Optogenetic release of norepinephrine from cardiac sympathetic neurons alters mechanical and electrical function

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Aims

Release of norepinephrine (NE) from sympathetic neurons enhances heart rate (HR) and developed force through activation of β-adrenergic receptors, and this sympathoexcitation is a key risk for the generation of cardiac arrhythmias. Studies of β-adrenergic modulation of cardiac function typically involve the administration of exogenous β-adrenergic receptor agonists to directly elicit global β-adrenergic receptor activation by bypassing the involvement of sympathetic nerve terminals. In this work, we use a novel method to activate sympathetic fibres within the myocardium of Langendorff-perfused hearts while measuring changes in electrical and mechanical function.

Methods and results

The light-activated optogenetic protein channelrhodopsin-2 (ChR2) was expressed in murine catecholaminergic sympathetic neurons. Sympathetic fibres were then photoactivated to examine changes in contractile force, HR, and cardiac electrical activity. Incidence of arrhythmia was measured with and without exposure to photoactivation of sympathetic fibres, and hearts were optically mapped to detect changes in action potential durations and conduction velocities. Results demonstrate facilitation of both developed force and HR after photostimulated release of NE, with increases in contractile force and HR of 34.5 ± 5.5 and 25.0 ± 9.3%, respectively. Photostimulation of sympathetic fibres also made hearts more susceptible to arrhythmia, with greater incidence and severity. In addition, optically mapped action potentials displayed a small but significant shortening of the plateau phase (25.5 ± 1.0 ms) after photostimulation.

Conclusion

This study characterizes a powerful and clinically relevant new model for studies of cardiac arrhythmias generated by increasing the activity of sympathetic nerve terminals and the resulting activation of myocyte β-adrenergic receptors.

Keywords

Optogenetic • Langendorff • Sympathetic activation • Arrhythmia

1. Introduction

Cardiac function is strongly influenced by sympathetic activity. A network of sympathetic neurons innervates cardiac tissue, including the SA node, the AV node, and the ventricles. Catecholamines, such as norepinephrine (NE), are released from sympathetic nerve terminals and activate β-adrenergic receptors within the sarcolemma of cardiac myocytes. In the heart, β-adrenergic stimulation elicits increases in heart rate and inotropy, responses that typically increase cardiac output and blood pressure.1 Tachycardia and arrhythmia can also occur as a result of β-adrenergic stimulation due to the formation of ectopic beats and conduction block.2 As such, β-adrenergic receptor blockers (e.g. propranolol) have been used as a standard treatment for heart diseases such as angina pectoris, infarction, and arrhythmia.1

Despite the widespread use of β-adrenergic receptor blockers in the treatment of arrhythmia, the mechanisms by which β-adrenergic stimulation increases the risk of ectopic beats and arrhythmia are not fully understood. Previous work has shown that β-adrenergic stimulation of isolated ventricular cardiomyocytes and trabeculae causes calcium overload and spontaneous calcium release from the SR as well as delayed after depolarizations, mechanisms that motivate premature ventricular contractions (PVCs).3–5 In whole hearts, subepicardial NE injections elicit localized β-adrenergic stimulation to cause synchronous SR Ca2+ release in enough cells to generate focal arrhythmias.6
In conscious animals, the cardiac effects of catecholamines are studied during exercise and during induced coronary occlusions using implanted sensor systems. Most cardiac studies of catecholamines activate β-adrenergic receptors in excised hearts via exogenous β-adrenergic receptor agonists using bath or perfusate additions of NE or isoproterenol, a non-selective β-adrenergic receptor agonist. Other studies have used direct bilateral sympathetic stimulation (BSS) to endogenously activate cardiac β-adrenergic receptors, but this approach is usually not suitable for excised heart experiments. Exogenous β-adrenergic receptor agonists and BSS elicit different responses that may depend upon the method of receptor stimulation, most likely due to sympathetic nerve heterogeneity and the density and location of adrenergic receptors within the myocardium. An aim of the present work was to study intrinsic activation of cardiac β-adrenergic receptors using an excised mouse heart preparation suitable for studies of electrophysiological and arrhythmic events.

In this manuscript, we present both a new approach for studying β-adrenergic activation in excised hearts and new results that contribute to the understanding of the cardiac sympathetic activation. The goal of our experiments was to photoactivate sympathetic fibres that innervate the myocardium to study the cascade of events from β-adrenergic receptor activation to changes in cardiac function to any resulting arrhythmic events. Activation of sympathetic cardiac nerves was achieved by cross-breeding mice to express channelrhodopsin-2 (ChR2) in sympathetic cardiac nerves using a Zeiss LSM 510 confocal imaging system, with dye-specific filter settings. A second round of immunohistochemistry verified that EYFP/ChR2 expression was not present in parasympathetic neurons. Heart slices were co-stained with rabbit anti-cholinergic acetylcholine transferase (ChAT, 1:200, Millipore) and chicken anti-GFP (1:1000, Abcam) to label sympathetic neurons and with chicken anti-GFP (1:1000, Abbam) to label EYFP/ChR2. Secondary antibodies were goat anti-rabbit Cy5 (1:1000, Jackson ImmunoResearch) and donkey anti-chicken Dylight 405 (1:300, Jackson ImmunoResearch). Z-stack images were acquired (11 μm) using a Zeiss LSM 510 confocal imaging system, with dye-specific filter settings.

2. Methods

2.1 Mice with sympathetic neurons that express ChR2

All animal protocols were approved by the George Washington University’s Animal Care and Use Committee and followed the National Institute of Health’s ‘Guide for the Care and Use of Laboratory Animals’. Mice having sympathetic neurons that express ChR2 were created by cross-breeding two parental strains. One parent (Stock No. 008601, Jackson Labs) expressed the tyrosine hydroxylase (TH) promoter to direct the expression of Cre recombinase to catecholaminergic cells. The other parent (Stock No. 012569, Jackson Labs) had ChR2 expression that was dependent upon Cre expression. Offspring expressing TH-Cre-ChR2 were confirmed via genotyping (Transnetx) of tail snips, and all mice without proper TH-Cre-ChR2 expression were excluded from data analysis.

2.2 Immunohistochemistry

Immunohistochemistry was used to identify EYFP/ChR2 expression specifically localized to sympathetic neurons, which express TH. Fresh frozen tissue sections were prepared from TH-Cre-ChR2 mice using OCT (Tissue-Tek) and liquid nitrogen. Heart slices (100 μm) were taken from the base of the heart and fixed using a standard 4% paraformaldehyde procedure. Heart slices were co-stained with rabbit anti-TH (1:200, Millipore) to label sympathetic neurons and with chicken anti-GFP (1:1000, Abbam) to label EYFP-expressing ChR2. Secondary antibodies were goat anti-rabbit Cy5 (1:1000, Jackson ImmunoResearch) and donkey anti-chicken Dylight 405 (1:300, Jackson ImmunoResearch). Z-stack images were acquired (11 μm) using a Zeiss LSM 510 confocal imaging system, with dye-specific filter settings.

2.3 Excised heart preparation

Mice were anaesthetized with ~4% isoflurane, after which a cessation of pain reflex was verified with a toe pinch before hearts were rapidly excised. The aorta of each heart was immediately cannulated, and hearts were retrogradely perfused via Langendorff at a constant pressure of 60 mmHg. The perfusate was a Krebs–Henseleit solution (118 NaCl, 3.3 KCl, 2.0 CaCl2, 1.2 MgSO4, 24.0 NaHCO3, 1.2 KH2PO4, 10.0 glucose, 2.0 sodium pyruvate, 10 HEPES, and 20.0 mg/L albumin) warmed to 37°C and oxygenated with 95% O2–5% CO2.

2.4 Experimental groups

Prior to experiments with TH-Cre-ChR2 hearts, a wild-type control group was initially tested to verify that blue light illumination did not cause any effects in hearts lacking the ChR2/EYFP fusion protein (n = 5, Figure 1A). Two separate sets of Langendorff-perfused experiments were then completed using blue light to activate ChR2. In each set, hearts were grouped

Figure 1 Per cent change in heart rate and contractile force with blue light stimulation (A) in wild-type hearts lacking the ChR2/EYFP protein (n = 5), and (B) in TH-Cre-ChR2 hearts at different light stimulation frequencies (1, 2, 5, and 8 Hz) and irradiances, 0.3 mW/mm² (n = 2) and 0.12 mW/mm².
as controls, with no blue light illumination (sham group, n = 16), or as experimental, with blue light illumination (photostimulation group, n = 16). In the first set (contraction experiments, n = 10), heart rate, contractile force, and the incidence of arrhythmias were measured before and after either sham or light illumination. In the second set (optical mapping experiments, n = 6), heart rate, optical action potentials (OAPs), and the incidence of arrhythmias were measured before and after either sham or light illumination.

2.5 Blue light illumination

After a period of initial stabilization (> 15 min) in both sets of experiments, hearts in the blue light groups were photostimulated by illuminating half the heart, including the RA and RV epicardial surface, with light from a high-power LED spotlight (peak excitation 470 nm, Mightex). During photostimulation, the spotlight was pulsed for 20 s (5 ms duration at 5 Hz). These light stimulation parameters are supported by a separate set of experiments that were conducted to examine the dose response to varying light frequencies (1, 2, 5, and 8 Hz, Figure 18). Maximal changes in heart rate and contractile force occurred at 5 Hz light pulsing, where light irradiance was computed to be 0.3 mW/mm². Light irradiance as low as 0.12 mW/mm² at 5 Hz pulsing frequency was sufficient to elicit the same response. After each photostimulation, measurements were monitored for ~5 min before a second period of photostimulation. The protocol was the same for hearts in the sham groups, except that the spotlight was not powered on.

2.6 Arrhythmia protocol

Hearts (n = 14) were burst paced to determine the susceptibility of TH-Cre-ChR2 hearts to arrhythmias during photo-stimulation. Burst trains lasted ~10 s and comprised multiple bursts that were 1–2 s long (5 ms pulses, 20 ms CL) with 3 s between bursts. These parameters are consistent with burst pacing protocols used to study arrhythmia induction in mouse hearts.18,19 Photostimulation was applied during half of the burst pacing phases, with the other half receiving burst pacing only. The sequential order was varied between studies. An arrhythmia scoring system was used to rate incidence of arrhythmia on a scale of 0–3, with the following designations: 0—no response, 1—at least 1 PVC, 2—more than one set of double or triple beats, and 3—ventricular tachycardia (VT).15 The highest score, corresponding to the most severe arrhythmia, determined the overall score for each study.

2.7 Contraction experiments

In the contraction experiments, hearts (n = 10) were vertically positioned for measuring changes in whole-heart contractile force.15,16 Contractile force was measured by attaching a small needle with silk suture to the tip of the apex. The suture was fed through a pulley and connected to a force transducer (FORT10 g, World Precision Instruments). An electrogram was recorded throughout the protocol using electrodes placed in the ventricular cavities; a ground electrode was placed within the perfusate fluid column. Force and electrogram signals were simultaneously acquired using a PowerLab/LabChart system (AD Instruments).

In multiple contraction experiments (n = 5), the β-adrenergic receptor agonist isoproterenol was administered at a 5 μM circulating concentration to compare the effects of blue light illumination to global exogenous β-adrenergic receptor activation. In other contraction experiments (n = 5), the β-adrenergic receptor antagonist propranolol was added, and hearts were subsequently illuminated with blue light to show blunting of a β-adrenergic response.

2.8 Optical mapping experiments

In optical mapping experiments, Langendorff-perfused hearts (n = 6) were horizontally positioned in a temperature controlled bath (37 °C) and mechanically arrested with blebbistatin (circulation concentration 5 μM) to prevent motion artefact. Blebbistatin is a myosin ATPase inhibitor that blocks contraction without affecting calcium cycling or electrical activity.17 Once contractions ceased, the potentiometric dye RH237 (30 μL in 1 mL of Krebs–Henseleit) was injected into the aorta to stain the tissue for imaging of OAPs. Light emitted from the epicardial surface was long pass filtered (680 nm) and imaged with a high-speed CCD camera (Andor iXon DU860E) at ~725 frames per second. There was no spectral overlap in light from the 530 nm spotlight used for optical mapping and the 470 nm spotlight used for photostimulation. A bipolar electrode was placed on the epicardial surface near the ventricular septum, and hearts were paced at a cycle length of 150 ms (400 b.p.m.) while imaging OAPs. Burst pacing was also applied using this electrode. OAPs were imaged before and after burst pacing, and when possible, sustained arrhythmias resulting from burst pacing were optically mapped. Sham studies were performed using the same protocol but without powering on the blue spotlight. A typical protocol is shown in Figure 2.

Similar to the contraction experiments, β-adrenergic receptor antagonist propranolol was added to a subset of optically mapped hearts (n = 3), with hearts subsequently illuminated with blue light, to show blunting of the β-adrenergic response. A lack of β-adrenergic response during illumination with 530 nm light was also confirmed in several hearts.

2.9 Data analysis and statistics

Apical force and electrograms were analysed using custom MATLAB (Mathworks) algorithms to compare changes in force and heart rate after photostimulation. Electrograms were systematically observed using LabChart to assign arrhythmia scores. Optical mapping data were pre-processed using

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**Figure 2** A typical protocol for optical mapping experiments. Protocols include a control phase and a photostimulation phase to map changes in OAPs as well as any arrhythmias that occur after burst pacing.
custom MATLAB algorithms to subtract background fluorescence from each image, to smooth each pixel signal using a mean spatiotemporal filter (three sample width), and to normalize signals at each pixel, as previously described. Ninety per cent action potential duration (APD90) and average conduction velocities during epicardial pacing were computed from optical mapping data using a standard approach. Activation times were computed at each pixel using the maximum first derivative of OAPs. Repolarization times were computed at each pixel as the time of 90% repolarization. The 90% action potential duration (APD90) was then calculated as the time between activation and repolarization. A region of interest on the RV epicardium was then selected for comparing APD90 before and after photostimulation. Isochronal activation maps were constructed to illustrate wavefront propagation during sinus rhythm, PVCs, and ventricular tachycardia. Statistical significance of differences between data sets was determined using a paired student’s t-test, where the time between activation and repolarization. The 90% action potential duration (APD90) was then calculated as the time between activation and repolarization. A region of interest on the RV epicardium was then selected for comparing APD90 before and after photostimulation. Isochronal activation maps were constructed to illustrate wavefront propagation during sinus rhythm, PVCs, and ventricular tachycardia. Statistical significance of differences between data sets was determined using a paired student’s t-test, where P < 0.05 was considered statistically significant.

3. Results

3.1 Immunohistochemistry

Sympathetic nerve segments with positive expression for both TH (red) and EYFP/ChR2 (blue) were observed within myocardial cross-sections of TH-Cre-ChR2 mice. Nerve segments with red and blue fluorescence were identified close to the epicardium and in deeper myocardial layers (green), as shown in Figure 3A. The co-localization of TH and EYFP fluorescence confirms the expression of EYFP/ChR2 within sympathetic nerve axons. Parasympathetic neurons with positive expression for ChAT (red) were also observed between cardiac myocytes (green). EYFP/ChR2 (blue) surrounded these neuron fibres, but it was never co-localized with ChAT (Figure 3B).

3.2 Force development and heart rate after photostimulation

At the beginning of all studies (contraction and optical mapping experiments), hearts were perfused without experimental perturbations for at least 15 min. This was done to ensure that heart rate and contractile force were stable. Figure 4A shows that heart rate and apical force fluctuated very little over a 15-min interval—with final values of heart rate and force being 94.3 ± 2.9 and 99.0 ± 2.6% of initial values, respectively (means ± SE). Before photostimulation, average baseline measurements of heart rate and apical force across studies were 192 ± 50 b.p.m. and 0.15 ± 0.03 g. The response to photostimulation is shown in Figure 4B, where heart rate increased 25.0 ± 9.3% and contractile force increased 34.5 ± 5.5%. Isoproterenol was added to the perfusate of a subset of studies to compare the photostimulation response to that of a known β-adrenergic receptor agonist. Isoproterenol elicited a steady-state increase in heart rate and apical force of 47.8 ± 13.8 and 58.8 ± 14.4%, respectively. In other studies, propranolol was administered to the perfusate to confirm that photoactivated increases in heart rate and contractile force were due to β-adrenergic receptor activation. Photoactivated responses were abolished in the absence of this β-adrenergic receptor antagonist (Figure 4B).

Typical responses after photostimulation are shown in Figure 4C and D. Changes in force and heart rate during two rounds of blue light photostimulation followed by infusion of isoproterenol are shown in Figure 4C, where photostimulation resulted in prolonged changes in contractile force and heart rate. Another typical response is shown in Figure 4D, where force and heart rate elevations were transient and returned to baseline between bouts of photostimulation. Administration of propranolol abolished any response to photostimulation. As expected, hearts from animals that did not express ChR2 in catecholaminergic nerves did not show any changes in heart rate or force during or after photostimulation.

3.3 Electrophysiological changes after photostimulation

APD90 was measured across the RV epicardial surface using optical mapping data sets acquired before and after photostimulation. On average, APD90 was reduced by 5.5 ± 1.0 ms after photostimulation (Figure 5A). This reduction was significantly greater than changes measured from sham studies (0.718 ± 0.711 ms, P = 0.002) as well as changes measured after administering propranolol before photostimulation (0.263 ± 0.693 ms, P = 0.03). Typical OAPs during sham and

Figure 3  Nerves in myocardial cross-sections of TH-Cre-ChR2 mice. (A) Sympathetic nerve fibre distribution near the epicardial surface showing selective expression of EYFP/ChR2 (blue) in TH-expressing sympathetic neurons (red) among cardiac myocytes (green); nerve fibres are indicated by arrows. (B) Parasympathetic neurons expressing ChAT (red) were present between cardiac myocytes (green), but lacked co-localization with light-activated EYFP/ChR2 fibres (blue). Scale bar is 20 μm.
photostimulation experiments are shown in Figure 5B. A reduction in Phase 2 of the OAP was observed after photostimulation (right) while no change was observed for sham OAPs (left). Figure 5C shows APD maps from a typical sham study, where activation times, APDs, and APD distribution (histogram) are shown before (top row) and after (bottom row) sham photostimulation. Changes in APD90 were not significant. Average epicardial conduction velocity for this study before and after sham photostimulation was measured to be 70.0 ± 5.0 and 70.7 ± 4.8 cm/s, respectively (P = NS). The same data for a photostimulation study is shown in Figure 5D, but with a significant reduction in APD90 after photostimulation. Average epicardial conduction velocity before and after photostimulation was 48.9 ± 2.1 and 49.1 ± 1.8 cm/s, respectively (P = NS). Across studies, the average epicardial conduction velocity for the TH-Cre-ChR2 mice was 59.7 ± 2.1 cm/s, which is within the range of accepted values for mouse hearts.21–23 For all non-sham optical mapping studies, heart rate increased 19.2 ± 5.3% from baseline after photostimulation, indicating a positive β-adrenergic response.

### 3.4 Photostimulation increases the incidence of arrhythmia

Electrogram signals from contraction and optical mapping studies were observed to identify the incidence of PVCs, bigeminy, salvos, and non-sustained VT/VF resulting from burst pacing with and without photostimulation. The Lambeth Conventions guidelines were used to devise an arrhythmia scoring system.14 Although these guidelines are typically used to analyse arrhythmias resulting from ischaemia/reperfusion and infarction, they were loosely applied to the data in these experiments to classify arrhythmia that potentially originated from sympathetic stimulation. Signals were classified by arrhythmia type and severity, with each study assigned a final score corresponding to the most severe type of arrhythmia. Figure 6 shows representative signals and isochronal maps for observed instances of each arrhythmia score. The lowest score of 0 was assigned to events that resulted in no arrhythmic response (Figure 6A), a score of 1 was assigned to the formation of at least one PVC (Figure 6B), a score of 2 was assigned to observation of either double (bigeminy) or triple (salvos) beats (Figure 6C), and the highest score of 3 corresponded to VT/VF (Figure 6D), the most serious classification of arrhythmia observed in these studies. Isochronal maps on the right side of Figure 6 show activation during normal sinus rhythm (Figure 6E), wavefronts resulting from a PVC (Figure 6F), and non-sustained VT (Figure 6G).

Table 1 shows that 9 out of 14 photostimulated hearts had an arrhythmia score of 1 or more, compared with 4 out of 14 control hearts. VT was also observed more frequently in photostimulated hearts; out of 14 hearts, 3 had VT, compared with just 1 in the non-photostimulation (control) group.

### 4 Discussion

This study presents a new animal model that has the potential to further elucidate the complex relationship between sympathetic activity and...
cardiac function. Using this model, we photoactivated sympathetic fibres in the heart that express the light-activated channel ChR2. We observed the following changes in cardiac function that were consistent with the release of NE and β-adrenergic stimulation: (i) an increase in contractile force and heart rate, (ii) reductions in APD, and (iii) a greater incidence of arrhythmia after burst pacing.

Increases in heart rate and contraction strength after epicardial photostimulation were consistent with changes expected upon sympathetic stimulation and subsequent NE release. Binding of NE to adrenergic receptors in cardiac myocytes results in an increased rate of L-type Ca\(^{2+}\) flux during depolarization, greater availability of SR Ca\(^{2+}\), and increased contractile strength.\(^{24,25}\) Sympathetic activation of the SA node results in a positive chronotropic effect, which we also observed in our studies. Both sustained and transient changes in heart rate and contractile force occurred within seconds of photostimulation of TH-Cre-ChR2 hearts. Similar, but more dramatic, force production and chronotropic effects were observed after isoproterenol infusion, confirming that photostimulation produced a response analogous to β-adrenergic activation. There could be several explanations for the observation of both sustained and transient evoked responses. One possibility is inherent dissimilarity in the Uptake-1 mechanism between heart populations. Uptake 1 is responsible for re-sequestration of the NE released by sympathetic nerves.\(^{26,27}\) Slight differences in reuptake kinetics could mean faster or slower removal of NE from the tissue, resulting in a transient or sustained response.

We observed APD reductions after photostimulation of TH-Cre-ChR2 hearts (Figure 5). This is consistent with previous studies that optically mapped action potentials from the surface of mouse hearts after the administration of isoproterenol.\(^{18}\) A slight shortening of APD is consistent with a higher concentration of NE release.\(^{28}\) This explanation is plausible considering that the entire RV, RA, and a sizeable portion of the LV were illuminated to induce NE release, and functional data demonstrate that the photostimulation response was approximately one half that of the global isoproterenol-induced response.

We also observed increased ectopic activity within all photostimulation experiments, as evidenced by the greater number of PVCs and double/triple beats (Table 1). Pro-arrhythmic effects of β-adrenergic stimulation are often attributed to altered SR Ca\(^{2+}\) sequestration, which can result in spontaneous Ca\(^{2+}\) release, as well as heterogeneous NE delivery, particularly in failing hearts.\(^{6,24}\) Burst pacing, to increase calcium loading in the heart, is often used in conjunction with a catecholamine, such as isoproterenol, during electrophysiological studies to induce arrhythmia.\(^{29}\) Burst pacing alone is often insufficient to induce ectopic activity, as we observed in the control studies. In photostimulation

Figure 5  Effect of blue light on APD90. (A) Average change in APD90 (ms) after photostimulation (black, n = 6), sham (grey, n = 6), and after administering propranolol (white, n = 3). (B) Representative signals showing APD changes after photostimulation in sham (left) and illuminated hearts (right). (C) Spatial maps showing activation time, APD, and APD histograms before (top) and after (bottom) the sham light protocol. (D) Spatial maps showing changes in activation time, APD, and APD histograms before (top) and after (bottom) 470 nm photostimulation. **P < 0.05, statistically different from baseline.
studies, not only did more hearts undergo arrhythmia after being photo-stimulated during fast pacing, but arrhythmias were often sustained and more severe than the control group (Table 1).

4.1 Limitations
Our studies focused on elucidating the β-adrenergic response of intrinsic release of NE within intact isolated perfused mouse hearts. As such, our studies did not address specific cellular mechanisms of the β-adrenergic response, such as increases in Ca^{2+} transient amplitude and SERCA activity. Such studies are left for future work. In multiple studies, we collected samples of coronary effluent before and after photostimulation and assayed the samples for NE. Detectable levels of NE were not found. Inability to detect NE in perfusate samples could be attributed to rapid NE degradation as well as a large extent of dilution that occurs when miniscule amounts of NE are released from sympathetic nerve fibres within a large volume of perfusate. This finding is consistent with previous reports: in healthy hearts 70—95% of NE released by cardiac sympathetic nerves is re-sequestered by the axon terminals.26,27,30 As such, the concentration of NE in the coronary effluent should be almost negligible.

While isolated hearts provide several benefits (control of metabolic fuels, fluorescence imaging without the presence of blood, and elimination of confounding variables, to name a few), future in vivo studies may be valuable for studying the effects of light-activated NE release within the context of the entire organism. Limitations of the TH-Cre-ChR2 mouse model for studying sympathetic-mediated arrhythmic events may involve the somewhat limited dynamic range resulting from the fast baseline sinus rhythm and short action potentials of mice. These characteristics can make it difficult to observe changes in heart rate or action potential characteristics, especially when sympathetic activity is not global.

4.2 Summary
We conclude that sympathetic axon activity is preserved in isolated perfused hearts and that an organ-level response associated with sympathetic activation can be produced using an optogenetic approach. Our findings support the hypothesis that the TH-Cre-ChR2 mouse is a useful model to study physiological changes upon photoexcitation of sympathetic nerves that innervate the heart. This new approach
overcomes several limitations of existing approaches, namely, preserving the pathway from nerve terminal to myocyte β-receptor and removing the complications of sympathetic nerve isolation. Preservation of the sympathetic pathway is critically important for studying the release and uptake of NE, which can be significantly altered during disease such as congestive heart failure. The results of our study provide an innovative and clinically relevant model as well as new results that contribute to the understanding of cardiac sympathetic activation.

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