Online data supplement

Supplemental Methods

Experimental model and optical mapping

The experimental model and procedures of optical mapping are essentially the same as reported previously.[1-3] Isolated rabbit hearts (*n*=27) were perfused on a Langendorff apparatus with modified Krebs-Ringer solution at 37°C. Complete atrioventricular (AV) block was produced by destruction of the His bundle. A 2-dimensional epicardial layer of ventricular myocardium (~1 mm thick) was prepared by a cryoprocedure at the endocardium. The tissue was stained with a voltage-sensitive dye, di-4-ANEPPS. 2, 3-butandione monoxime (BDM) was applied to minimize motion artifacts, unless otherwise specified. Bipolar electrograms were used to monitor ventricular activation.

The hearts were illuminated by bluish-green light emitting diodes (LEDs) and the fluorescence was recorded with a high-speed digital video camera to acquire 10 bit gray scale images (256x256 pixels) at a sampling rate of 1,000 frames/s. The image covered the anterolateral surface (~30x30 mm) of the left ventricle (LV). To reveal the action potential signal, the background fluorescence was subtracted and low-pass filtering was applied. Spatial resolution after filtering was ~0.1 mm. Isochrone maps of 1-5 ms intervals were generated from the filtered image. From normalized action potential signals, time points at 10% depolarization and 90% repolarization were identified, and the interval in between was defined as action potential duration (APD). Wave propagation patterns during ventricular tachycardia/fibrillation (VT/VF) were analyzed by the phase mapping method.[4]

Measurement of conduction velocity (CV) and action potential duration (APD)

Global CV and APD were measured during constant (S1) stimulation from the anterior center of the LV free wall at basic cycle lengths (BCLs) of 180-400 ms. A unipolar electrode made of teflon-coated platinum wire (diameter, 0.1 mm) was used for stimulation. The pulses were 2 ms in duration and had an intensity of 1.2 times diastolic threshold. CV was calculated from the slope of a linear least-square fit of the activation time plotted against the distance in the direction perpendicular to activation isochrones.

Induction of VT/VF

VT/VF (lasting ≥3 beats) was induced by modified cross-field stimulation. Nineteen basic (S1) stimuli (BCL, 400 ms) were applied to the LV apex through a pair of contiguous bipolar electrodes, and a 20-ms monophasic DC pulse of constant voltage (DS2, 20 V) was delivered through a pair of Ag-AgCl paddle electrodes (7 mm in diameter) placed on the lateral surfaces of both ventricles. The S1-DS2 coupling interval was shortened progressively in 10 ms steps to cover the whole vulnerable window of the S1 excitation.

References

1. **Yamazaki M, Honjo H, Nakagawa H, et al.**: Mechanisms of destabilization and early termination of spiral wave reentry in the ventricle by a class III antiarrhythmic agent, nifekalant. *Am J Physiol Heart Circ Physiol* 2007;292:H539-H548.
2. **Harada M, Honjo H, Yamazaki M, et al.**: Moderate hypothermia increases the chance of spiral wave collision in favor of self-termination of ventricular tachycardia/fibrillation. *Am J Physiol Heart Circ Physiol* 2008;294:H1896-H1905.
3. **Ishiguro YS, Honjo H, Opthof T, et al.**: Early termination of spiral wave reentry by combined blockade of Na^+ and L-type Ca^{2+} currents in a perfused two-dimensional epicardial layer of rabbit ventricular myocardium. *Heart Rhythm* 2009;6:684-692.
4. **Gray RA, Pertsov AM, Jalife J.**: Spatial and temporal organization during cardiac fibrillation. *Nature* 1998;392:75-78.

Figure and Movie Legends

Online Supplement Fig. 1.

Rotors during VT induced in the absence and presence of regional cooling (RC). ***A***, monomorphic VT under control conditions (in the absence of RC). Top, bipolar electrogram; bottom, 4-ms isochrones activation map. A counterclockwise rotor circulated around a functional block line (FBL, pink). ***B,C***, short polymorphic VT induced during RC. ***B***, top, bipolar electrogram; *bottom*, isochrones maps of 2 consecutive beats prior to VT termination. A counterclockwise rotor circulating around a long FBL (pink) in the vicinity of the RC region (yellow circle) changes its circuits and terminated by collision with the atrioventricular (AV) groove (gray bar). ***C***, left, optical action potential signals (a-f in the isochrones map) prior to VT termination. *Right*, phase maps of the last 2 beats. White circle, a phase singularity (PS) of clockwise rotation; black solid line in the right bottom panel, PS trajectory; dotted circle, RC region.

Online Supplement Fig. 2

Termination of VT by mutual annihilation of counter-rotating rotors in the RC region. ***A***, a bipolar electrogram shows that VT induced during RC self-terminated. ***B***, *left,* 4-ms isochrone map of the last beat prior to VT termination. FBLs of figure-of-eight reentry (pink) were located in the periphery of the RC region (yellow circle) and wave propagation in the central common pathway was blocked in the RC region. *Right*, optical action potential signals (*a-i* in the isochrone map). ***C***, phase maps of the last beat prior to VT termination. Black and white circles, PSs of clockwise and counterclockwise rotation, respectively. Black lines in the bottom right panel, trajectory of PSs.

Online Supplement Fig. 3.

Failure and success of cardioversion by DC-shocks in the absence of RC. ***A***, failure of cardioversion by medium-intensity shock resulting from transformation from VT to VF. *Left*, phase maps before (a) and after 30-V DC-shock application (b). Multiple PSs showing irregular meandering were generated by the shock. *Right*, superimposed action potential signals from 3 sites. ***B***, success of cardioversion by high-intensity shock resulting from phase resetting. *Left*, phase maps before (a) and after 50-V DC-shock application (b). A single clockwise rotor around a PS (black circle) disappeared promptly after the shock. *Right*, superimposed action potential signals from 5 sites.

Online Supplement Fig. 4

Termination of VT/VF in a 3-D heart by DC-shock combined with RC. ***A***, a phase map of a VF induced under control conditions (in the absence of RC). Six PSs, 3 PSs of clockwise rotation (black circle) and 3 PSs of counterclockwise rotation (white circle), showing irregular meandering are recognized. The VF was not cardioverted by biphasic DC-shocks at the maximum intensity (100 V). ***B***, phase maps of a VF that cardioverted by DC-shock combined with RC. Application of a 25-V DC-shock to the VF, which had been resistant to cardioversion by RC alone, resulted in delayed termination in the presence of RC. PSs in the periphery of the RC region (dotted circle) decreased in number and finally disappeared probably through collision. ***C***, action potential signals recorded from * in ***B***.

Online Supplement Fig. 5.

Termination of sustained-VT in a 2-D heart by RC in the absence of BDM. ***A***, monomorphic VT was maintained by a stationary rotor under control conditions. *Top*, bipolar electrogram; *bottom*, phase maps of 4 consecutive beats during the VT. ***B***, termination of sustained-VT ~10 s after application of RC (dotted circle). *Top*, bipolar electrogram; *bottom*, phase maps of 4 beats prior to VT termination. Trajectory of PSs is illustrated in the *bottom right* panel. White and black circles, PSs of counterclockwise and clockwise rotation, respectively; white bars in the phase maps, AV groove.

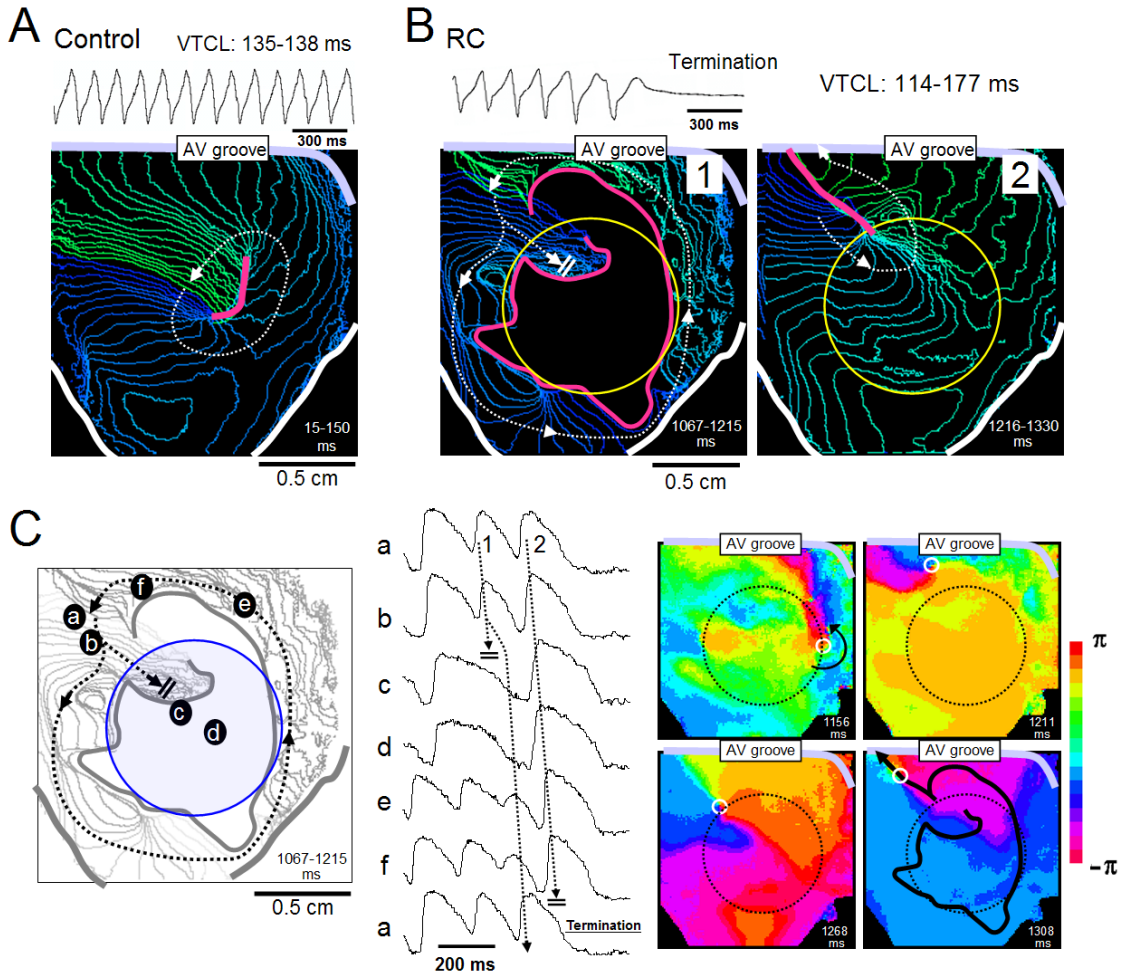
Online Supplement Movie 1

Representative phase movie during sustained-VT without RC. A stable and stationary rotor was observed in the anterior left ventricular free wall. Each color corresponds to a different phase of the action potential as illustrated in Figure 2A

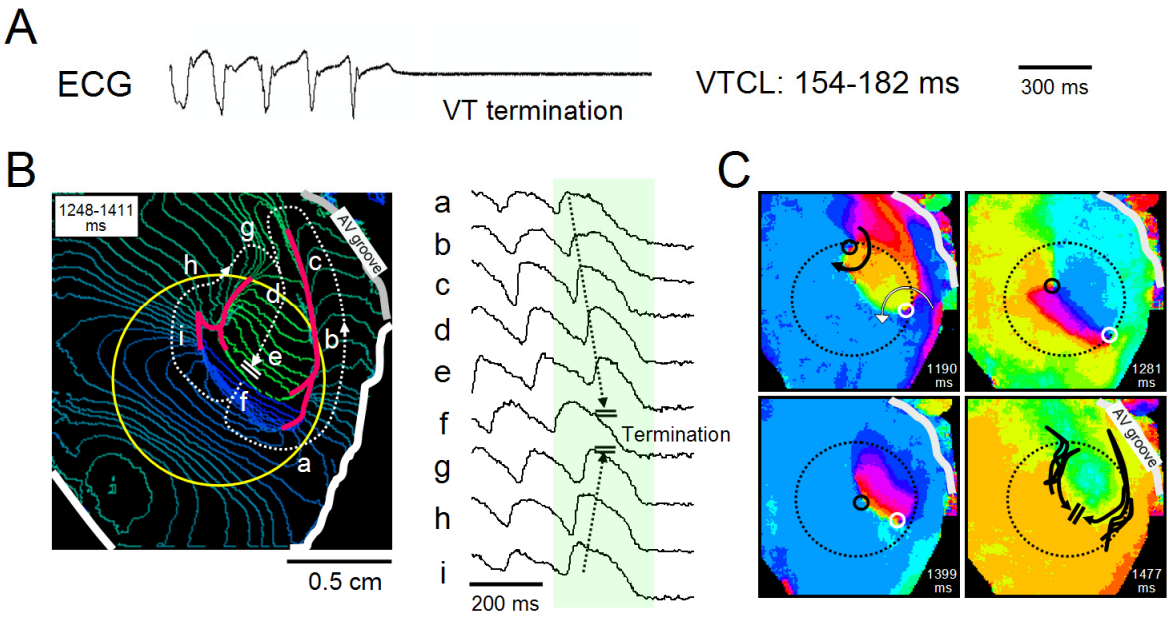
Online Supplement Movie 2

Representative phase movie during sustained-VT with RC prior to VT termination. Phase maps show that a single PS moved along the periphery of the RC region and collided with the atrioventricular groove.

Online Supplement Fig. 1



Online Supplement Fig. 2



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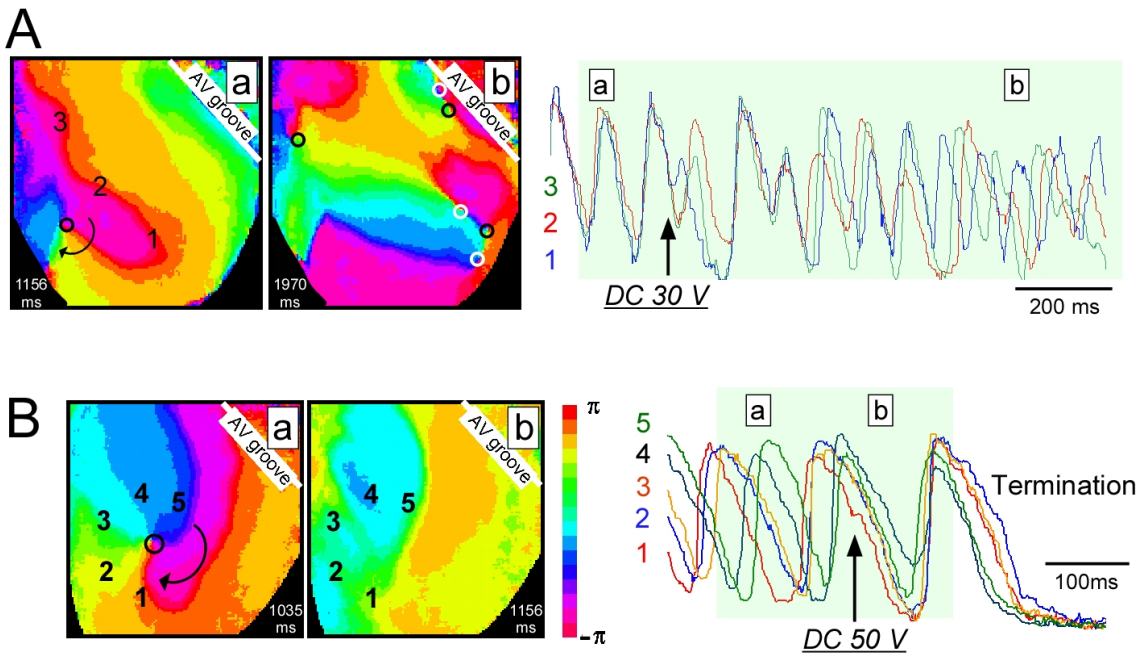
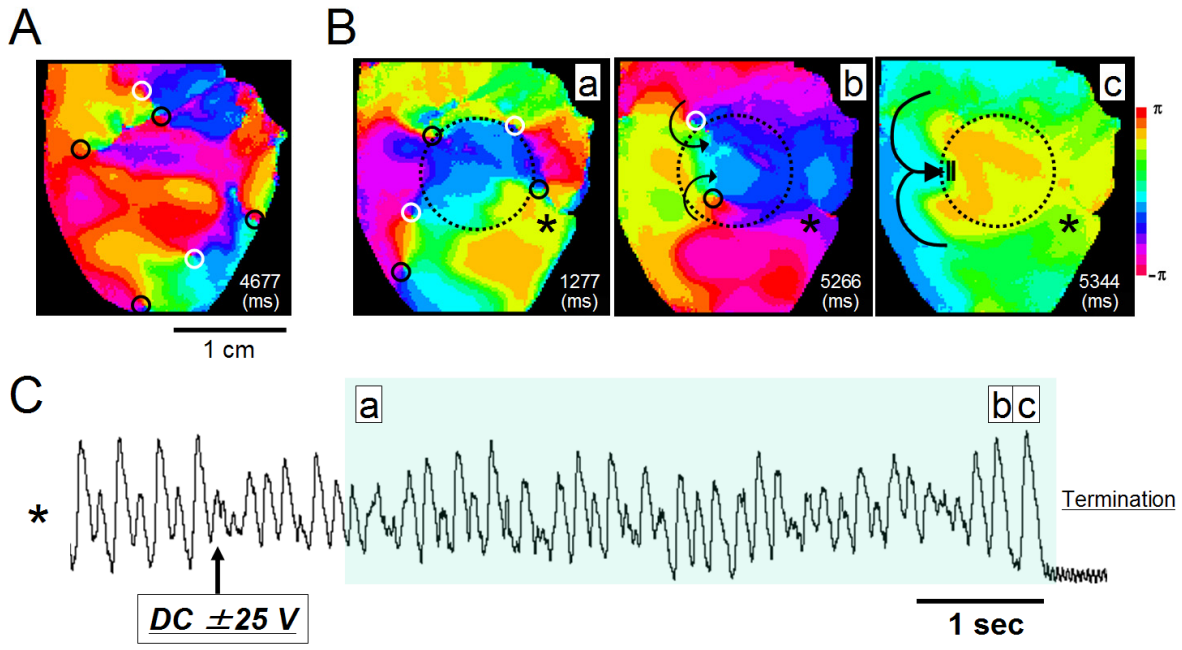


Online Supplement Fig. 4



Online Supplement Fig. 5