubmitted 5 September 2018  
Accepted 17 January 2019  
Published 27 February 2019  
10.1126/scitranslmed.aau6447

**Citation:** E. C. A. Nyns, R. H. Poelma, L. Volkers, J. J. Plomp, C. I. Bart, A. M. Kip, T. J. van Brakel, K. Zeppenfeld, M. J. Schalij, G. Q. Zhang, A. A. F. de Vries, D. A. Pijnappels, An automated hybrid bioelectronic system for autogenous restoration of sinus rhythm in atrial fibrillation. *Sci. Transl. Med.* **11**, eaau6447 (2019).

## An automated hybrid bioelectronic system for autogenous restoration of sinus rhythm in atrial fibrillation

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*Sci. Transl. Med.*, 11 (481), eaau6447.

### Atrial fibrillation treatment lightens up

Atrial fibrillation is an irregular, rapid heartbeat that interrupts normal blood flow and increases risk of stroke and clots. Normal heart rhythm can be restored by electroshock (electrical cardioversion), but implantable cardioverter defibrillators can cause pain and damage myocardial tissue. Rather than supply exogenous current, Nyns *et al.* combined atrial gene painting to deliver viral vectors encoding light-activatable ion channels with an implantable light source and rhythm detector, developing an autogenous arrhythmia termination system. This optogenetic approach restored normal heart rhythm in rat hearts *ex vivo* and *in vivo* under closed-chest conditions. Further testing in larger animal models is necessary, but results suggest that this could be a pain-free alternative to electroshock.

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