An Innovative Toolkit to Investigate the Complex Mechanisms of Cardiac Arrhythmias

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Editorial

Arrhythmias are very common and clinically highly relevant; however, current treatment remains largely insufficient since most therapeutic strategies are only focusing on symptoms rather than targeting the causal mechanisms of arrhythmias. Thus, a better understanding of the complex disease mechanisms is urgently needed to develop novel, innovative, and causal treatments.

The aim of the current JoVE methods collection is to provide an unbiased and diverse set of protocols allowing to comprehensively investigate various aspects of arrhythmogenesis.

An altered calcium homeostasis is one of the hallmarks of arrhythmogenesis but remains challenging to investigate since multiple aspects need to be considered, each requiring specific techniques to study[1,2]. In this methods collection, Tomsits et al. provide a protocol that allows the isolation of atrial and ventricular cardiomyocytes from the same mouse and the study of both the L-type calcium current and calcium transients simultaneously[3]. Thus, this protocol enables researchers to investigate calcium disturbances more globally and to reduce animal numbers at the same time, which represents a significant scientific and ethical improvement.

Nevertheless, patching isolated cells remain complex, labor-intensive, and low-throughput experiments[4]. To address this, Seibertz et al. provide a protocol using a voltage-sensitive dye to measure action potentials in human induced pluripotent stem cell-derived cardiomyocytes[5]. As the technique does not need extensive training (as needed for patch clamping) and can be applied by using a relatively low-cost experimental setup, this protocol has broad applications.

Besides cardiomyocytes, other cell types (e.g., fibroblasts or macrophages) also play an important role in electrophysiology[6,7,8]. To investigate cell-type specific effects in the conduction system, Xia et al. provide a step-by-step guide to identify the sinus node (SAN) and the atrioventricular node (AVN) in mice[9,10] followed by microdissection and fluorescent activated cell sorting (FACS) to isolate and study macrophages[9] or by whole-mount

immunofluorescence imaging. This allows the identification of different cell types, the evaluation of the three-dimensional structure, and the study of cell-type specific protein expression within the SAN/AVN[10].

Furthermore, multi-electrode array (MEA) recordings can be performed on such whole-mount preparations as presented by Kumar et al.[11]. This method is an excellent addition to the currently used techniques as it is relatively fast and simple and also provides superior spatial resolution. It allows pharmacologic interventions and can be easily applied to other specific regions of the heart (e.g., the AVN).

The autonomic nervous system plays an essential role in electrophysiology[12,13,14] and there exists treatment strategies, such as sympathectomy[15,16,17,18] or ablation of cardiac ganglia[19]. Nevertheless, the mechanistic basis is still largely unknown, especially since anatomic structures of the autonomic nervous system are not easily accessible in mice. In this issue, Scherschel et al. provide a protocol that allows the identification and dissection of the stellate ganglia in mice to study gene and protein expressions in various cell types[20]. This will allow the study of novel aspects of autonomic remodeling.

Studying electrophysiology at a cellular level is critical to study the fundamental mechanisms. However, *in vivo* studies in animal models are still needed to validate cellular findings and to confirm (patho-)physiologic relevance of identified target structures[21]. Mice are most widely used, but especially the generation of transgenic mice is complex. In this issue, Shimura et al. present a protocol using CRISPR-Cas9 gene editing to specifically study the complex regulatory roles of truncated proteins and their effects *in vivo*. For this, they generated a transgenic mouse model harboring a single point mutation in the GJA1 gene encoding connexin-43. This results in a regular expression of the full-length Cx43 but a reduced expression of a truncated Cx43[22].

One of the major limitations of *in vivo* studies is the need to anesthetize animals during experiments, which may affect various electrophysiologic parameters and may thus not represent the 'true' situation[23,24]. Telemetric devices, on the other hand, allow the study of awake and freely moving mice over long periods of several weeks to months[25,26]. Rötzer et al. describe how to implant telemetric devices to record heart rate and blood pressure simultaneously in mice, which specifically allows investigation of the autonomous nervous system and the baroreflex sensitivity[27].

Although telemetry devices are very powerful tools, a major challenge remains: how to analyze these large datasets. To address this issue, Tomsits et al. develop a protocol to analyze telemetric ECG recordings in a standardized semi-automatic manner[28]. Following their protocol, researchers can obtain fundamental ECG parameters (e.g., heart rate or PR interval) and screen for individually defined arrhythmias (e.g., pauses, brady-/tachycardia). Importantly, they also discuss challenges and pitfalls of recording and analyzing telemetric ECGs, including some guidance to improve signal quality.

Newly developed medical devices, such as prosthetic valves, are thoroughly tested in animal models prior to their application in human patients[29]. However, studies in healthy animals may not perfectly translate to human patients, especially those who have undergone a surgery previously[30,31]. To overcome such limitations, Grab et al. provide a protocol for 3D printing of individual, patient-specific heart models[32]. Their models are elastic and resemble biomechanical properties as well as hemodynamic characteristics similar to human patients; these models allow

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the teaching of physicians, the testing of novel interventional procedures or devices, and the planning of individualized treatment strategies, especially in patients with complex anatomy.

The protocols provided in this JoVE methods collection will support many researchers in studying electrophysiology and arrhythmias. The major strength of this collection is that it provides improvements and innovations for traditional technologies but also demonstrates novel approaches to study various aspects of arrhythmogenesis. This collection will thus help to broaden our understanding of these complex diseases and ultimately to develop innovative therapies for patients with arrhythmias.

Disclosures

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