

Thermogenetics for cardiac pacing

Supplementary information

Frequency, Hz	Pulse width, ms	Average power, mW	The number of successfully paced cells	The number of cells failed to be paced
2	50	560	5	14
	75	600	1	
	100	140	4	
	250	300	3	
7	20	330	3	15
	30	500	6	
	50	510	1	
Total:			23	29

Table S1. Statistics over recordings of successful cardiomyocyte pacing experiments. Highlighted lines demonstrate the most promising stimulation modes for 2 and 7 Hz pacing. The diversity of experimental conditions is presented in Fig. S2 in a more detailed way.

Heart answer \ Stimulation freq.	Number of recordings (Number of animals)			
	2–3 Hz	4 Hz	5 Hz	6 Hz
Locking 1:1 (ventriculum)	3 (2)	7 (3)	4 (3)	5 (3)
Locking 1:1 (atrium)	2 (1)	1 (2)	2 (1)	4 (1)
Incomplete phase locking	0 (0)	0 (0)	3 (2)	2 (1)
Locking 3:2 (atrium)	0 (0)	1 (1)	0 (0)	1 (1)
Locking 4:3 (atrium)	0 (0)	0 (0)	0 (0)	4 (2)
Total number of animals:	4			
Total number of recordings:	37			

Table S2. Statistics over recordings of successful heart pacing experiments.

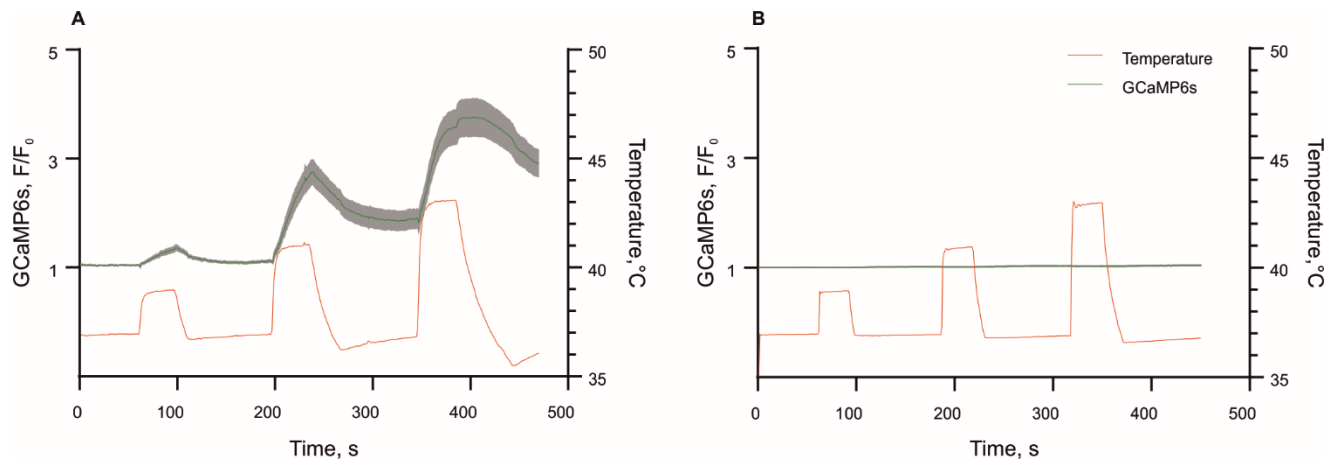


Figure S1. Calcium dynamics upon heating in HEK293 cells transfected with hTRPV1(-109aa) together with GCaMP6s (A) and GCaMP6s only (B). For **A** n=50 and for **B** n=54. Grey areas show SEM.

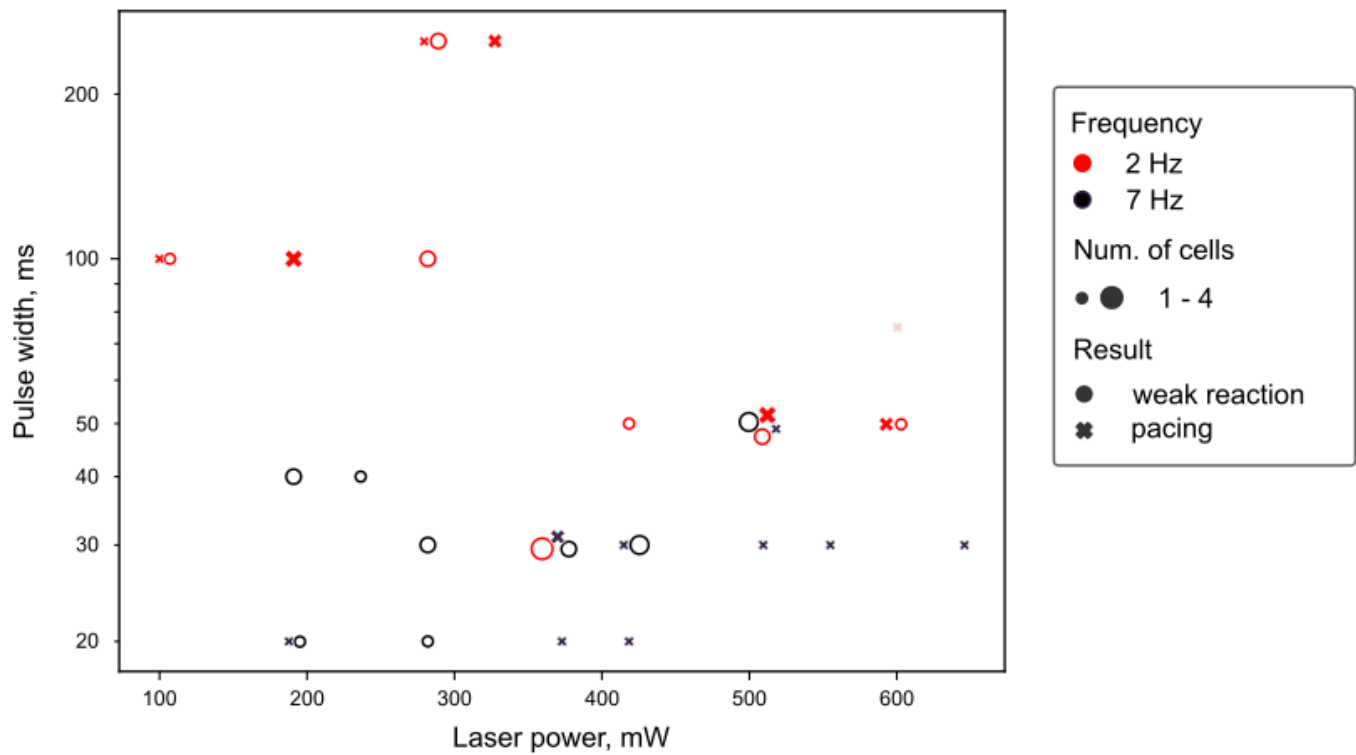


Figure S2. Distribution of successful and unsuccessful pacing experiments with cardiomyocytes. Data is presented depending on stimulation parameters: frequency, pulse width, and laser power.

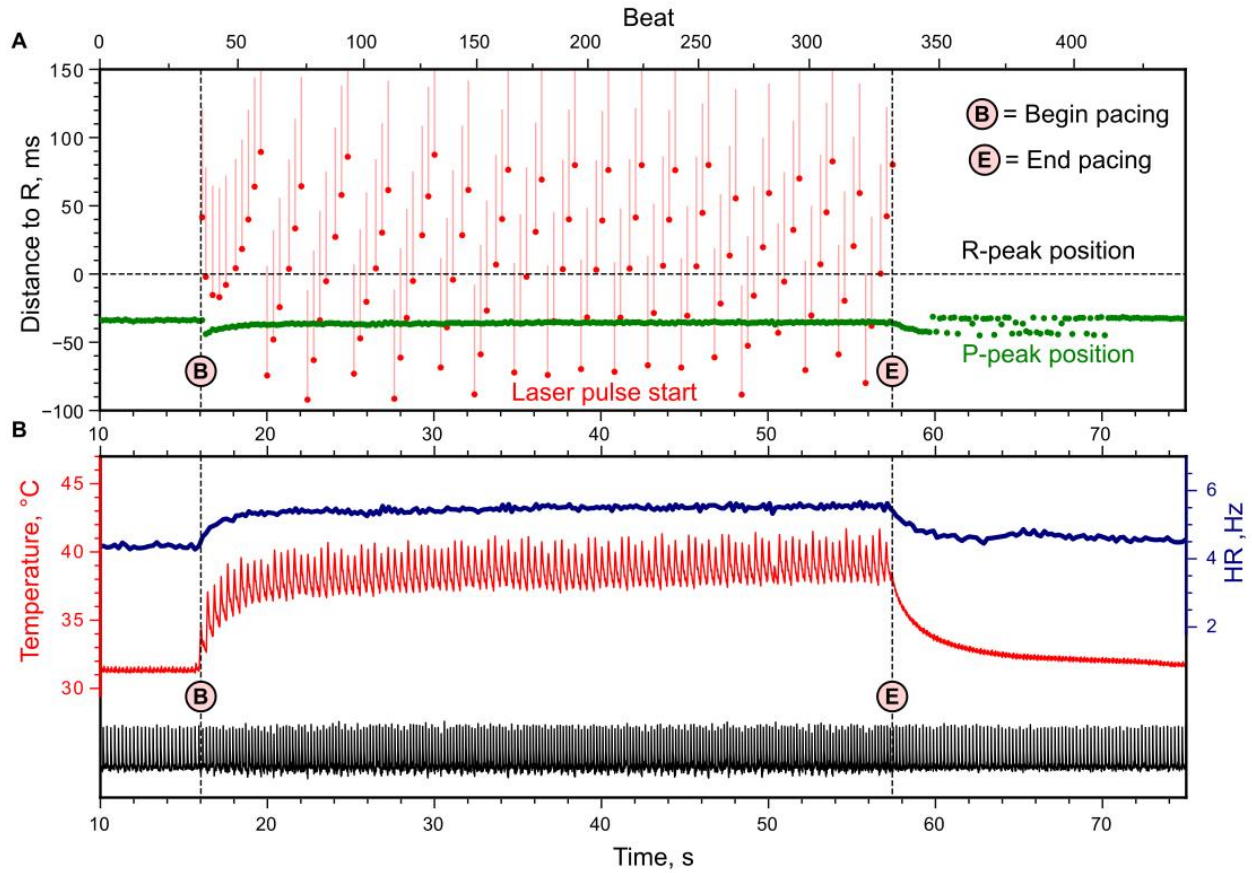


Figure S3. Reaction of the heart beating to pulse heating of the atrium in a control mouse. Pacing was at 2.5 Hz with 80-ms pulses, initial HR ~4Hz. **A.** Position of the P-peak and laser pulse in relation to the R-peak in ms. Laser pulses don't synchronize with the heart beat and are located at different time shifts in relation to R-peak zero line. **B.** Change of ECG signal (black curve), heart rate (blue curve), and temperature (red curve) during the experiment. Temperature was measured using an ultrathin thermocouple probe mounted under the atrium.

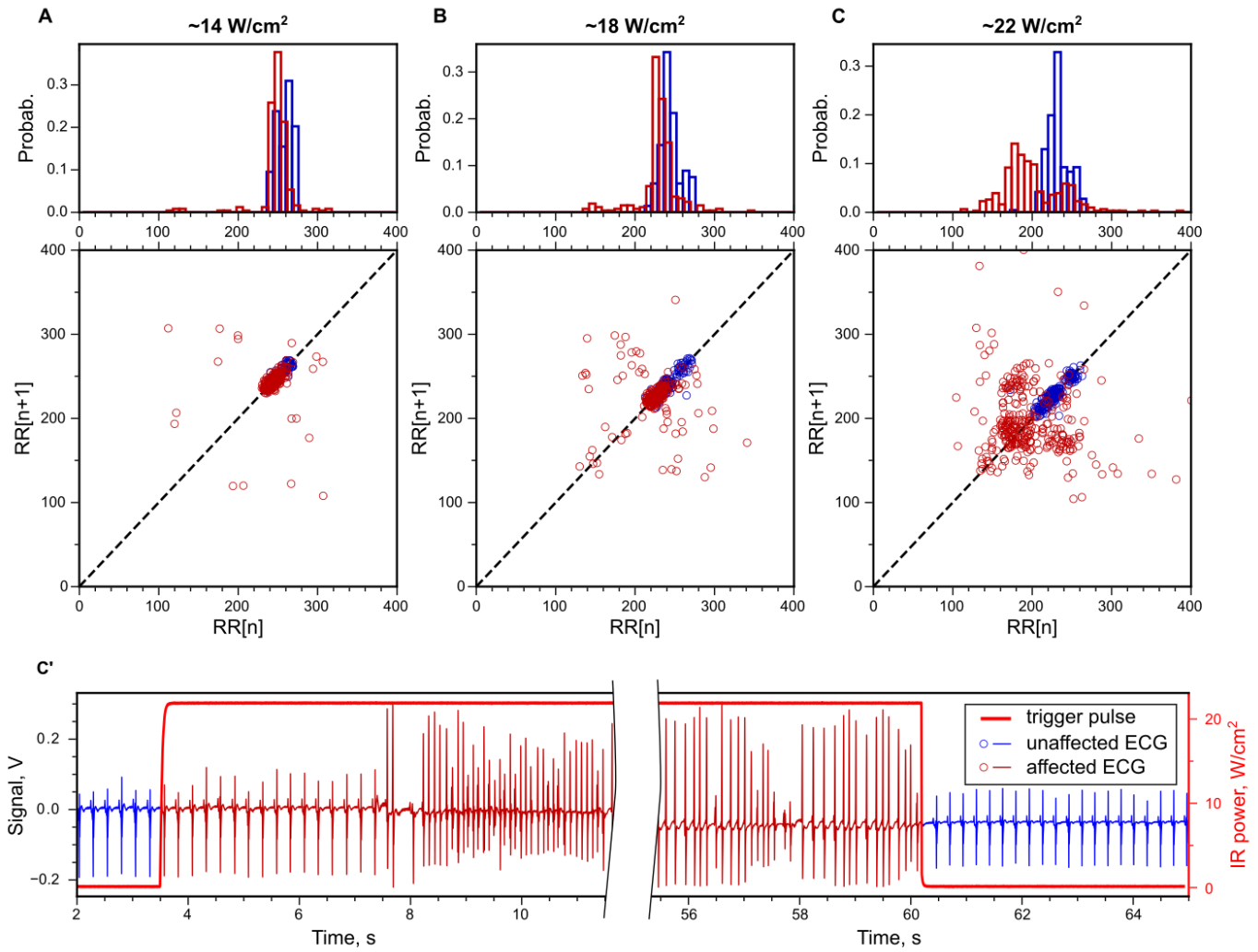


Figure S4. Effect of continuous heating of the atrium with three different IR laser intensities on heart rhythm. A–C (top row). Distribution of RR interval lengths plotted separately for intervals before and after heating (unaffected ECG, blue), and for intervals during heating (affected ECG, red). **A–C (bottom row).** Poincaré plots, where each pair of consecutive R-R intervals is a point on a graph. **C'.** Denoised ECG corresponding to the 3rd case with the middle part removed.

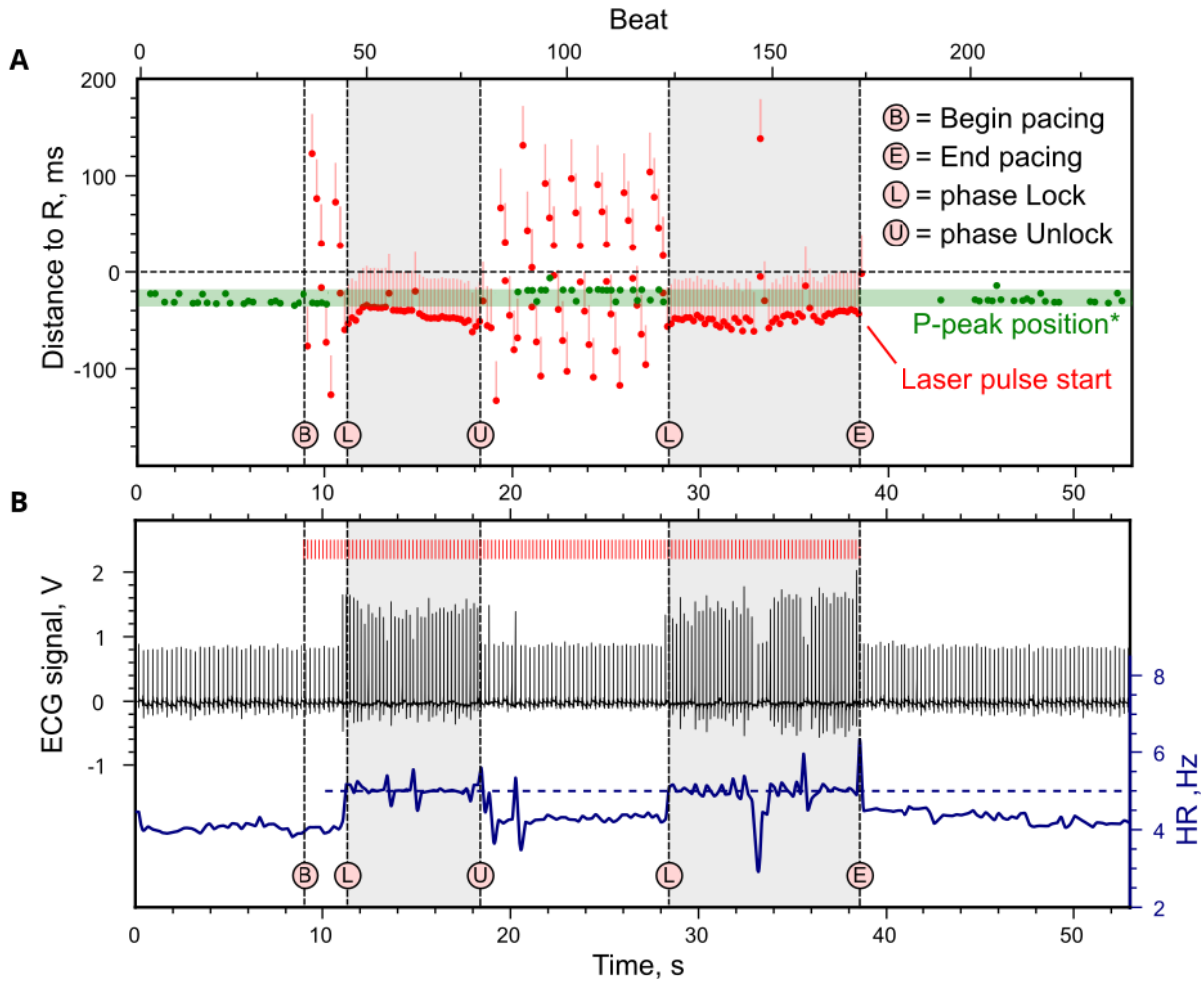


Figure S5. Lost and recovery of phase locking during left ventricle pacing with 5-Hz 20-ms trigger pulses. **A.** Position of the P-peak and laser pulse in relation to the R-peak in ms. **B.** Change of complex shape (black curve) and heart rate (blue curve) during the experiment. Vertical red lines demonstrate trigger pulses. Critical events are marked with 'B'/'L'/'U'/'E' letters (see the legend).

* — In this sample atrium beat was not synchronized with laser pulses that's why it was hard to separate P-peaks from the noise during the period of synchronization.

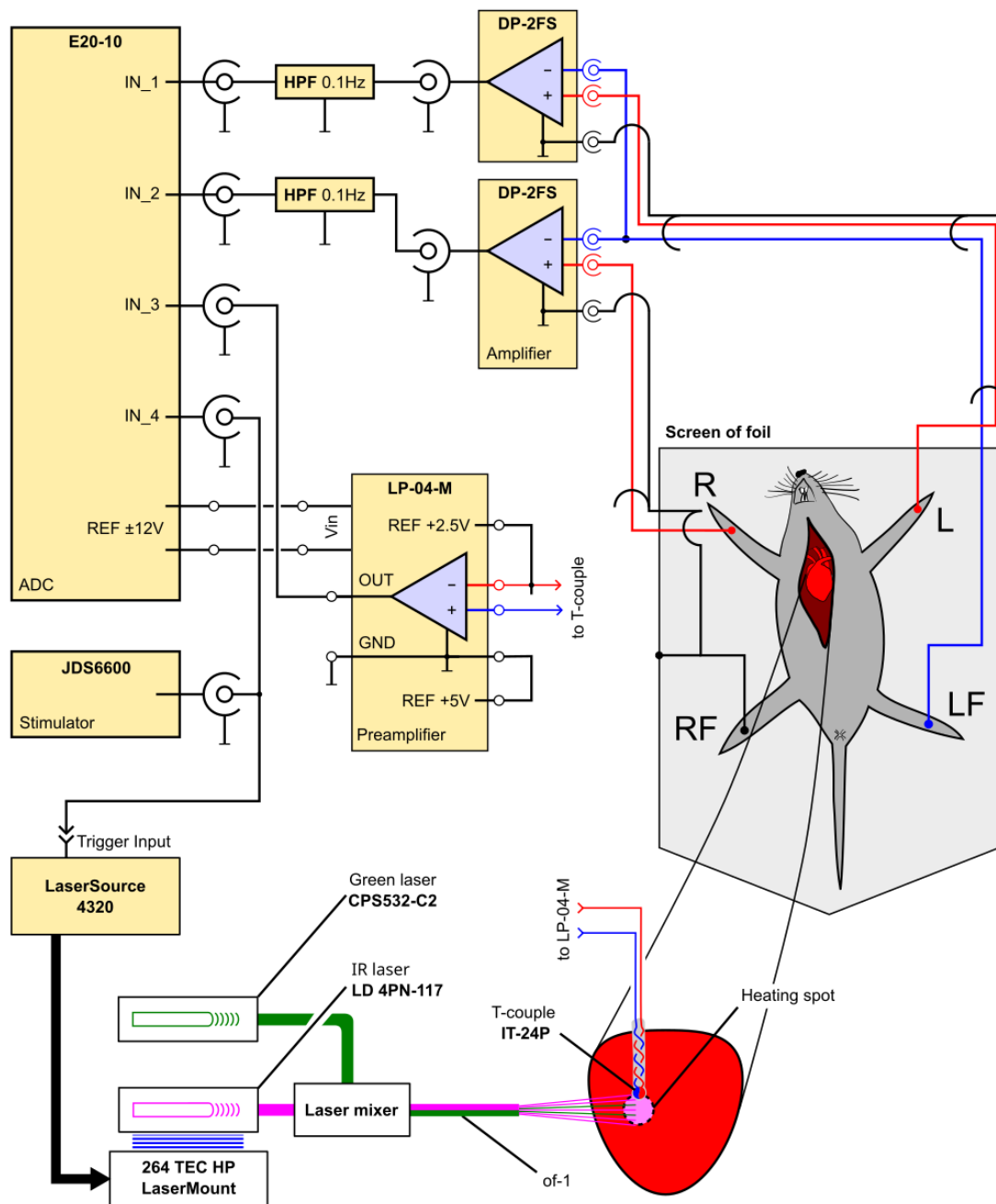


Figure S6. Interconnected electrical components allowing precise monitoring of heart rhythm and IR laser pulses. Signals from ECG electrodes at four leads (R, L, RF, and LF) were processed with two **DP-2FS** band differential amplifiers (shown). The two resulting amplified single-ended signals (**R-LF** and **L-LF**) were processed with a simple high-pass filter (**HPF**, shown) and fed to the 1st and 2nd inputs of the **E20-10** ADC (shown), which was connected to a PC by a USB interface. The **JDS6600** stimulator (shown) generated trigger pulses and operated laser diode driver **4320** (shown); these pulses were recorded by the 4th input of the ADC. The driver fed IR laser diode **4PN-117** (shown), whose light was mixed with a visible green light from **CPS532-C2** source (shown) by means of dichroic mirror (**Laser mixer**) and focused into the optical fiber (**of-1**). The fiber was mounted on a holder in close proximity to the heart, allowing the **heating spot** (shown) to be rather small. The ultrasmall thermocouple **IT-24P** (shown) was inserted into the myocardium at the location of the heating spot; its signal was processed by an **LP-04-M** preamplifier (shown) and fed into the 3rd input of the ADC.

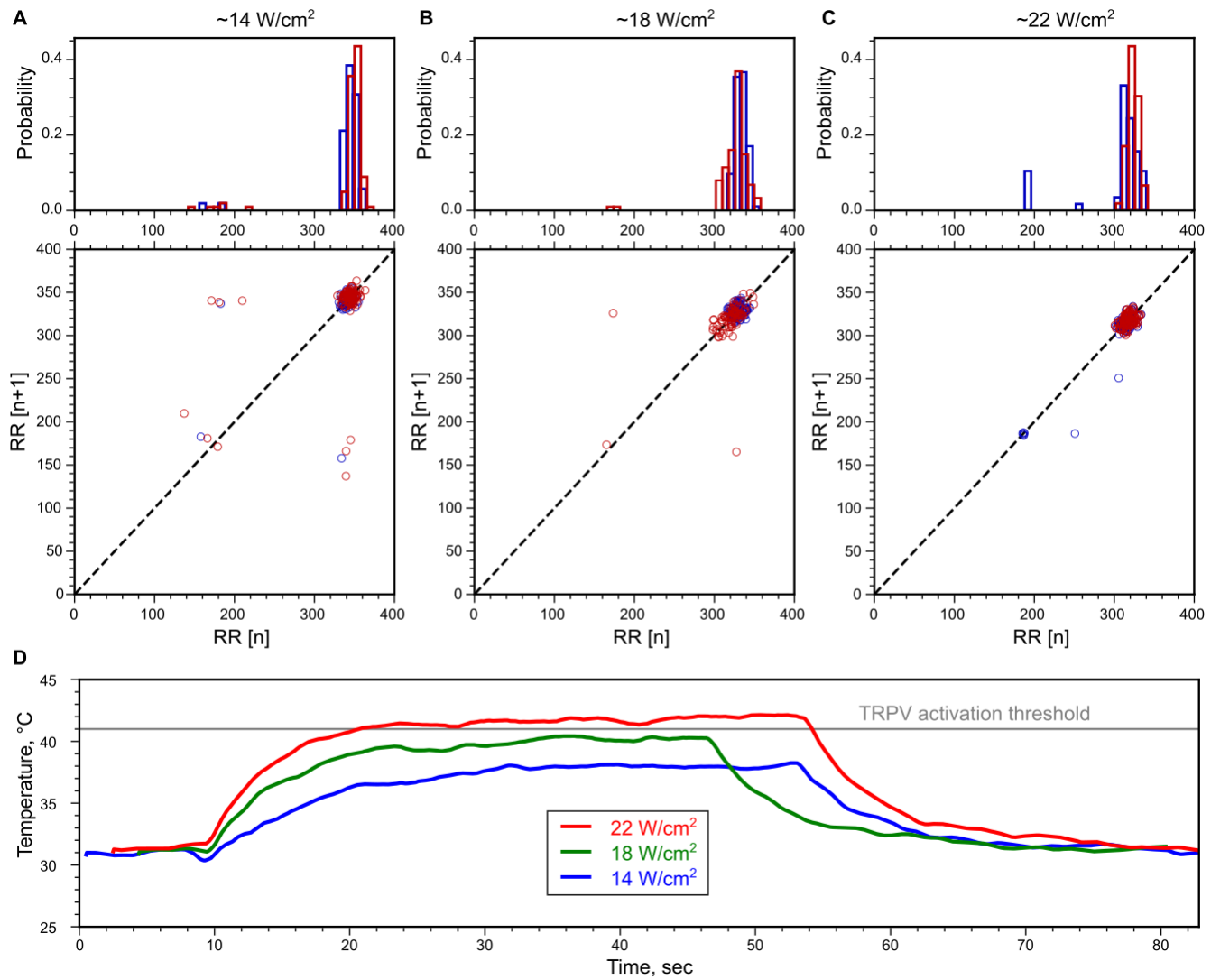


Figure S7. Reaction of the heart rate on the constant heat of heart ventriculum. A–C (top row). Distribution of RR interval lengths plotted separately for intervals before and after heating (unaffected ECG, blue), and for intervals during heating (affected ECG, red). **A–C (bottom row).** Poincaré plots, where each pair of consecutive R-R intervals is a point on a graph. **D.** Temperatures during experiments A–C measured using an ultrathin thermocouple probe mounted inside the myocardium.

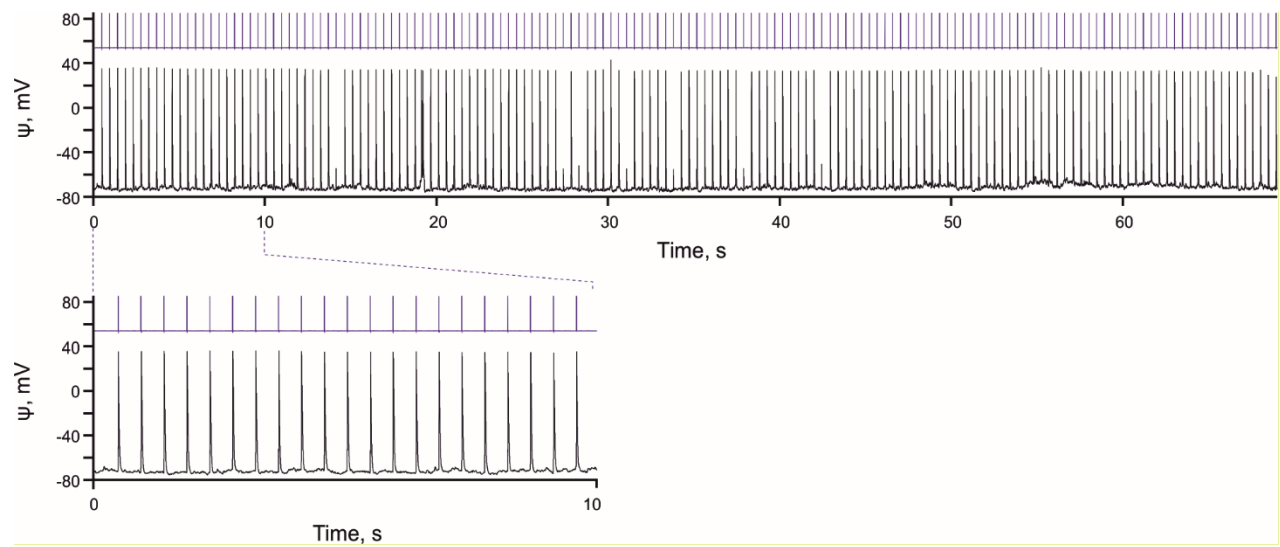


Figure S8. Pacing of control mouse neonatal cardiomyocyte with electric pulses. The membrane potential is shown in black, electric pulses are shown in blue with arbitrary amplitude. Cardiomyocyte recorded in current clamp mode, with 5 ms duration current injections of 30pA.