Changes in Cardiac Function Upon Light-Activated Release of Norepinephrine from Sympathetic Neurons Expressing Channelrhodopsin

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BACKGROUND

β-adrenergic stimulation and arrhythmia. A network of sympathetic neurons innervates the heart at locations such as the SA node, the AV node, and the ventricles (**Figure 1**)[1].

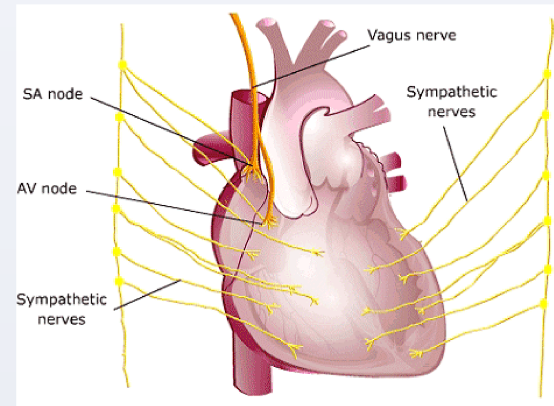

Figure 1. Sympathetic neurons of the heart.

Catecholamines, such as **norepinephrine (NE)**, are released from sympathetic nerve endings to activate β-adrenergic receptors on cell surfaces in the heart. Effects of β-adrenergic stimulation in the heart due to NE include increased heart rate and muscle contraction. However, **arrhythmia** can also occur as a result of β-adrenergic stimulation[3]. As such, β-blockers (e.g., propranolol) have been used as a standard treatment for arrhythmia[2]. Despite the widespread use of β-blockers, the exact mechanisms that implicate β-adrenergic stimulation in the formation of arrhythmic activity are not well understood.

RESEARCH APPROACH

Optogenetics and channelrhodopsin-2. Previously, sympathetic responses in hearts have been temporally and spatially mediated by exogenous mechanisms, typically using drugs such as isoproterenol to activate β receptors. We have developed a novel technique to precisely control the endogenous release of NE from sympathetic neurons in mouse hearts. Mice have been bred to express the light-gated ion channel protein channelrhodopsin-2 (ChR2) in catecholaminergic neurons so that stimulation with blue light results in release of NE from these neurons. This "combination of genetics and optics to control well-defined events within specific cells of living tissue"[4] is called **optogenetics**.

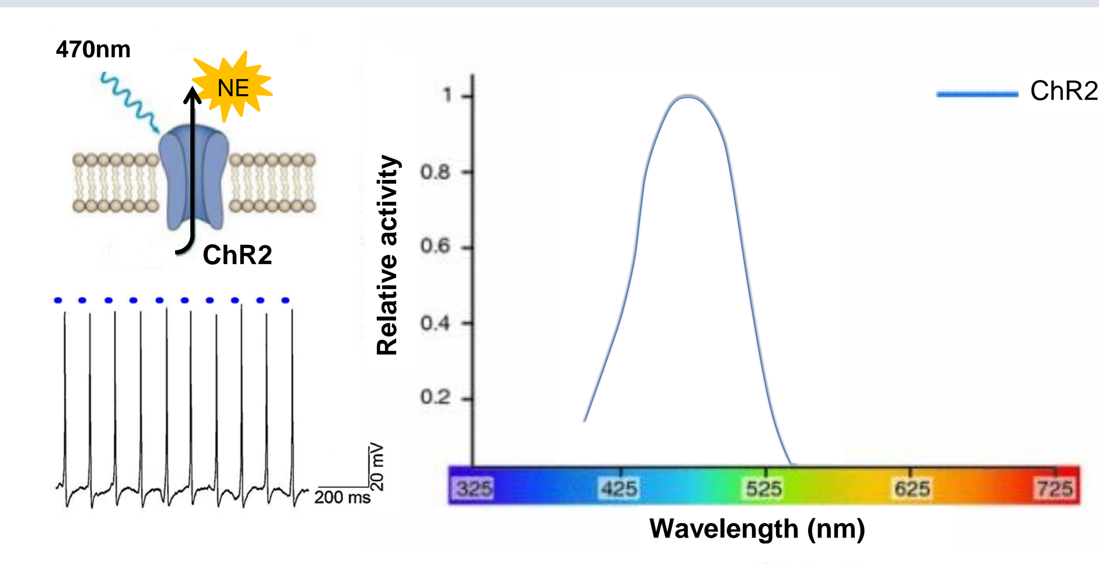

Figure 2. Top left: Light stimulates ChR2 to release NE from sympathetic neurons. Bottom left: Blue light excites neural action potentials. Right: ChR2 peak excitation is at 470nm (blue light).

Light-gated ion channels such as channelrhodopsin (ChR) are popular in optogenetic studies, and since 2005, mice have been bred to express ChR in neurons to study brain activity[5]. On the whole heart level, however, the use of optics to control NE release from sympathetic neurons is an entirely new concept. Our studies utilize ChR2, which has peak excitation at 470nm (**Figure 2**).

MATERIALS & METHODS

Mice expressing ChR2. ChR2 mice were created by breeding mice expressing the tyrosine hydroxylase (TH) promoter directing expression of Cre recombinase to catecholaminergic cells with mice that express channelrhodopsin dependent upon Cre expression (Stock Numbers: 008601 & 012569, Jackson Labs). Dr. Mendelowitz's lab was able to see ChR2 expression in paraventricular nucleus of the hypothalamus (PVN) neurons and in distal axons in the brain stem of these ChR2 mice (**Figure 3**)[5].

Characterization of epicardial neurons. Hearts were imaged using confocal microscopy to look at the distribution of nerve fibers and to estimate cardiac neuronal density. This estimation is useful for determining the potential amount of neurons activated by light.

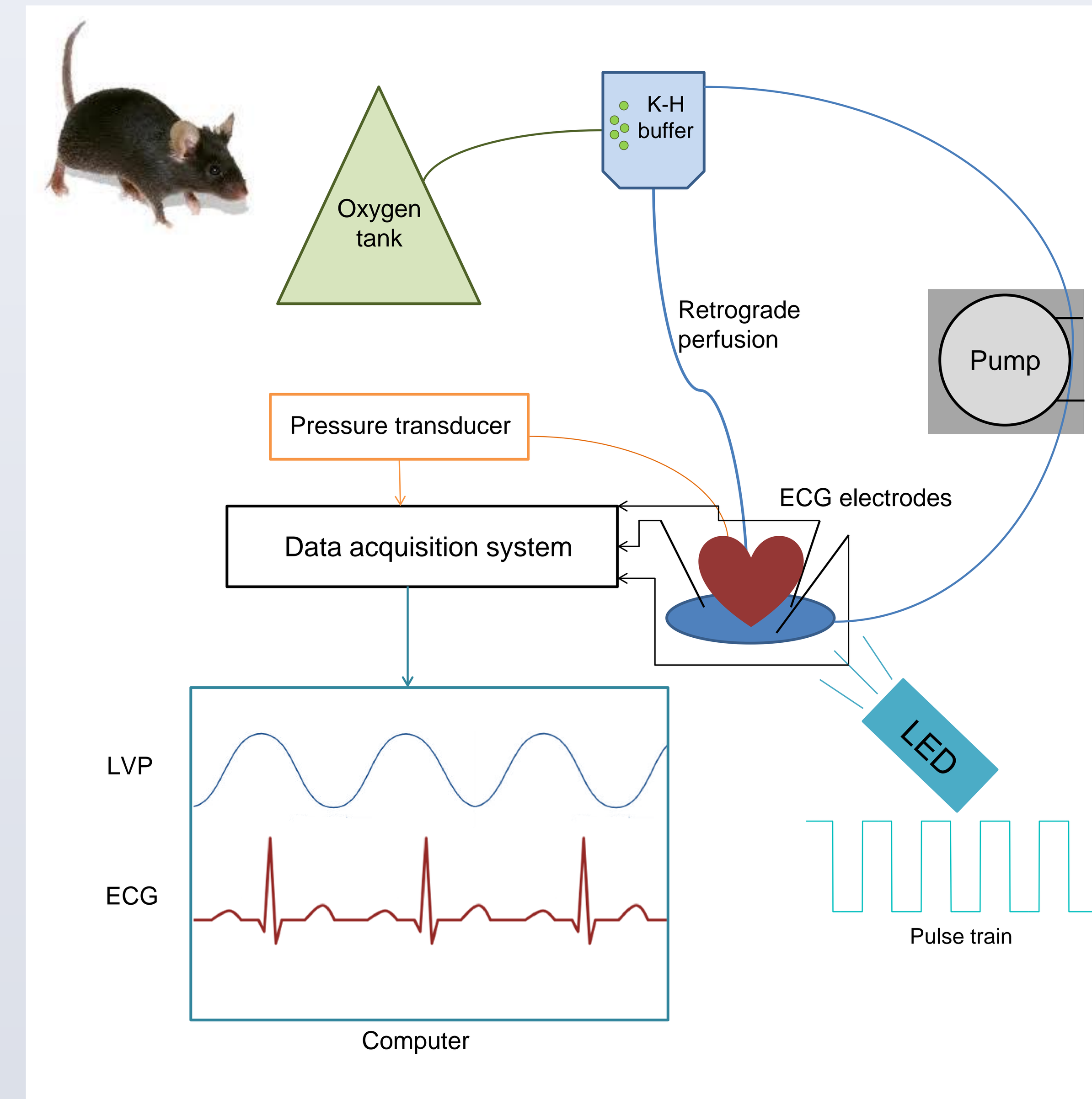

Figure 4. Experimental setup. The isolated mouse heart is perfused retrograde with O_2-bubbled Krebs-Henseleit buffer warmed to 37°C while a pump returns perfusate for recirculation. A balloon attached to a pressure transducer is carefully inserted into the LV for measurement of LVP, and ECG electrodes are placed around the heart. After the heart has stabilized, a 470nm LED illuminates the epicardial surface for stimulation of ChR2 and subsequent NE release. Changes in HR and LVP are captured by the ECG electrodes and pressure transducer connected to the data acquisition system, which sends signals to a computer for recording.

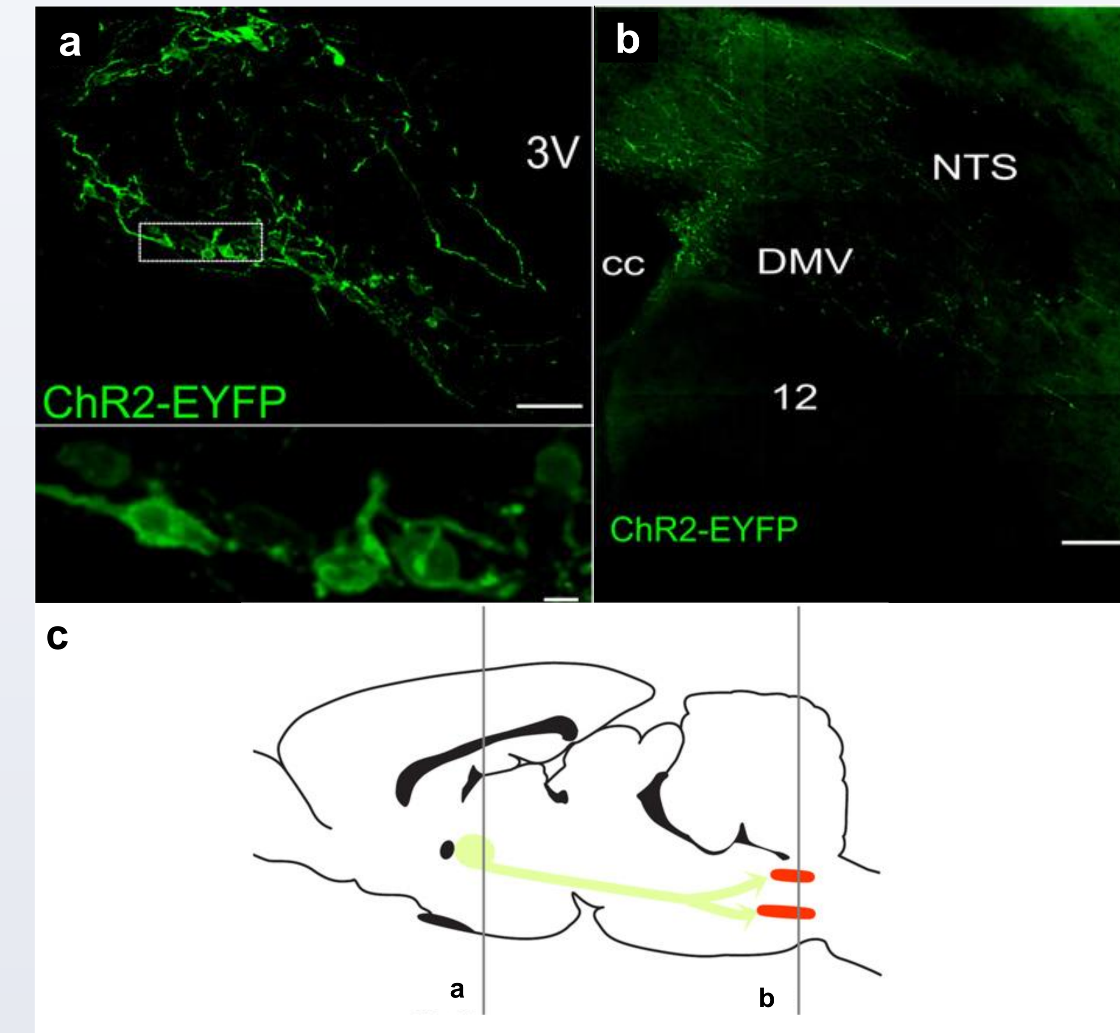

Figure 3. a) ChR2-EYFP expression in PVN neurons and distal axons in the brain stem; top scale bar 90μm, bottom 10μm. b) 150 μm. c) Schematic depiction of a rat brain indicating where slices were taken[5].

Using blue light to stimulate release of NE. Genetic testing confirmed expression of ChR2 in mice (n=3) used for the functional pilot study. A 470nm LED (1000mA, 450mW, Mightex, Pleasanton, CA) illuminated the epicardium of isolated hearts (5 ms duration pulses, 1 and 2 Hz frequency) for 10 seconds. At the end of each study, β-agonist isoproterenol (1 μM) was added to confirm that β-adrenergic receptors could be stimulated.

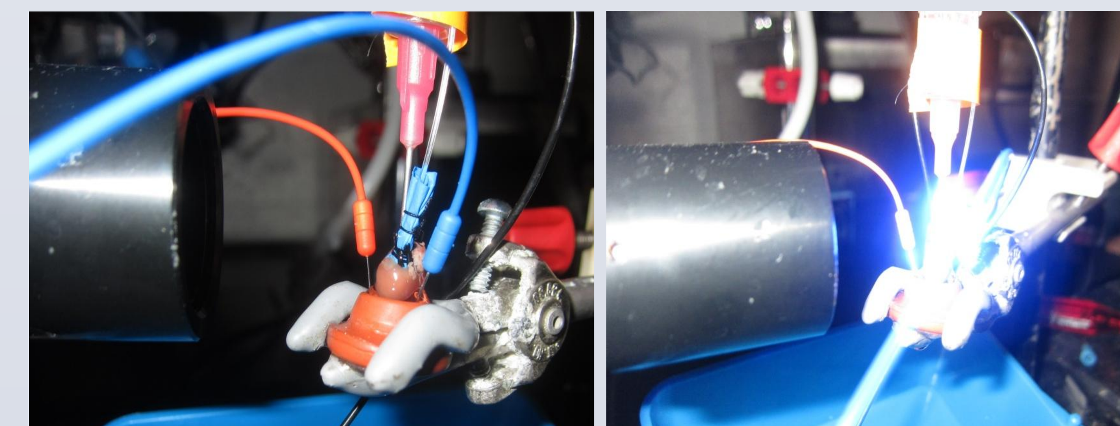

Figure 5. Illumination of the epicardial surface with blue light. A Langendorff-perfused heart is depicted in both panels. Left: The balloon used to measure changes in LVP and the ECG electrodes used to monitor changes in HR can be seen. Right: A 470nm LED is shining on the heart.

IMAGING RESULTS

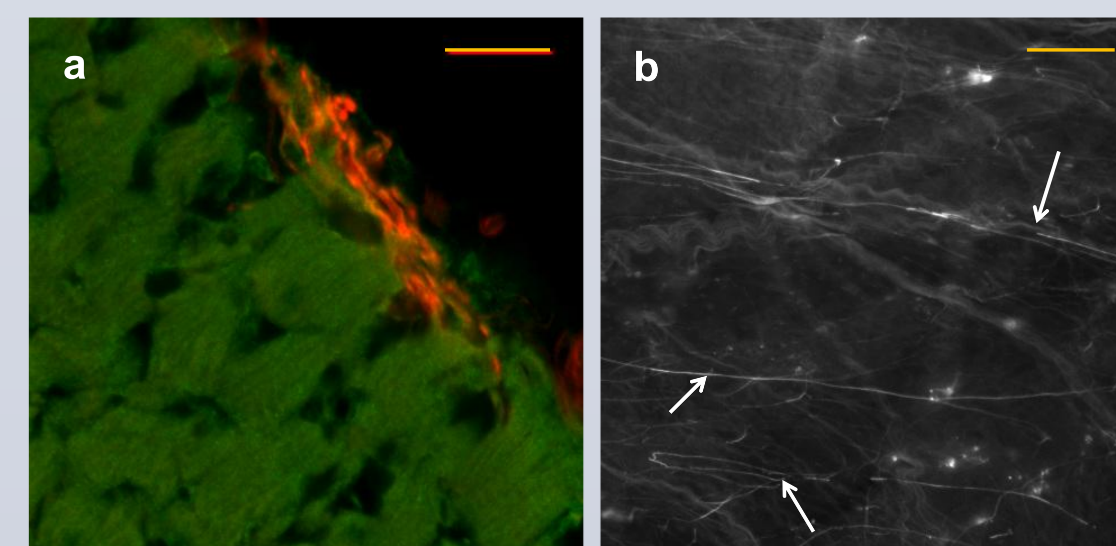

Figure 6. **a)** Mouse TH-positive neurons (red) and cardiac myocytes (green); scale bar is 20 μm. **b)** Sympathetic neurons on the epicardial surface of a rat heart; scale bar is 50 μm.

Distribution of cardiac neurons. The investigator observed densely packed arrays of nerve fibers on the epicardial surfaces of heart slices (**Figure 6a**). The dense arrays of neurons were never more than 20 μm deep, a finding which provided confidence that light would stimulate the multitude of sympathetic neurons that lie so close to the epicardial surface.

Epicardial neuronal surface area estimation. From **Figure 6b** we were able to roughly estimate the average neuronal surface area on the epicardium. This neuronal mass is estimated to be approximately 40% of the total epicardial surface area (we assumed axon diameter to be 3 μm[6]). A typical mouse heart surface area is about 100mm[2,7].

EXPERIMENTAL RESULTS

LVP and HR measurements. An increase in HR (18±9 bpm) as well as LVP (up to 75% increase) was observed in all hearts after stimulation with blue light. Addition of isoproterenol further increased HR (22±2 bpm) and LVP (up to 21% increase).

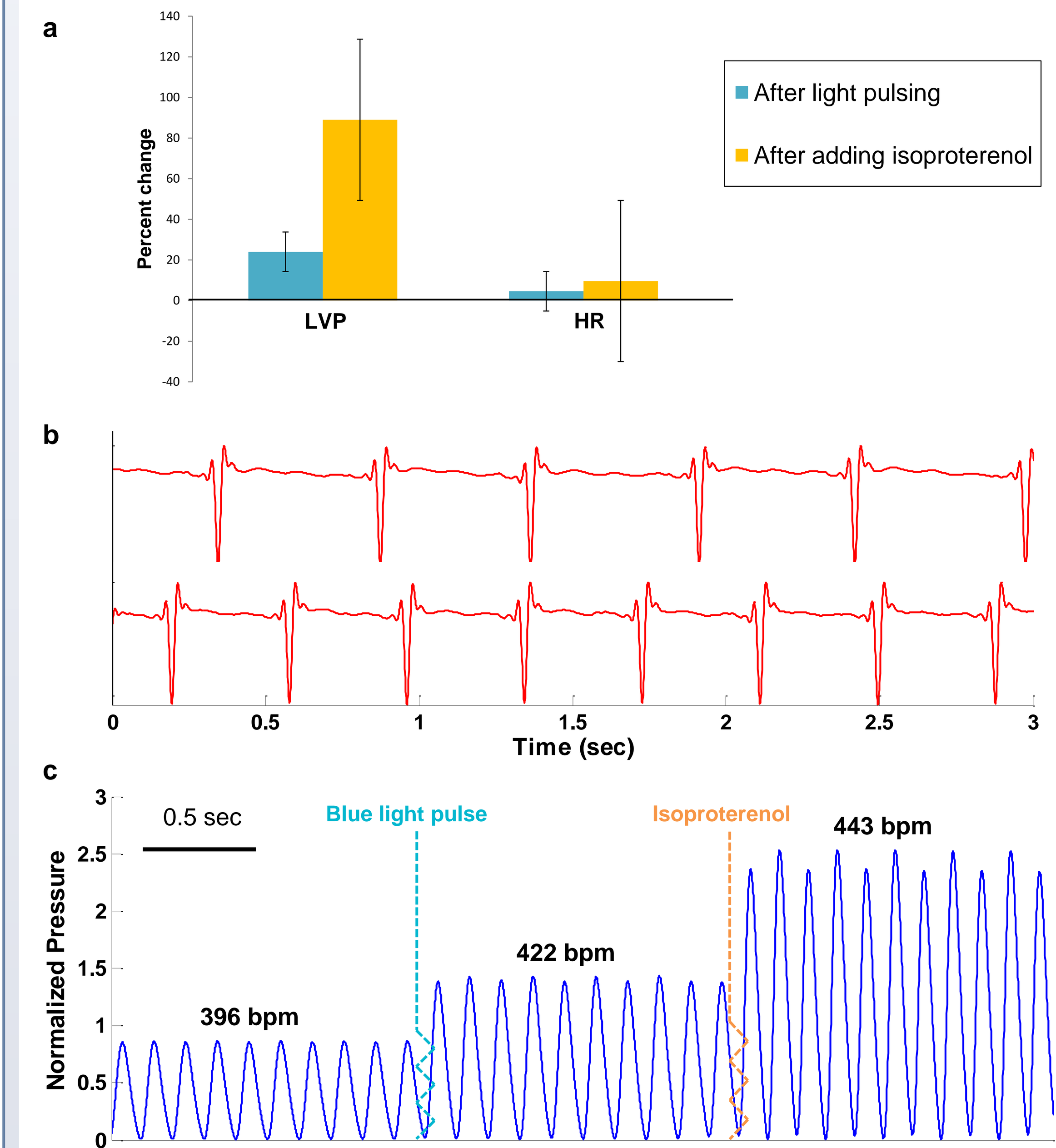

Figure 7. a) Percent change in LVP and HR after light pulsing and the administration of isoproterenol. b) Sample ECG signals. ECG signals were used to calculate changes in HR after perturbations. c) The effects of blue light and isoproterenol on normalized LVP.

CONCLUSIONS

Blue light pulsing increased left ventricular pressure and heart rate, confirming the release of norepinephrine from catecholaminergic cells expressing ChR2 in isolated mouse hearts. This experiment introduces a new and physiologically relevant model for the study of cardiac arrhythmias that arise from the activation of intrinsic sympathetic fibers and local β-adrenergic stimulation.

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