Gary Tse*, Sheung Ting Wong, Vivian Tse and Jie Ming Yeo

Monophasic action potential recordings: which is the recording electrode?

Abstract: The aim of this article is to provide an overview of current debate on the monophasic action potential (MAP) recording technique, specifically whether the depolarizing or the reference electrode is responsible for recording the MAP waveform. A literature search was made using key words including monophasic action potential, MAP, electrophysiological basis, recording electrode, depolarizing electrode, contact electrode, indifferent electrode, and reference electrode. References from articles were screened for additional relevant papers. Articles published by the different experimental groups claim that depolarizing electrode, but not reference electrode, records MAPs from the myocardium. This can be more accurately described when considering biophysical theory, which states that MAP is a bipolar signal with contributions from not only the depolarizing electrode but also remote activation at the reference electrode. It is not meaningful to claim that one is the recording electrode because potential differences must be measured between two points in space. Nevertheless, the MAP technique is useful for assessing the local electrical activity of the myocardium in contact with the depolarizing electrode. It is important to have the recording electrode in close proximity with the reference electrode to minimize contamination from far-field signals.

Keywords: cardiac electrophysiology; contact electrode; indifferent electrode; monophasic action potential; recording electrode.

*Corresponding author: Dr. Gary Tse, School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, SAR, P.R. China, Phone: +852 53322823, Fax: +852 2817 0857, E-mail: gary.tse@doctors.org.uk
Sheung Ting Wong and Jie Ming Yeo: School of Medicine, Imperial College London, London, UK
Vivian Tse: Department of Physiology, McGill University, Montreal, Quebec, Canada
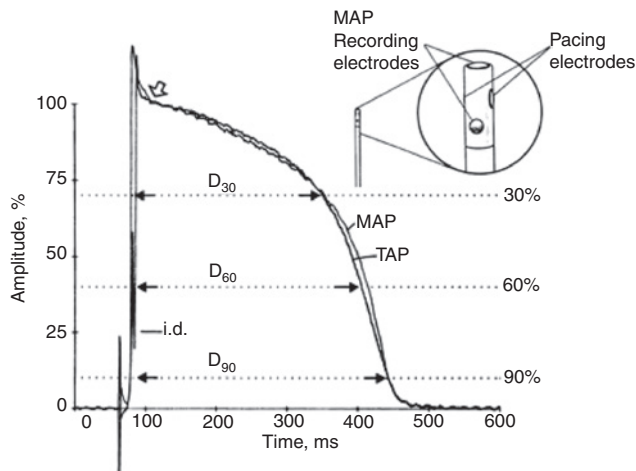
Introduction

In clinical cardiac electrophysiology, there are two main methods for obtaining electrical recordings from the heart: monophasic action potential (MAP) and electrogram recordings, which include bipolar electrograms (BEGs) and surface electrocardiograms (ECGs). Of these, the MAP technique has been the most controversial regarding the genesis of its waveforms. This review aims to provide an introduction to MAP recordings and summarize current understanding on its underlying biophysics.

History of MAP recordings

MAPs are extracellular recordings that reflect the time courses of transmembrane action potentials with a high-fidelity correlation [1–4] (Figure 1, reproduced from Moore and Franz, with permission [4]). The first MAP was obtained from experiments that recorded the potentials generated by frog hearts using suction electrodes [5, 6]. These authors placed an electrode on the epicardial surface and another at an injury site, which was produced by cutting into the myocardium. Transitory monophasic potentials with only one polarity were recorded. Before this, multiphasic potentials with both positive and negative polarities had been recorded by electrodes. It was believed that monophasic potentials could only be recorded by tissue injury, and the resulting currents were therefore termed monophasic injury currents [7]. The waveform of the MAPs they recorded was very similar to that of the transmembrane action potentials recorded using intracellular microelectrodes [8].

Later, the suction electrode technique was developed [9] and subsequently used to record MAPs from the ventricular endocardium in situ [10]. This permitted measurement of MAPs without requiring specific injury, as a lesion was already produced by the suction electrode. This technique was later improved and refined and extended to record MAPs from the atrium [11–13]. An alternative technique involved injection of potassium to inactivate the myocardium, which created tissue injury that resulted in

Figure 1: Simultaneous recordings of intracellular transmembrane action potential (TAP) and extracellular monophasic action potential (MAP) show high-fidelity correlation.
Repolarization is labeled at 30%, 60%, and 90% of the return from plateau to resting potential. Open arrow shows 0% repolarization. The distal tip records the MAP waveform, while the close proximal electrode (5 mm from the tip) eliminates far-field potential. Pacing electrodes are positioned orthogonally between tip and reference MAP electrodes. Figure and figure legend reproduced with permission from Moore and Franz [4].

the recording of MAPs [14]. This method provides similar information to the contact method described below [15].

The contact electrode technique was the first non-traumatic method that allowed the recording of MAPs, and its use simply involved pressing the electrode against the surface of the toad ventricular epicardium [16]. This was later adapted for use in clinical practice to obtain MAPs from both the epicardium and endocardium of human hearts [17, 18], with catheters and probes developed later [19]. In clinical electrophysiological studies, the stimulating and recording electrodes are combined in a single catheter, whereas in experimental animal models, the electrodes are physically separated on opposite sides of the heart [20]. An advantage of the contact method over the suction method is that it produces little myocardial injury, meaning that MAPs remain stable and can be recorded over several hours. This would in turn allow the electrophysiologist to assess the effects of anti-arrhythmic agents or basic cycle length changes on local myocardial depolarization and repolarization over an extended period of time [18].

Franz's contact MAP method

Traditionally, the explanation of the MAP waveforms offered by Franz is as follows [21]. When the MAP

electrode is pressed against the myocardium, it causes depolarization in the group of cells under the electrode to –20 mV, with respect to the extracellular reference potential. The sodium channels are inactivated at these depolarized potentials. These cells are therefore unexcitable and cannot participate in the depolarization and repolarization processes that occur in the adjacent, normal myocardium. The potential of the depolarized cells is clamped at –20 mV, whereas that of the adjacent normal cells can vary. An electrical gradient is established between the normal and depolarized myocardium, producing a local current flow. In the resting state, this gradient results in a source current emerging from the normal cells and a sink current arriving at the depolarized cells. This sink current produces a negative electrical field that is proportional to the current amplitude, which depends on the voltage gradient and the number of cells contributing to the interface between the depolarized cells and normal cells. When the myocardium is activated, normal cells undergo depolarization, with the membrane potential reaching +30 mV. In contrast, the already depolarized cells under the MAP electrode remain refractory and have a potential of –20 mV. This means the previous current sink is now the current source, producing an electric field of opposite polarity. Thus, the MAP recording reflects the time course of the voltage of the normal cells that surround the volume of cells depolarized by the pressure of the contact electrode. Taken together, both the depolarized cells with a clamped membrane potential and the adjacent normal and active cells contribute to the genesis of the MAP.

Controversies and bringing it all together

There has been an ongoing heated debate regarding the genesis of the MAP. The main controversy is whether the electrode in contact with the injured (depolarized) myocardium, or the electrode in contact with uninjured myocardium, is the recording electrode [1, 18]. On the one hand, Schaefer argued that MAPs can only be obtained when there is an injury present, and therefore, the electrode in contact with the injured myocardium is the recording electrode [22, 23]. On the other hand, others contended that injured cells are electrically inactive and, therefore, the signals recorded must originate from the uninjured cells [24–26]. Now, it has been shown that both the injured and the normal cells contribute to the genesis of MAPs, as discussed below [27].

Based on the experiments performed on frog hearts using the suction method, Schütz hypothesized that the MAP recorded the voltage drop between the electrode in contact with the injured myocardium and the electrode in contact with the uninjured myocardium and that there is a flow of leak current between the two sites [7, 9]. In such a scheme, MAP results from a voltage source with the voltage generators in parallel. In addition, the extracellular resistance is proportional to the MAP amplitude. Thus, if the extracellular resistance increases, which occurs when the surface of the heart is dried, then the MAP amplitude is increased. This was indeed observed. Short-circuiting between the injured myocardium and the extracellular space is assumed to be minimal by a tight seal between the recording electrode and the surrounding myocardial tissue.

However, Franz showed that Schultz's hypothesis only applied to MAPs recorded using the suction electrode method but not those recorded using the contact method [27]. Instead, he proposed that the contact electrode MAP results from a current source and is governed by volume conductor theory with voltage generators in series, for the following reasons. Firstly, in clinical practice, the contact electrode obtains endocardial MAPs while being surrounded by blood in the chamber cavity and has no tight seal surrounding the tissue, suggesting that extracellular resistance did not influence the MAP amplitude. Secondly, the number of cells (voltage generators) contributing to the MAP appears to be important because increased pressure between the tip electrode and the myocardium increases the MAP amplitude [18]. Finally, MAPs have greater amplitudes when they are recorded from the ventricular myocardium than those from the atrial myocardium, or when recorded from larger hearts compared to smaller hearts. This suggests that the thickness of the myocardial wall beneath the tip electrode is an important determinant of MAP amplitude.

Antzelevitch's group proposed an alternative hypothesis stating that the depolarizing electrode acts as a stationary ground and the recording electrode is the distant, indifferent electrode with a wide field of view [28, 29]. Other investigators pointed out that the contact and indifferent electrodes were placed on the same piece of ventricular myocardium and that the MAP waveforms recorded were in fact not MAPs but hybrid signals with the MAP waveform originating from the contact electrode with superimposition of the repolarizing T-wave from the unipolar electrogram [30, 31].

Potential differences measured between two sites are independent of the paths taken. As previously pointed out by Vigmond but largely overlooked by the experimental

groups, Antzelevitch simply followed the intracellular core conductor route, whereas Franz took the extracellular route [32]. Nevertheless, the answer as to which is the recording electrode was resolved experimentally in 2006 [33]. When the depolarizing electrode was placed on the myocardium, local activation was recorded with a superimposed electrogram due to remote activation from the reference electrode [33]. When the depolarizing electrode was placed on the atrium in a dog with AV block, the MAP recorded was an atrial MAP, which was distinct from the ventricular electrogram. Similar findings were observed in separate experiments conducted by Zhang and Mazgalev [34].

Practical considerations and limitations

Clearly, MAP recordings are extracellular potentials obtained from groups of cells, but they closely track the time courses of the transcellular action potential obtained from single cells with a good fidelity. The MAP technique has been extensively used in a clinical setting and experimentally for examining arrhythmic behavior using animal systems. Figure 2 illustrates an experimental rig that has been used to investigate electrophysiological properties in mouse hearts. The stimulating and recording electrodes are on opposite sides of the heart, which was secured to the apparatus using a micro-aneurysm clip. Here, oxygenated perfusing solution was delivered to the aorta through the cannula at a constant flow rate in the Langendorff-perfusion mode. Using this experimental system, stable MAP recordings can be obtained from different epicardial sites over a prolonged period of time. Note that in mice, MAP waveforms are triangular and without a plateau phase found in humans (Figure 3). This MAP technique is sufficiently sensitive to detect alterations in conduction velocity (CV), action potential duration (APD), and
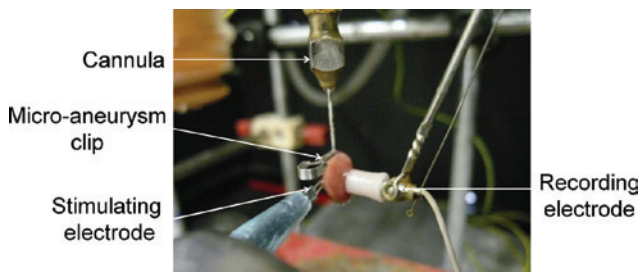

Figure 2: An experimental rig used for investigating electrophysiological properties in Langendorff-perfused mouse hearts.

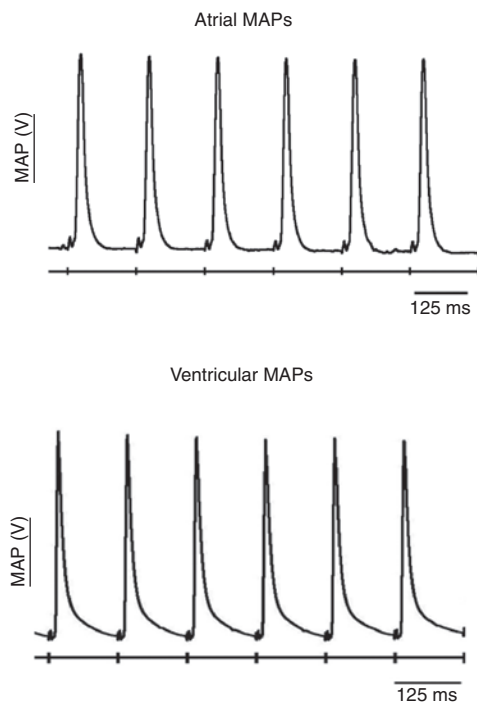## Atrial MAPs

Ventricular MAPs

Figure 3: Examples of atrial (top) and ventricular (bottom) monophasic action potential recordings obtained from mouse hearts.

effective refractory period (ERP) with high reproducibility [20, 35]. Moreover, the recording of MAP waveforms in conjunction with using a number of different stimulation protocols, such as extrasystolic and dynamic pacing, has further improved our understanding of cardiac dynamics, particularly how repolarization alternans leads to wavebreak and re-entrant arrhythmias [36–41]. For example, restitution-dependent and restitution-independent mechanisms for the production of the alternans can be examined [42–45].

However, there are several limitations of the MAP method that we must bear in mind when interpreting MAP data. The absolute amplitude of the action potential or the maximum upstroke velocity (V_{max}) cannot be determined, but information from relative changes is helpful because decreasing amplitude would reflect impairment of sodium channel function [45]. The baseline amplitude of the MAP is unknown, but it will always be less than that of the cellular action potential, because the peak is smoothed by averaging the potentials, as pointed out previously [46]. Early after-depolarization (EAD) events have been recorded by MAP electrodes [47, 48], but movement artifacts can also generate EAD-like activity [15, 21]. Furthermore, an initial hyperpolarizing notch can be observed immediately before the upstroke of a MAP, which can be related to the depth of the tissue [46]. A second notch,

occurring after the end of depolarization, has erroneously been attributed to the transient outward current (I_{to}) when in fact, it represents an intrinsic deflection that could be observed in surface electrogram recordings before contact pressure is applied [21]. Another disadvantage of the MAP method is the relatively localized area that is covered by the recording electrode. Novel methods such as non-contact mapping reconstructs signals from unipolar electrograms over a wide area and therefore allows global view of repolarization characteristics over the entire endocardial surface in a clinical setting [49]. Moreover, because of the size of the MAP electrode relative to the small size of mouse hearts, it is difficult to obtain and detect differences in depolarization or repolarization properties of neighboring regions. In this case, optical mapping using a microelectrode array would be a better technique [50]. Furthermore, the MAP electrode would cover the entire mouse atrial surface for studying atrial electrophysiological properties, but MAPs have been successfully recorded from the mouse atria [51]. Other methods such as bipolar electrograms can also be used to determine repolarization properties [51, 52].

Some questions on the MAP technique need to be clarified. Firstly, gap junction uncoupling is predicted to decrease both MAP amplitude and V_{max} [53]. Based on Vigmond's theory, abolition of intercellular communication by complete uncoupling of gap junctions should abolish MAP recordings [46]. It is unclear as to the minimum degree of intercellular coupling needed for obtaining MAP recordings and the effects of impaired gap junction function on the MAP waveform. Experimental studies in mouse hearts have demonstrated stable MAP recordings obtained from myocardium when gap junctions were uncoupled by the agent heptanol at a concentration of 0.1 mM [20, 35, 51]. At a higher concentration of 2 mM, conduction velocity was progressively reduced and MAP amplitudes were decreased, eventually leading to conduction block and abolition of the MAP waveform. However, it is unclear whether this reflected dose-dependent uncoupling of gap junctions or additional inhibition of sodium channels. Secondly, Franz's theory states that the region under the contact electrode contributes actively by acting as either a current source or sink [21], yet theoretical work argued that the myocardium beneath the depolarizing electrode does not produce a net current [46]. Readers who are interested in the biophysical theory underlying MAP recordings should be directed to excellent review articles here [32, 46].

In summary, MAP is a bipolar signal with contributions from not only the depolarizing electrode but also remote activation at the reference electrode [32]. This

highlights the importance of having both the depolarizing and reference electrode in close proximity to each other. This is crucial in arrhythmogenic conditions such as Long QT and Brugada syndromes, where there are regional differences in depolarization and repolarization properties [44].

References

1. Hoffman BF, Cranefield PF, Lepeschkin E, Surawicz B, Herrlich HC. Comparison of cardiac monophasic action potentials recorded by intracellular and suction electrodes. Am J Physiol 1959;196:1297–301.
2. Ino T, Karagueuzian HS, Hong K, Meesmann M, Mandel WJ, Peter T. Relation of monophasic action potential recorded with contact electrode to underlying transmembrane action potential properties in isolated cardiac tissues: a systematic microelectrode validation study. Cardiovasc Res 1988;22:255–64.
3. Franz MR, Burkhoff D, Spurgeon H, Weisfeldt ML, Lakatta EG. In vitro validation of a new cardiac catheter technique for recording monophasic action potentials. Eur Heart J 1986;7:34–41.
4. Moore HJ, Franz MR. Monophasic action potential recordings in humans. J Cardiovasc Electrophysiol 2007;18:787–90.
5. Burdon-Sanderson J, Page FJ. On the electrical phenomena of the excitatory process in the heart of the frog and of the tortoise, as investigated photographically. J Physiol 1884;4:327–86.
6. Burdon-Sanderson J, Page FJ. On the time-relations of the excitatory process in the ventricle of the heart of the frog. J Physiol 1880;2:384–435.
7. Schütz E. Elektrophysiologie des Herzens bei einphasischer Ableitung. Ergebn Physiol Exper Pharmakol 1936;38:493–620.
8. Woodbury LA, Woodbury JW, Hecht HH. Membrane resting and action potentials of single cardiac muscle fibers. Circulation 1950;1:264–6.
9. Schütz E. Weitere Versuche mit einphasischer Aufzeichnung des Warmblüter-Elektrokardiogramms. Z Biol 1934;95:78–90.
10. Korsgren M, Leskinen E, Sjöstrand U, Varnauskas E. Intracardiac recording of monophasic action potentials in the human heart. Scand J Clin Lab Invest 1966;18:561–4.
11. Olsson SB. Monophasic action potentials from right atrial muscle recorded during heart catheterization. Acta Med Scand 1971;190:369–79.
12. Olsson SB. Atrial repolarization in man. Effect of beta-receptor blockade. Br Heart J 1974;36:806–10.
13. Brorson L, Olsson SB. Atrial repolarization in healthy males. Studies with programmed stimulation and monophasic action potential recordings. Acta Med Scand 1976;199:447–54.
14. Nesterenko VV, Antzelevitch C. Spatial resolution of newly developed MAP recording techniques: experimental evaluation in highly heterogeneous myocardium in vitro. Pacing Clin Electrophysiol 1998;21(S2):857A.
15. Kadish A. What is a monophasic action potential? Cardiovasc Res 2004;63:580–1.
16. Jochim K, Katz LN, Mayne W. The monophasic electrogram obtained from the mammalian heart. Am J Physiol 1935;111:177–86.
17. Franz M, Schöttler M, Schaefer J, Seed WA. Simultaneous recording of monophasic action potentials and contractile force from the human heart. Klin Wochenschr 1980;58:1357–9.
18. Franz MR. Long-term recording of monophasic action potentials from human endocardium. Am J Cardiol 1983;51:1629–34.
19. Franz MR, Flaherty JT, Platia EV, Bulkley BH, Weisfeldt ML. Localization of regional myocardial ischemia by recording of monophasic action potentials. Circulation 1984;69:593–604.
20. Tse G, Tse V, Yeo JM. Ventricular anti-arrhythmic effects of heptanol in hypokalaemic, Langendorff-perfused mouse hearts. Biomed Rep 2016;4:313–24.
21. Franz MR. Current status of monophasic action potential recording: theories, measurements and interpretations. Cardiovasc Res 1999;41:25–40.
22. Schaefer H. Theorie des potentialabgriffs beim elektrokardiogramm, auf der grundlage der 'membrantheorie'. Pflug Arch Eur J Physiol 1942;245:72–97.
23. Schaefer H, Peña A, Schölmerich P. Der monophasische aktionsstrom von spitze und basis des warmblüterherzens und die theorie der T-welle des EKG. Pflug Arch Eur J Physiol 1943;246:728–45.
24. Sugarman H, Katz LN, Sanders A, Jochim K. Observations on the genesis of the electrical currents established by injury to the heart. Am J Physiol 1940;130:130–43.
25. Cranefield PF, Eyster JA, Gilson WE. Electrical characteristics of injury potentials. Am J Physiol 1951;167:450–7.
26. Eyster JA, Gilson WE. The development and contour of cardiac injury potential. Am J Physiol 1946;145:507–20.
27. Franz MR. Method and theory of monophasic action potential recording. Prog Cardiovasc Dis 1991;33:347–68.
28. Kondo M, Nesterenko V, Antzelevitch C. Cellular basis for the monophasic action potential. Which electrode is the recording electrode? Cardiovasc Res 2004;63:635–44.
29. Weissenburger J, Nesterenko VV, Antzelevitch C. Transmural heterogeneity of ventricular repolarization under baseline and long QT conditions in the canine heart in vivo: torsades de pointes develops with halothane but not pentobarbital anesthesia. J Cardiovasc Electrophysiol 2000;11:290–304.
30. Franz MR. Letters to the editor. J Cardiovasc Electrophysiol 2000;11:946.
31. Jungschleger JG, Vos MA. Hybrid action potential etiology. J Cardiovasc Electrophysiol 2000;11:946–8; author reply 8–51.
32. Vigmond EJ. The electrophysiological basis of MAP recordings. Cardiovasc Res 2005;68:502–3.

33. Coronel R, de Bakker JM, Wilms-Schopman FJ, Opthof T, Linnenbank AC, Belterman CN, et al. Monophasic action potentials and activation recovery intervals as measures of ventricular action potential duration: experimental evidence to resolve some controversies. Heart Rhythm 2006;3:1043–50.

34. Zhang Y, Mazgalev TN. Site of origin of the monophasic action potential: which electrode, the "potassium" or the "indifferent," records monophasic action potential? Heart Rhythm 2009;6:561–3.

35. Tse G, Hothi SS, Grace AA, Huang CL. Ventricular arrhythmogenesis following slowed conduction in heptanol-treated, Langendorff-perfused mouse hearts. J Physiol Sci 2012;62:79–92.

36. Osadchii OE. Effects of ventricular pacing protocol on electrical restitution assessments in guinea-pig heart. Exp Physiol 2012;97:807–21.

37. Osadchii OE, Larsen AP, Olesen SP. Predictive value of electrical restitution in hypokalemia-induced ventricular arrhythmogenicity. Am J Physiol Heart Circ Physiol 2010;298:H210–20.

38. Osadchii OE. Mechanisms of hypokalemia-induced ventricular arrhythmogenicity. Fundam Clin Pharmacol 2010;24:547–59.

39. Osadchii OE, Olesen SP. Electrophysiological determinants of hypokalaemia-induced arrhythmogenicity in the guinea-pig heart. Acta Physiol (Oxf) 2009;197:273–87.

40. Osadchii OE, Bentzen BH, Olesen SP. Chamber-specific effects of hypokalaemia on ventricular arrhythmogenicity in isolated, perfused guinea-pig heart. Exp Physiol 2009;94:434–46.

41. Tse G, Wong ST, Tse V, Yeo JM. Restitution analysis of alternans using dynamic pacing and its comparison with S1S2 restitution in heptanol-treated, hypokalaemic Langendorff-perfused mouse hearts. Biomed Rep 2016 (in press).

42. Tse G, Wong ST, Tse V, Lee YT, Lin HY, Yeo JM. Cardiac dynamics: alternans and arrhythmogenesis. J Arrhythmia 2016 (in press). DOI: 10.1016/j.joa.2016.02.009.

43. Kalb SS, Dobrovolny HM, Tolkacheva EG, Idriss SF, Krassowska W, Gauthier DJ. The restitution portrait: a new method for investigating rate-dependent restitution. J Cardiovasc Electrophysiol 2004;15:698–709.

44. Tse G. Mechanisms of cardiac arrhythmias. J Arrhythmia 2015;32:75–81.

45. Tse G, Yeo JM. Conduction abnormalities and ventricular arrhythmogenesis: the roles of sodium channels and gap junctions. IJC Heart Vasculature 2015;9:75–82.

46. Vigmond EJ, Leon LJ. Electrophysiological basis of mono-phasic action potential recordings. Med Biol Eng Comput 1999;37:359–65.

47. Yang SG, Kittnar O. New insights into application of cardiac monophasic action potential. Physiol Res 2010;59:645–50.

48. Shimizu W, Ohe T, Kurita T, Kawade M, Arakaki Y, Aihara N, et al. Effects of verapamil and propranolol on early afterdepolarizations and ventricular arrhythmias induced by epinephrine in congenital long QT syndrome. J Am Coll Cardiol 1995;26:1299–309.

49. Yue AM, Paisey JR, Robinson S, Betts TR, Roberts PR, Morgan JM. Determination of human ventricular repolarization by noncontact mapping: validation with monophasic action potential recordings. Circulation 2004;110:1343–50.

50. Ghazanfari A, Rodriguez MP, Vigmond E, Nygren A. Computer simulation of cardiac propagation: effects of fiber rotation, intramural conductivity, and optical mapping. IEEE Trans Biomed Eng 2014;61:2041–8.

51. Tse G, Tse V, Yeo JM, Sun B. Atrial anti-arrhythmic effects of heptanol in Langendorff-perfused mouse hearts. PLoS One 2016;11:e0148858.

52. Vigmond EJ, Tsoi V, Yin Y, Page P, Vinet A. Estimating atrial action potential duration from electrograms. IEEE Trans Biomed Eng 2009;56:1546–55.

53. Tranquillo JV, Franz MR, Knollmann BC, Henriquez AP, Taylor DA, Henriquez CS. Genesis of the monophasic action potential: role of interstitial resistance and boundary gradients. Am J Physiol Heart Circ Physiol 2004;286:H1370–81.