Universiteit Leiden
The Netherlands

Optogenetic investigation of cardiac arrhythmia mechanisms

Feola, I.

Author: Feola, I.
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Appendix 2

RESPONSE BY FEOLA *ET AL* TO LETTER REGARDING ARTICLE, "LOCALIZED OPTOGENETIC TARGETING OF ROTORS IN ATRIAL CARDIOMYOCYTE MONOLAYERS"

Iolanda Feola[1], MSc; Linda Volkers[1], PhD;
Rupamanjari Majumder[1], PhD; Alexander Teplenin[1], MSc;
Martin J. Schalij[1], MD, PhD; Alexander V. Panfilov[1,2], PhD;
Antoine A.F. de Vries[1], PhD; Daniël A. Pijnappels[1], PhD.

[1] Laboratory of Experimental Cardiology, Department of Cardiology, Heart Lung Center Leiden; Leiden University Medical Center, the Netherlands.
[2] Department of Physics and Astronomy, Ghent University, Ghent, Belgium.

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IN RESPONSE

We thank Houston *et al* for their interest in our study. In their letter, they raise the question whether the rotors and accompanied spiral waves observed in our study represent microreentrant circuits anchored to lines of conduction block/slowing (*i.e.* anatomical reentry), instead of reentrant activity around an unexcited, yet excitable core region (*i.e.* functional reentry). Their comment is based on a movie published on their website, showing high-resolution mapping, in an HL-1 culture, of reentrant activity that seems anchored to microregions of conduction abnormalities. Although appraisal of these data is difficult without a detailed description of methods and results, we still would like to add our thoughts about the distinction between functional and anatomical reentry on a cellular level. Heterogeneities, such as gradients in excitability, refractoriness, and nonhomogeneous distribution of cardiac fibroblasts may have a destabilizing effect on functional reentry by contributing to drift, meandering, and breakup of spiral waves.[1,2] Furthermore, reentrant activity may undergo alternating transitions between functional and anatomical reentry by pinning to or unpinning from an anatomical obstruction.[3–6] These transitions depend on several factors (*e.g.* size of the obstacle and tissue excitability)[5,6] and highlight the difficulty to determine which type of reentry underlies arrhythmic activity at a given location and time. To deal with the complex dynamic nature of reentrant activity, we used confluent monolayers of optogenetically modified neonatal rat atrial cardiomyocytes and patterned illumination to induce and target a single stable rotor. The resolution of optical voltage mapping allowed us to visualize electrical activity in the entire monolayer and to investigate the effects of rotor targeting by light-controlled induction of conduction blocks once a rotor had been established at a predefined location through light-based cross-field stimulation.[7]

Although any involvement of microreentry cannot be ruled out completely, we found evidence that rotor stability was related to the absence of the aforementioned heterogeneities. Our cultures showed uniform and fast activation with little dispersion in action potential duration on 1-Hz electric stimulation. Moreover, by changing the timing and location of the second light stimulus, stable rotors at different, yet predefined locations could be induced. Finally, we found spiral wave drifting by creating a line of block away from the core region, followed by post-illumination rotor stabilization. Such drifting was also evident in other cases where light-induced conduction block did not cause effective reentry termination. The reentrant wave described by Houston *et al* was able to follow a path of block, despite the poor excitability and coupling of the medium, because of the permanent expression of the oncogenic SV40-LT-ag (simian virus 40–large T-antigen) in HL-1 cells (*e.g.* conduction velocity of 4.1±0.1 cm/s)[8,9] and the sharp corners (*e.g.* U-turns) in the trajectory. These conditions would normally favor unpinning and subsequent transition into functional reentry, sparking curiosity about the underlying biophysical mechanism(s).[3,4]

Clinically, it remains to be determined whether and how a strict distinction between functional and anatomical reentry, on a cellular level, could further improve the treatment of reentry-driven arrhythmias.[10] We encourage the authors to publish an in-depth study on microreentry dynamics in HL-1 cultures.

DISCLOSURES

None.

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REFERENCES

1. Pertsov AM, Davidenko JM, Salomonsz R, Baxter WT, Jalife J. Spiral waves of excitation underlie reentrant activity in isolated cardiac muscle. Circ Res.1993;72:631–650.

2. Fast VG, Kléber AG. Role of wavefront curvature in propagation of cardiac impulse. Cardiovasc Res. 1997;33:258–271.

3. Pandit SV, Jalife J. Rotors and the dynamics of cardiac fibrillation. Circ Res. 2013;112:849–862.

4. Cabo C, Pertsov AM, Davidenko JM, Baxter WT, Gray RA, Jalife J. Vortex shedding as a precursor of turbulent electrical activity in cardiac muscle. Biophys J. 1996;70:1105–1111.

5. Lim ZY, Maskara B, Aguel F, Emokpae R Jr, Tung L. Spiral wave attachment to millimeter-sized obstacles. Circulation. 2006;114:2113–2121.

6. Ikeda T, Yashima M, Uchida T, Hough D, Fishbein MC, Mandel WJ, Chen PS, Karagueuzian HS. Attachment of meandering reentrant wave fronts to anatomic obstacles in the atrium. Role of the obstacle size. Circ Res. 1997;81:753–764.

7. Feola I, Volkers L, Majumder R, Teplenin A, Schalij MJ, Panfilov AV, de Vries AAF, Pijnappels DA. Localized optogenetic targeting of rotors in atrial cardiomyocyte monolayers. Circ Arrhythm Electrophysiol. 2017;10:e005591.

8. Dias P, Desplantez T, El-Harasis MA, Chowdhury RA, Ullrich ND, Cabestrero de Diego A, Peters NS, Severs NJ, MacLeod KT, Dupont E. Characterisation of connexin expression and electrophysiological properties in stable clones of the HL-1 myocyte cell line. PLoS One. 2014;9:e90266.

9. Claycomb WC, Lanson NA Jr, Stallworth BS, Egeland DB, Delcarpio JB, Bahinski A, Izzo NJ Jr. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. Proc Natl Acad Sci USA. 1998;95:2979–2984.

10. Nattel S, Xiong F, Aguilar M. Demystifying rotors and their place in clinical translation of atrial fibrillation mechanisms. Nat Rev Cardiol. 2017;14:509–520.

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