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Formation of virtual isthmus: new scenario of spiral wave death after decrease of excitability

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Termination of rotating (spiral) waves or reentry is crucial when fighting with the most dangerous cardiac tachyarrhythmia. To increase the efficiency of the antiarrhythmic drugs as well as finding new prospective ones it is decisive to know the mechanisms how they act and influence the reentry dynamics. The most popular view on the mode of action of the contemporary antiarrhythmic drugs is that they increase the core of the rotating wave (reentry) to that extent that it is not enough space in the real heart for the reentry to exist. Since the excitation in cardiac cells is essentially change of the membrane potential, it relies on the functioning of the membrane ion channels. Thus membrane ion channels serve as primary targets for the substances which may serve as antiarrhythmics. At least, the entire group of antiarrhythmics class I (modulating activity of sodium channels) and partially class IV (modulating activity of calcium channels) are believed to destabilize and terminate reentry by decreasing the excitability of cardiac tissue. We developed an experimental model employing cardiac tissue culture and photosensitizer (AzoTAB) to study the process of the rotating wave termination while decreasing the excitability of the tissue. A new scenario of spiral wave cessation was observed: an asymmetric growth of the rotating wave core and subsequent formation of a virtual isthmus, which eventually caused a conduction block and the termination of the reentry.

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Introduction. Excitation waves are known to play an important role in the self-organization of a wide variety of nonlinear media, including biological, chemical, and ecological systems [1]. The complex patterns usually arise when propagating fronts lose stability and can be eventually broken [2]. The wave breaks give birth to rotating spiral waves, or vortices. Disorder of the excitation propagation may lead to circulating excitation, or reentry, a selfperpetuating mechanism involved in the initiation and maintenance of the majority of tachyarrhythmia [3]. It is widely recognized that rotating spiral waves or reentries underlie the most dangerous cardiac arrhythmia often leading to the fibrillation and sudden death [4–6]. Prevention and termination of the rotating waves, thus underlie the majority of life-saving procedures, such as applied defibrillation shock, and also determine the selection of prospective antiarrhytmic drugs. Now a wide agreement exists between biophysicists and cardiologists that extinguishing of reentry is based on the pharmacological increase of the minimal reentry path, or in other words, an increase of the rotating wave core [7–11]. Then, it is supposed that this increase of the characteristic size of reentry should lead to one of two termination scenarios.

The first and the simplest, one-step scenario, suggests that the rotating wave simply hit its own refractory tail and fails to propagate in a non-recovered tissue. This effect can be easily described in a quasi one-dimensional case, as the rotation of the excitation wave in a virtual ring [5, 8, 12, 13]. In a real heart it would mean that the entire heart size is not sufficient for the reentry rotation.

Another, more complex, two-step scenario, relies on the fact that free rotating spiral wave tends to migrate in the inhomogeneous excitable medium and the only stably rotating reentry waves are those anchored to some local heterogeneities [14–17]. While migrating (or drifting), rotating wave practically inevitably hits the border of the excitable tissue and ceases to exist [18–20]. In the real heart it may be the boundary of vessels, unexcitable connective tissue, etc., in the experiments with two-dimensional tissue culture, it is the boundary of the sample.

Thus, the main role of the antiarrhythmia drug is on the first step, to destabilize (unpin) the anchored spiral wave and convert it to the free rotating one, which at the second step, quickly drifts away. The unpinning would

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happen while decreasing the excitability and/or making spiral wave core size significantly larger than the size of the anchor heterogeneity [10, 21, 22]. However the result would be unclear if the effective size of the anchor heterogeneity would also rise under the decreasing excitability, as it could happen, for example, for an obstacle with the sink-like boundary conditions. This case we address in the present study.

Experimental setup. Primary cell cultures of neonatal rat ventricular myocytes were prepared as described in [23]. The cells were seeded into 22-mm diameter glass coverslips coated with fibronectin (16.7 μg/ml) at a cell density of $2.6 \cdot 10^3$ cells/mm^2.

The modulation of cardiomyocyte excitability was done by illumination, with the aid of AzoTAB and photo-control setup [24], Fig. 1.

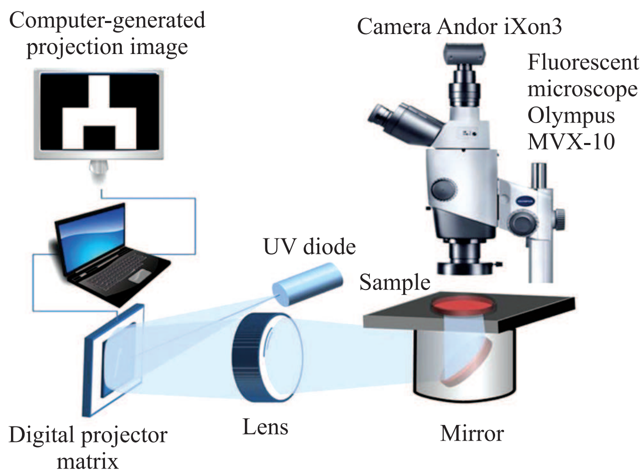

Fig. 1. (Color online) The experimental setup. The variable intensity UV light (365 nm) controlling the excitability of the tissue layer was projected below through the custom made illumination system. The constant intensity light exciting fluorescence and turning AzoTAB to *trans*-form was projected uniformly from top on the sample through the optical system of MVX-10 (not shown). The fluorescence intensity of the Ca-dye was monitored by the Andor camera

AzoTAB (azobenzene trimethylammonium bromide) was reported recently as a tool for controlling of excitation in cultured cardiomyocytes by light irradiation with the proper wavelength [24, 25]. In the dark, thermally relaxed, or obtained by irradiation with blue light (> 440 nm) *trans*-isomer of AzoTAB reversibly reduces the occurrence of spontaneous activity, as well as decreases the speed of propagating excitation waves, up to their total suppression. The excitation waves may be reactivated after irradiation with near-UV light ($\sim$ 365 nm), which produces prevalence of *cis*-isomer of AzoTAB. The cycle of suppression – reactivation of the excitation waves may be repeated several times without observable damage to the tissue culture. Simultaneous irradiation by light with two wavelengths allows fine-tuning the excitability in the tissue culture by varying the intensities ratio [24].

In our experiments, 100 mM AzoTAB was added to the sample incubating medium during the experiment and the excitability of cells controlled by changing the intensity of UV irradiation (at the wavelength 365 nm). The excitation waves in cardiac tissue caused calcium upstroke and, accordingly were monitored and recorded with the aid of Ca^{2+} sensible dye Fluo-4 AM (Invitrogen) [23]. Images were acquired using a high-speed imaging setup (an Olympus MVX-10 Microscope equipped with EM-CCD camera, Andor iXon3) at 68 frames per second. Image processing was performed in Image J. To induce an excitation wave in some experiments we used electrical stimulation. The rectangular pulses with 1.5 to 4.0 V amplitudes and 20 ms duration were delivered via a 1 mm non-polarizing platinum electrode. The anode was placed around the glass, along the border of a Petri dish. Spiral waves were generated by applying overdrive pacing [23].

Results. First, the setup was calibrated by measuring the dependence of excitation wave speed on the intensity of UV irradiation. At the lower UV intensities, the *trans*-isomer of AzoTAB prevails lowering the excitability of the cardiac cells [25]. The plot of the dependence of wave speed on the irradiation intensity is shown on Fig. 2. At UV intensities greater than 2 mW/cm^2 the cell culture was excitable and conducted excitation waves.

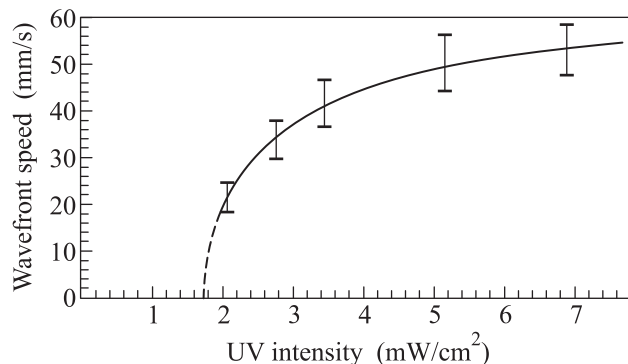

Fig. 2. The dependence of wavefront speed on the intensity of UV irradiation. At UV intensity less than 2 mW/cm^2 cell culture was unexcitable

By decreasing the intensity of the UV irradiation we could efficiently decrease the excitability of the tissue sample, thus simulating the addition of the antiarrhythmic drug.

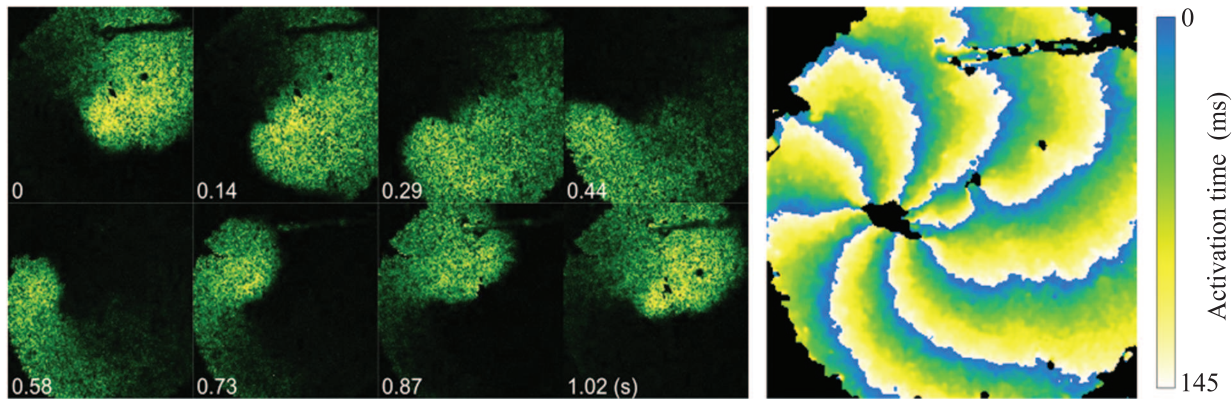

Fig. 3. (Color online) Spiral wave pinned to obstacle. Left – time sequence of spiral wave rotation. Right – activation map. The shadow on the top of the images is due to excitation electrode

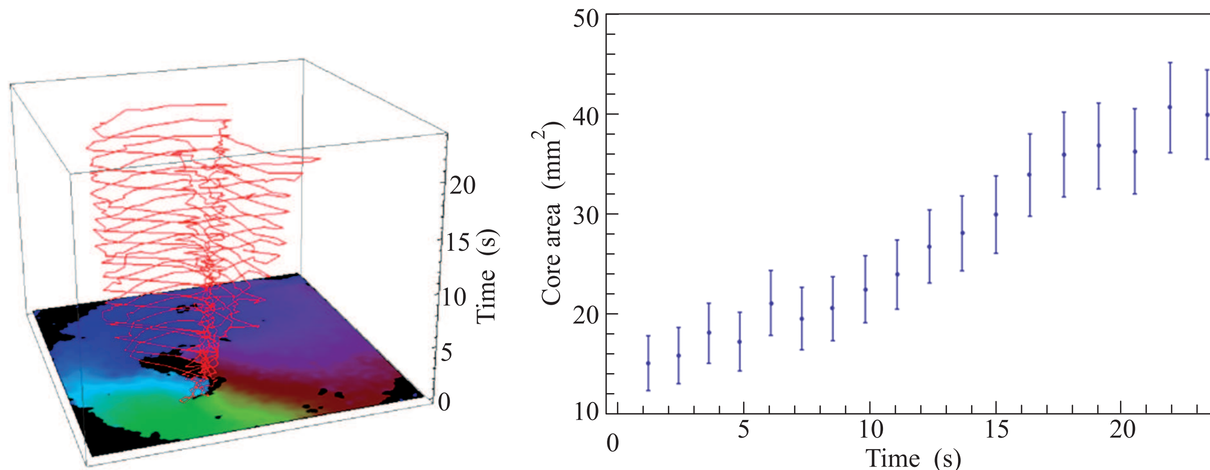

Fig. 4. (Color online) The growth of spiral wave core after the decrease UV irradiation. Left – trajectory of the spiral wave tip. Right – plot of the core area versus time

The experiment on rotating wave termination was conducted as follows. First, the area free of cells was created in the layer of cardiomyocytes by careful removing cells with the sharp tip of the syringe needle controlled by the micromanipulator. This area, typically, about 2–3 mm in diameter, served as an obstacle, anchoring rotating wave. Then, the initial UV irradiation intensity was set at $7\,\mathrm{mW/cm^2}$, and rotating wave was obtained by the overdrive pacing. In about 50 % cases formed reentry spontaneously attached to the obstacle, if not – it was shifted by the pacing [23] until the attachment happened. The resulting spiral wave is shown on Fig. 3. The obtained rotating wave was observed for about 10 min to make sure it is stable. After that, the UV intensity was decreased to $2.5\,\mathrm{mW/cm^2}$ and rotation wave dynamics was recorded until it disappeared. At the end, after termination of the spiral wave, the cell culture excitability was tested by the electrical initiation of the excitation, to make sure that the termination resulted from the spiral wave dynamics under the decrease excitability and not from the total excitability suppression.

After the decrease of UV intensity we observed the growth of the spiral wave core – the region the spiral wave tip is moving around. Fig. 4 shows the trajectory of the spiral wave tip as well as the plot of the growth of core area versus time.

The growth of the spiral wave core has been usually asymmetrical, most probably due to the natural heterogeneities of the cell culture. Typically, growing core approached on one side the peripheral heterogeneity or the edge of the sample forming distinct narrowing in the excitation propagation path. Thus, the “virtual” isthmus in the rotating wave pathway formed (Fig. 5). When the isthmus became critically narrow, the failure of wave propagation was observed, similar to described

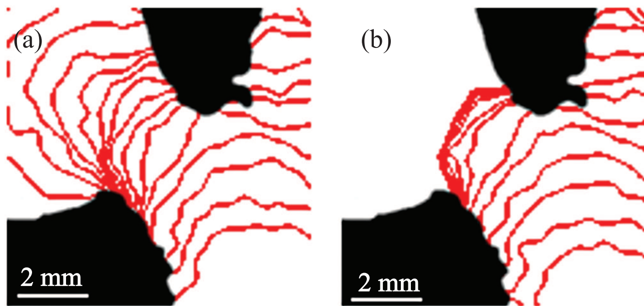

Fig. 5. (Color online) The death of the wave in the virtual isthmus. (a) – Sequence of wavefronts shows how the spiral wave passed through the virtual isthmus during the next to last turn. (b) – Sequence of fronts during the propagation failure

earlier [26, 27], resulting from the so called source-sink mismatch. In other words, the size of the segment of the propagating wave front becomes smaller than the minimal size of the excitation area from which excitation wave may propagate (similar to the critical nucleation in phase transitions) [1].

Thus, we have shown, that reduced excitability of the cardiac monolayer with pinned and stable spiral wave, first leads to the growth of the spiral core, and then to the termination of the spiral wave due to the critical isthmus formation between the core and the borders of the sample. Earlier, it was shown that a preset narrow isthmus, obtained by cutting tissue layer as in [26], cannot block excitation in a healthy and uninhibited cardiac tissue, while when decreasing the excitability of the tissue (by blocking some portion of membrane voltage-gated sodium channels) it is possible to observe the conduction block even at relatively macroscopic scales of the isthmus size (tens of millimeters and millimeters). Our data are in complete agreement with these earlier findings: we observe isthmus formation and the conduction block only when the excitability of the tissue layer is essentially reduced.

Table 1

Sample	Experiments	Termination success	IS (isthmus scenario)
AM6	2	1	–
AM9	9	6	–
AM11	9	1	–
AM12	5	3	3
AM14	4	3	3
Total	22	14 (64 %)	6 (27 %)

The last question is how frequently the above scenario could be observed? In our case, the "isthmus scenario" (IS) of spiral wave termination was observed in 6 experiments out of 22, performed on 6 different samples of tissue culture, while in 14 cases in total, rotating wave was successfully terminated, Table 1. We should note that the sample exhibited IS had one thing in common: the core of the rotating wave was placed asymmetrically, closer to the one side of the sample.

Conclusions. In the present study, we show the existence of a new mechanism leading to the rotating wave termination: origination of the virtual isthmus under the decreasing excitability of the cardiac tissue. This scenario was observed in approximately 27 % of cases (42cases) and its likelihood increased if the core of rotating wave was placed closer to the border of the tissue.

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