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Supplementary Materials for

A coupled-clock system drives the automaticity of human sinoatrial nodal pacemaker cells

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
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Supplementary Figure S1

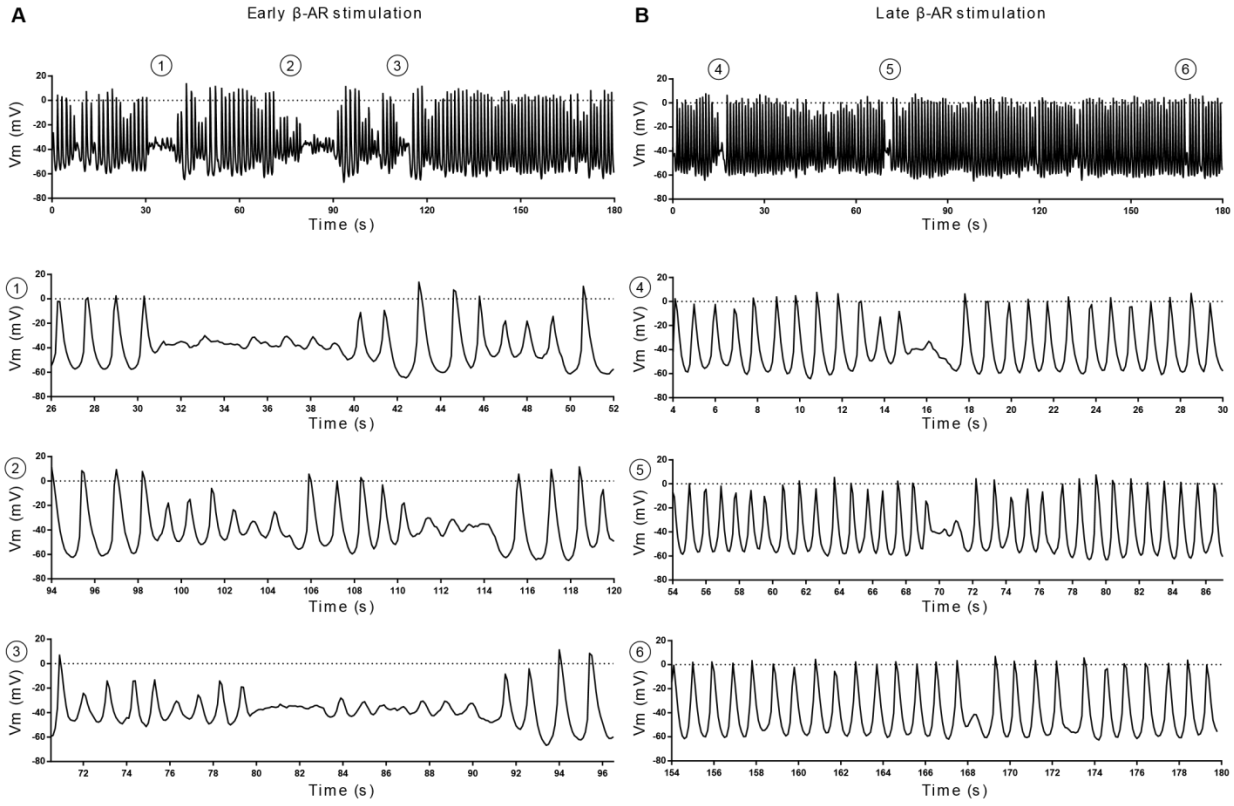

Fig. S1. Action potential repolarization failure in the transition from arrest to spontaneous action potential firing in response to β-AR stimulation.

After a variable number of action potentials were fired, the action potential membrane potential failed to repolarize (i.e. repolarization failure) and returned to the same membrane potential level as in the arrested state. Spontaneous oscillations of membrane potential about this arrest state heralded eventual hyperpolarization to the maximum diastolic potential and resumption of action potential firing (**panel A**). As exposure time to β-AR stimulation continued (**panel B**), both the number of repolarization failures and the time at which the cell remained depolarized was reduced (**panel B**).

Supplementary Figure S2

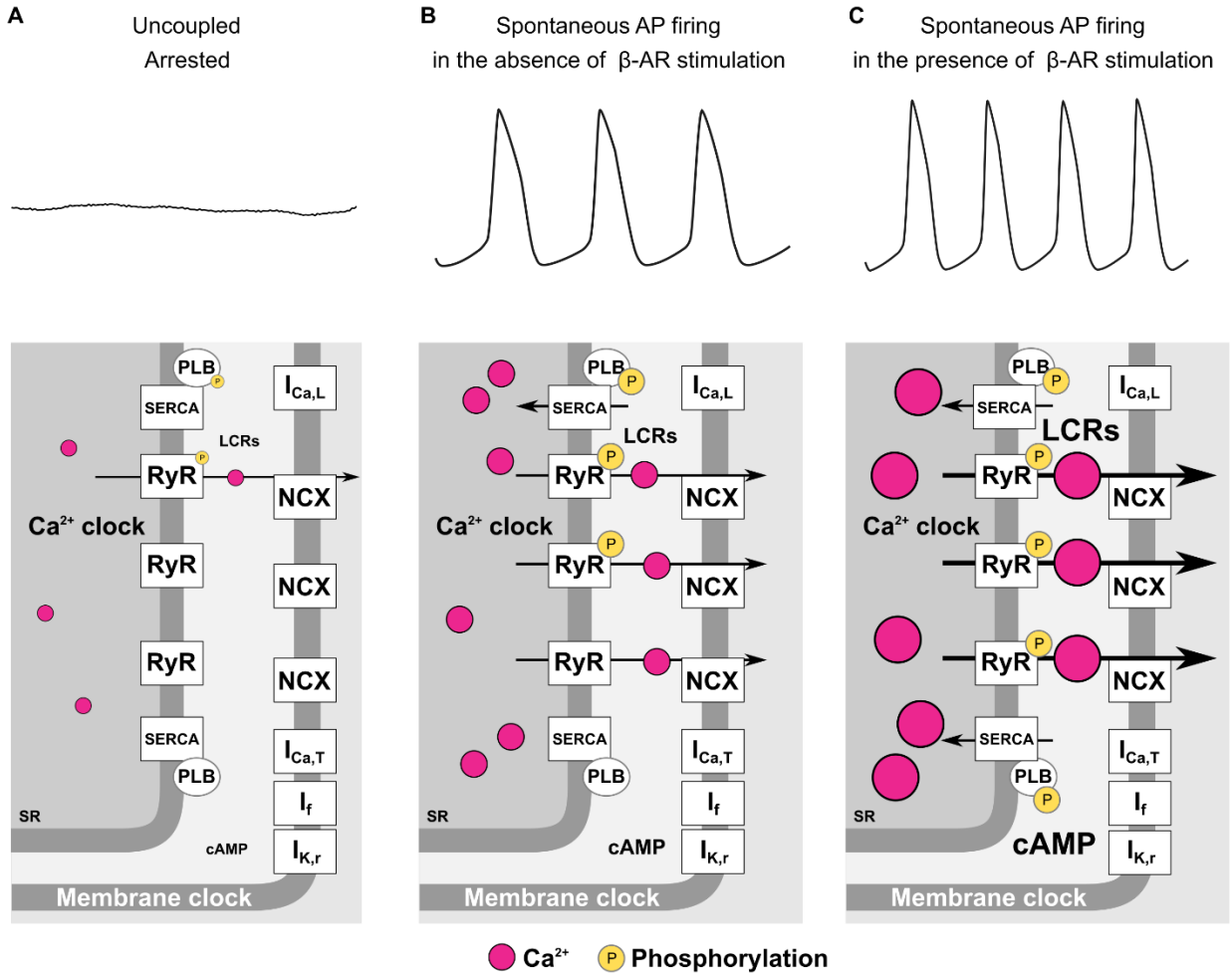

Fig. S2. A schematic of the clock coupling in SANCs.

The degree to which the Ca^{2+}- and M-clocks of SANC are coupled determines the spontaneous AP firing rate and rhythmicity. Local Ca^{2+} releases generated by the roughly periodic Ca^{2+} clock become electrogenic only when released Ca^{2+} activates NCX to generate a net inward current by exchanging a Ca^{2+} ion with 3 Na^{+} ions. (**A**) SANC arrest observed in the present study can be envisioned as a manifestation of severe clock uncoupling in which membrane potential is depolarized, the SR Ca^{2+} load is small, and small and random local Ca^{2+} releases occur but do not produce an ensemble Ca^{2+} signal to sufficiently activate I_{NCX}. Spontaneous APs do not occur. (**B**) During spontaneous action potential firing, both cAMP and cAMP-dependent PKA-mediated phosphorylation modulate Ca^{2+} and M-clock functions to generate spontaneous action potentials. At a hyperpolarized membrane potential and a larger SR Ca^{2+} load, a larger local Ca^{2+} release ensemble Ca^{2+} signal, and I_f activation generate inward current contributing to diastolic depolarization. (**C**) β-AR stimulation enhances M- and Ca^{2+} clock coupling that increases local Ca^{2+} release synchronization and augments I_f to increase diastolic depolarization rate, resulting in an increase in the spontaneous action potential firing.

Supplementary Table 1

Table S1. Characteristics of the donor hearts used in the present study.
The hearts were from patients aged 26-65 years. None of the donors had a history of major cardiovascular diseases. LVEF: left ventricular ejection fraction. OD, drug overdose, CVA, cerebrovascular accident, CP, cardioplegic.

	Gender	Age	LVEF (%)	Time in CP solution	Cause of death
Heart 1 (2016 Nov)	Male	28	50	~8h	OD
Heart 2 (2016 Dec)	Male	45	N/A	~8h	CVA
Heart 3 (2017 Jan)	Male	26	65	4h	OD
Heart 4 (2017 Jul)	Female	65	60	5h	Head trauma

Supplementary Table 2

Table S2. Time-dependent evolution of action potential parameters in the transition state of an initially arrested human SANC.

Note that V_m were not recorded in a few minutes during the transition. AP, action potential, TIP, time to ignition point.

β-AR stimulation time (s)	Cycle length (ms)	AP amplitude (mV)	TIP (ms)
30-60	1710±190	60±3	1570±190
60-90	1752±103	62±3	1516±60
90-120	1425±89	56±2	1258±98
120-150	1428±77	67±2	1260±99
150-180	1113±14	61±2	920±33
600-630	955±20	62±2	765±34
630-660	944±16	54±2	734±31
660-690	956±33	60±2	765±38
690-720	953±33	58±3	777±49

Supplementary Movie 1. A freshly isolated single spontaneously beating human SANC from heart 3.

Supplementary Movie 2. LCR detection software on a spontaneously beating human SANC from heart 4. Spontaneous APs were recorded in tandem with AP-induced Ca^{2+} transients. AP and AP-induced Ca^{2+} transients with most similar CL are overlaid in the figure (black line).

Supplementary Movie 3. Simultaneous measurements of membrane potential and a 2D Ca^{2+} signal. Measurements in an initially arrested responder SANC were made prior to and during β-AR stimulation. The recorded 2D Ca^{2+}-signal (grayscale) and detected LCRs (white areas encircled in colors) are shown in the lower panel. A reconstructed plot in the upper panel shows membrane potential (black), whole-cell Ca^{2+} signal (magenta), detected individual local Ca^{2+} signals (gray in patterns) and the Ca^{2+} signal of the LCR ensemble (blue).