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Sustained spiral calcium wave patterns in rat ventricular myocytes

Michal Cagalinec [a] *, Dusan Chorvat Jr. [b], Anton Mateasik [b], Ljuba Bacharova [b]

[a] Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina, Bratislava, Slovakia
[b] International Laser Center, Ilkovicova 3, Bratislava, Slovakia

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Ca^{2+} waves, propagating in non-stimulated isolated cardiac myocytes under physiological conditions, are described as planar waves traveling along the longitudinal cell axis [1]. Other patterns of Ca^{2+} wave propagation – circular or spiral Ca^{2+} waves [2, 3] – have been observed in physiological conditions as well. Lipp & Niggli, 1993 [2], Ishida *et al.*, 1999 [3] and Engel *et al.*, 1994 [4] have described spiral calcium waves as individual events. We demonstrate hereby existence of repetitive spiral calcium wave patterns in adult rat ventricular cardiomyocytes.

The work was done on 20-week old Wistar rats. Myocytes were isolated with retrograde perfusion of the heart with proteolytic enzymes [5] and loaded with calcium indicator Fluo-3/AM (1 μmol/l). The fluorescence emission was recorded with laser scanning confocal microscope LSM 510 Meta, Zeiss (frame rate from 8 to 14 frames/s, excitation/emission 488/515 nm), equipped with C-Apochromat 40 × / NA = 1.2 water immersion objective.

We observed both planar, circular (Fig. 1A, B) and spiral calcium waves (Fig. 1C) in the same myocyte. Figure 1C shows four subsequent spiral waves, demonstrating repetitive propagation of the spiral wave burning from the same origin. We observed an average of 8 spiral waves per cell, ranged 1–49. The median burning time of the spiral wave was 0.9 s, ranged from 0.4 to 13.7 s (n = 133 spiral calcium waves, 12 cells). The percentage of the spiral waves duration with respect to the entire recording time (ranged from 131 to 227 seconds, n = 12 cells) was 10.8 %, ranged from 0.6 to 26.5 %. The repetitive spiral waves (defined as waves progressed further after reaching its initial position) were detected in 56 cases from all spiral waves observed (n = 133). The spiral wave pattern formation was a reversible process, as we observed also return to planar-type burning. No blebs or hypercontractures were observed when the recording of the cell was finished.

In contrast to the smaller amplitude of the spiral waves described by Ishida *et al.*, 1999 [3], we observed no difference in relative amplitudes of the calcium waves: planar wave preceding the spiral wave (4.1, range 2.4–6.1), spiral (4.0, range 2.4–6.2) and subsequent planar wave (4.2, range 2.2–5.8, n = 9 waves analyzed) were not significantly different. Moreover, we observed no decrease in the amplitude of the repetitive wave when compared the starting and ending point. This contributes to the stabilization of the spiral burning and allows its long-time propagation.

We suggest that the repetitive spiral waves can be considered as a temporary equilibrated state in myocyte calcium wave spreading. As calcium waves are capable of traversing to the adjacent cells *via* gap

*Correspondence to: Michal CAGALINEC
Faculty of Mathematics, Physics and Informatics,
Comenius University, Mlynska dolina, 842 48 Bratislava, Slovakia.
Tel.: ++421 2 654 21 575
Fax: ++421 2 654 23 244
E-mail: misoc@ilc.sk

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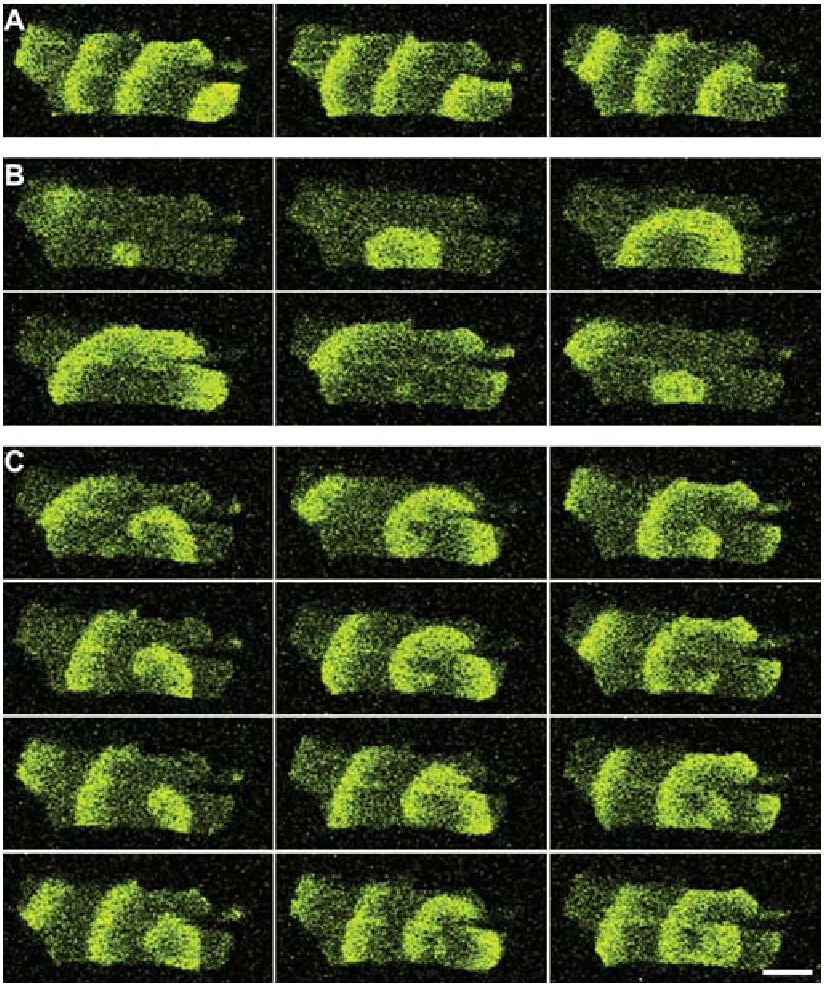

Fig. 1 Recording of the (**A**) planar, (**B**) circular and (**C**) spiral calcium waves in an isolated rat left ventricular myocyte after staining with calcium indicator Fluo-3/AM imaged with confocal laser scanning microscopy. Frame interval: 113 ms. Scale bar: 20 μm.

junctions [4], a cell with repetitive spiral bursts may negatively impact functioning of the adjacent myocytes.

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