Ncardia
Stem cell experts

Cardiac safety assessment during electrical pacing using Pluricyte® Cardiomyocytes

in combination with Axion BioSystems Maestro E Stim+ MEA technology

Application note
Version 2.0

Contents

1. Introduction

Pluricyte® Cardiomyocytes are highly suitable for Axion Maestro MEA assays

Pluricyte® Cardiomyocytes are fully functional human-induced pluripotent stem cell (hiPSC) derived ventricular cardiomyocytes that are particularly suitable for electrophysiology-based microelectrode array (MEA) assays for predictive safety pharmacology, toxicity testing and efficacy screening in early drug discovery and development. Pluricyte® Cardiomyocytes' well-pronounced depolarization and repolarization peaks permit the easy detection of electrophysiological parameters (e.g. depolarization/repolarization peak amplitudes, beat rate, field potential duration) and facilitate efficient data analysis and interpretation of studies performed with the Axion Maestro MEA system.

The combination of Pluricyte® Cardiomyocytes with the Axion Maestro MEA system enables detailed electrophysiological detection of cardioactive/proarrhythmic effects of test compounds at 48- or 96-well plate formats.

Pluricyte® Cardiomyocytes strengths and characteristics

Pluricyte® Cardiomyocytes exhibit a relatively high level of maturity, when compared to other human stem cell-derived cardiomyocytes and present the following unique characteristics:

- High purity of ventricular cardiomyocytes
- Low resting membrane potentials (~-78 mV)
- Fast upstroke velocities and high action potential amplitudes
- Organized sarcomere structures
- Monolayer field potential containing well-pronounced depolarization and repolarization peaks that enable easy detection of field potential durations in MEA assays
- Relatively low spontaneous beat rate (20-30 BPM), making the cardiomyocytes highly suitable for pacing applications: a broad window of pacing rates can be applied

Benefits of pacing in Pluricyte® Cardiomyocyte Maestro assays

Axion BioSystems (Axion BioSystems, Atlanta, GA, USA) developed a new pacing system, the Maestro E-Stim+ Classic MEA plate, that delivers high-quality MEA results with superior stimulation capacity. Pluricyte® Cardiomyocytes readily adapt to electrical stimulation ('pacing') applied through Axion Maestro E-Stim+ MEA technology. Controlling the beat rate by pacing provides several advantages and enables the user to do a more precise and more in-depth analysis of cardioactive/proarrhythmic compound effects. These advantages include:

- Assessment of cardiac safety at user-defined beat rates
- Detection of beat rate dependent effects (use-dependence)
- Increased physiological relevance
- Reduced well-to-well variability
- Reduced assay-to-assay variability

This application note describes the assessment of pro-arrhythmic compound effects in stimulated Pluricyte® Cardiomyocytes. Electrical stimulation of Pluricyte® Cardiomyocytes provides a reproducible and relevant in vitro assay to record the field potential signal under a stable and defined beat rate to study cardiac safety profile of novel drug candidates.
For more data and information on how to use Pluricyte® Cardiomyocytes in combination with Axion Maestro E-Stim+ MEA technology, please refer to our **User Guide**.

Technical support

Our scientists are ready to help you with any questions you may have regarding this application note or our Pluricyte® Cardiomyocytes. In addition, in-lab training is available upon request. For further information please visit our website **www.ncardia.com**, or contact us directly by e-mail (**support@ncardia.com**).

2. Assessment of pro-arrhythmic effects using pluricyte® cardiomyocytes during electrical stimulation in Maestro E-Stim+ MEA plates

Pluricyte® Cardiomyocytes field potential measurements during stimulation using Maestro E-Stim+ MEA technology

The Axion BioSystems Maestro system enables the recording of Pluricyte® Cardiomyocyte extracellular field potentials in real-time using proprietary microelectrode array (MEA) technology. Combined with Maestro E-Stim+ Classic 48 MEA plates, which have a dedicated pacing electrode in each well, the field potential of cardiomyocytes can be measured during pacing.

In the pacing assay described here ventricular cardiomyocytes adapt to beat rates applied by external stimuli, resembling the in vivo situation where the ventricles rely on external input from pacemaker cells. The low spontaneous beating rates of Pluricyte® Cardiomyocytes enables pacing at a wide range of beating rates, allowing the user to pick a beating rate that is relevant for the application. Additionally, pacing of Pluricyte® Cardiomyocytes increases performance of cardiac safety assays by eliminating any variability caused by variation in beat rate. Another advantage of pacing is that (reverse) use-dependence of drugs can be tested. This phenomenon, described as a different magnitude of effect at different beat rates is observed with several types of ion channel blockers. Compounds in which a higher beat rate increases their effect, are known as use-dependent compounds. Use-dependence is typically observed in sodium channel blockers[1]. On the other hand, hERG channel blockers are typically more effective at lower beat rates, which is referred to as reverse use-dependence[2]. Reverse use-dependence is one of the pillars of TRIaD (Triangulation, Reverse use-dependence, Instability, and Dispersion) of ventricular repolarization. It is believed that augmentation of TRIaD provides the proarrhythmic substrate, individual parameters are thus of interest for the assessment of the pro-arrhythmic potential of a compound[3].

Parameter measurements that can be analyzed using the AxIS software may include the depolarization amplitude, the beat period and the field potential duration (the time period between the depolarization and repolarization peaks), as described in our **User Guide**. Furthermore, analysis may also include the number of wells in which capture of the pacing stimulus is lost, for example due to compound effects on excitability or conductivity.

2.1 Experimental design to study acute drug effects during electrical stimulation

Pluricyte® Cardiomyocytes were cultured on Maestro E-Stim+ Classic MEA 48 plates in Pluricyte® Cardiomyocyte Medium for 8 days. The set of pro-arrhythmic drugs (Table 2.1) was dissolved in DMSO at a concentration of 10 mM and then diluted in Pluricyte® Cardiomyocyte Medium in serial dilutions. The Pluricyte® Cardiomyocytes were then treated with this set of pre-diluted pro-arrhythmic drugs in a cumulative dose response experiment (Table 2.2). Acute-drug effects were measured while pacing at different rates using the Maestro system. Compound concentrations were increased by 2-fold (Table 2.2) for each separate recording step. After each compound addition, the cardiomyocytes were paced during steady state at several rates (Table 2.3). The data were analyzed using AxIS (version 2.4.1) to determine the compound effects on the field potential duration and spike amplitude and to determine the capture of the stimulus at each of the pacing rates.

Drug class	Drug	Expected effects on hIPSC-Cardiomyocytes electrophysiology
hERG channel blocker (I_{Kr})	E4031 Dofetilide	Reverse use-dependent delay of the repolarization phase by blocking the hERG channel, resulting in prolonged FPD and ultimately arrhythmias
Sodium channel blocker (I_{Na})	TTX	Use-dependent decrease of the depolarization amplitude by blocking sodium channels
Calcium channel blocker ($I_{Ca,L}$)	Diltiazem	Shortening of the FPD by blocking calcium influx through L-type calcium channels
Slow delayed rectifier channel blocker (I_{Ks})	JnJ303	Small FPD prolongation, without occurrence of arrhythmias at higher concentrations

Table 2.1. List of pro-arrhythmic drugs and their expected effects on hiPSC-derived cardiomyocytes

Compounds	t=0*	t=30 min.	t=60 min.	t=90 min.	t=120 min.	t=150 min.
E4031	0 nM	1 nM	2 nM	4 nM	8 nM	16 nM
Dofetilide	0 nM	1 nM	2 nM	4 nM	8 nM	16 nM
JnJ303	0 nM	50 nM	100 nM	200 nM	400 nM	800 nM
TTX	0 nM	750 nM	1500 nM	3000 nM	6000 nM	12000 nM
Diltiazem	0 nM	100 nM	200 nM	400 nM	800 nM	1600 nM
DMSO	0%	0.01%	0.02%	0.04%	0.08%	0.16%

*t=0 is start of recording, after stabilization (see Table 2.3)

Table 2.2. Final compound concentrations used

Time after compound addition	Activity
0-10 minutes	Wait for field potential signals to stabilize
11-12 minutes	1 minute recording of spontaneous activity
12-30 minutes	Record activity during steady state at various pacing rates ranging from 0.5 Hz to 1.75 Hz

Table 2.3. Measurement and pacing schedule for each compound addition

2.2 Results

Analysis of the different field potential parameters before and after application of compounds showed that Pluricyte® Cardiomyocytes show expected results to several ion channel blockers during pacing. Figures 2.1 and 2.2 provide an overview of the different cardioactive compound effects on average field potential traces (Figure 2.1A-E) as well as on field potential duration, sodium spike amplitude and stimulus capture during pacing at 1 Hz (Figure 2.2A-E). Additionally, analysis of compound effects at different pacing rates revealed that hERG blockers and sodium channel blockers exhibited, as expected, reverse use-dependence and use-dependence, respectively (Figure 2.3).

hERG potassium channel (I_{Kr}) blockers block the rapid component of the delayed rectifier outward potassium current (I_{kr}), thereby delaying the repolarization phase. This results in an increase in field potential duration and flattening of the repolarization peak. At higher concentrations, blocking of the hERG channel may lead to Torsade de Pointe (TdP)-like arrhythmias[4]. Figures 2.1A-B and 2.2A-B show prolongation of the field potential duration (FPD) of Pluricyte® Cardiomyocytes induced by hERG potassium channel blockers E4031 and dofetilide. Additionally, analysis of the FPD at different pacing rates after treatment with E4031 showed that the inverse relationship between FPD and beat rate was reverse use-dependent (Figure 2.3A), a phenomenon that is well known to occur during in vivo hERG channel blockade[2]. For examples of TdP-like arrhythmias caused by hERG channel blockers at higher concentrations than presented here, please refer to our **User Guide** for standard Axion BioSystems Maestro MEA plates.

Calcium channel ($I_{Ca,L}$) blockers affect the plateau phase between the depolarization and repolarization phase, resulting in a shortening of the field potential duration[5]. As shown in Figures 2.1C and 2.2C, the L-type calcium channel blocker diltiazem shortens the field potential duration of Pluricyte® Cardiomyocytes during pacing at 1 Hz in a concentration-dependent manner.

Kv7.1 channel (I_{Ks}) blockers block the slow component of the delayed rectifier outward potassium current (I_{Ks}), resulting in a delay of the repolarization phase. In human cardiomyocytes this current is known to be relatively small compared to the I_{Kr} current[6], resulting in less severe FPD prolongations. Also, blockade of this channel does not result in TdP-like arrhythmias in human cardiomyocytes. As expected, the $K_{v7.1}$ channel blocker JnJ303 induced a small but robust prolongation of the FPD during pacing at 1 Hz (Figures 2.1D and 2.2D). Furthermore, no TdP-like arrhythmias were observed after application of this blocker.

Sodium channel (I_{Na}) blockers affect the depolarization phase of the field potential, resulting in a decrease in sodium spike amplitude[7]. The effect of sodium channel blockers on the $Na_{v1.5}$ channel is known to be use-dependent[1]. Figures 2.1E and 2.2E show that sodium channel blocker TTX indeed decreases the sodium spike amplitude in Pluricyte® Cardiomyocytes in a concentration-dependent manner. Additionally, the loss of excitability due to sodium channel blockade was noticed as a loss of stimulus capture. At concentrations higher than 6 µM, stimulation was not possible at all. Furthermore, analysis of the reduction in sodium spike amplitude at different beat rates showed that the effect of

TTX exhibits use-dependence: the reduction in sodium spike amplitude tended to be larger at higher beat rates (Figure 2.3B).

2.3 Concluding Remarks

Pluricyte® Cardiomyocytes in combination with Axion Maestro E-Stim+ MEA technology are highly suitable for next generation of cardiac safety screening assays. Using this technology, Pluricyte® Cardiomyocytes readily adapt their electrophysiology to various pacing rates and still exhibit their unique strengths and characteristics including field potential signals containing well-pronounced depolarization and repolarization peaks.

Pluricyte® Cardiomyocytes, while electrically paced using specialized E-Stim+ MEA plates, showed the expected pharmacological responses, including (reverse) use-dependent drug effects in a reproducible manner.

The combination of Pluricyte® Cardiomyocytes with E-Stim+ MEA technology for the Maestro system provides a highly relevant in vitro assay to study cardiac safety profiles of compounds under stable and defined pacing rates at an early stage of drug development. Compared to assays using spontaneous beating cardiomyocytes, this next generation assay provides several advantages: increased biological relevance, reduction of variability and detection of (reverse) use-dependence.

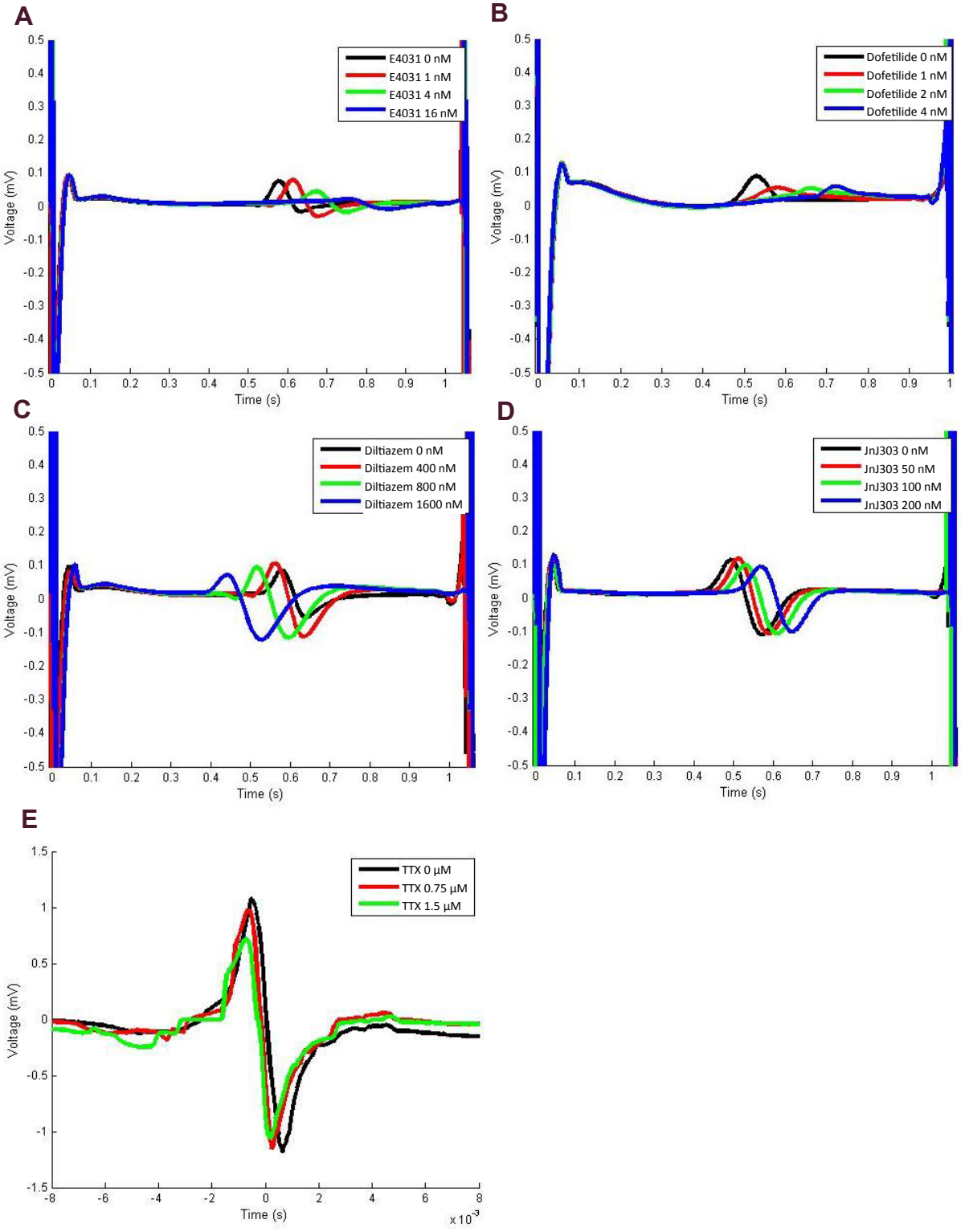

Figure 2.1. Effects of different compounds on field potential traces during pacing at 1 Hz. Each figure represents the average traces per concentration of 1 representative well from a 1 minute recording (A) FPD prolongation and repolarization peak flattening by 1-16 nM of the hERG blocker E4031 during pacing at 1 Hz. (B) FPD prolongation and repolarization peak flattening by 1-4 nM of the hERG blocker Dofetilide during pacing at 1 Hz. (C) FPD shortening by 400-1600 nM of L-type calcium channel blocker Diltiazem during pacing at 1 Hz. (D) FPD prolongation by 50-200 nM of the IKs blocker JnJ303 during pacing at 1 Hz. (E) Reduction in sodium spike amplitude after treatment of 0.75-1.5 µM TTX. 3 µM TTX is not shown, since capture was lost in most wells.

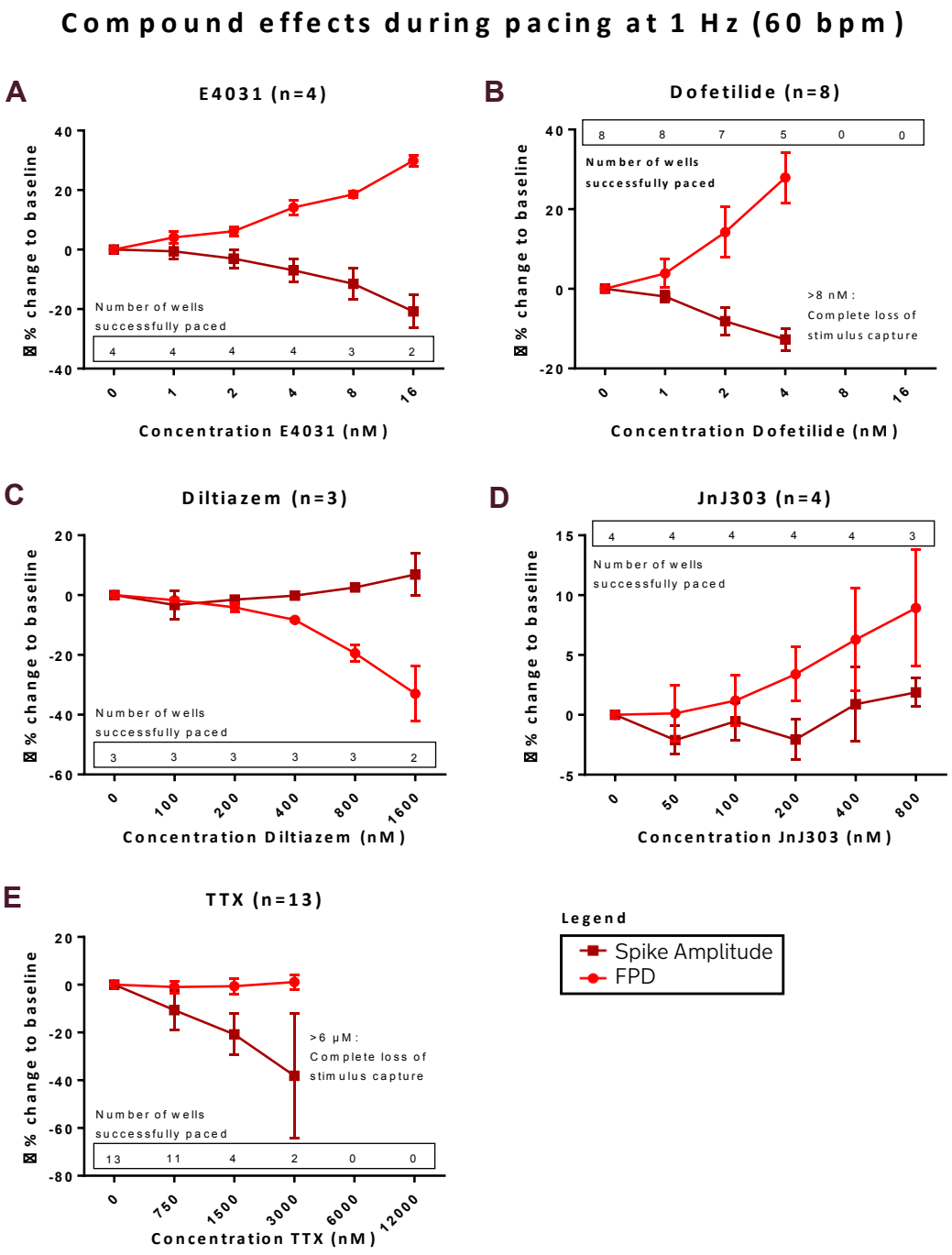

Figure 2.2. Effects of different compounds on field potential parameters during pacing at 1 Hz. Y-values are plotted as Δ% change, meaning that the values are normalized to the control wells by subtracting the average % change in DMSO-treated control wells. Error bars represent standard deviations. (A) FPD prolongation by 1-16 nM of hERG blocker E4031 during pacing at 1 Hz. (B) FPD prolongation by 1-4 nM of hERG blocker Dofetilide during pacing at 1 Hz. At concentrations of 8 nM or higher, stimulation at 1 Hz was no longer possible. (C) FPD shortening by 400-1600 nM of L-type calcium channel blocker Diltiazem during pacing at 1 Hz. (D) Tendency of FPD prolongation by 50-200 nM of IKs blocker JnJ303 during pacing at 1 Hz. (E) Reduction in spike amplitude and stimulus capture after treatment of 750-3000 nM TTX. At concentrations of 6 µM or higher, stimulation at 1 Hz was no longer possible. For TTX, experiment was repeated and data was pooled to compensate for the reduction of stimulus capture typically seen after sodium channel blockade.

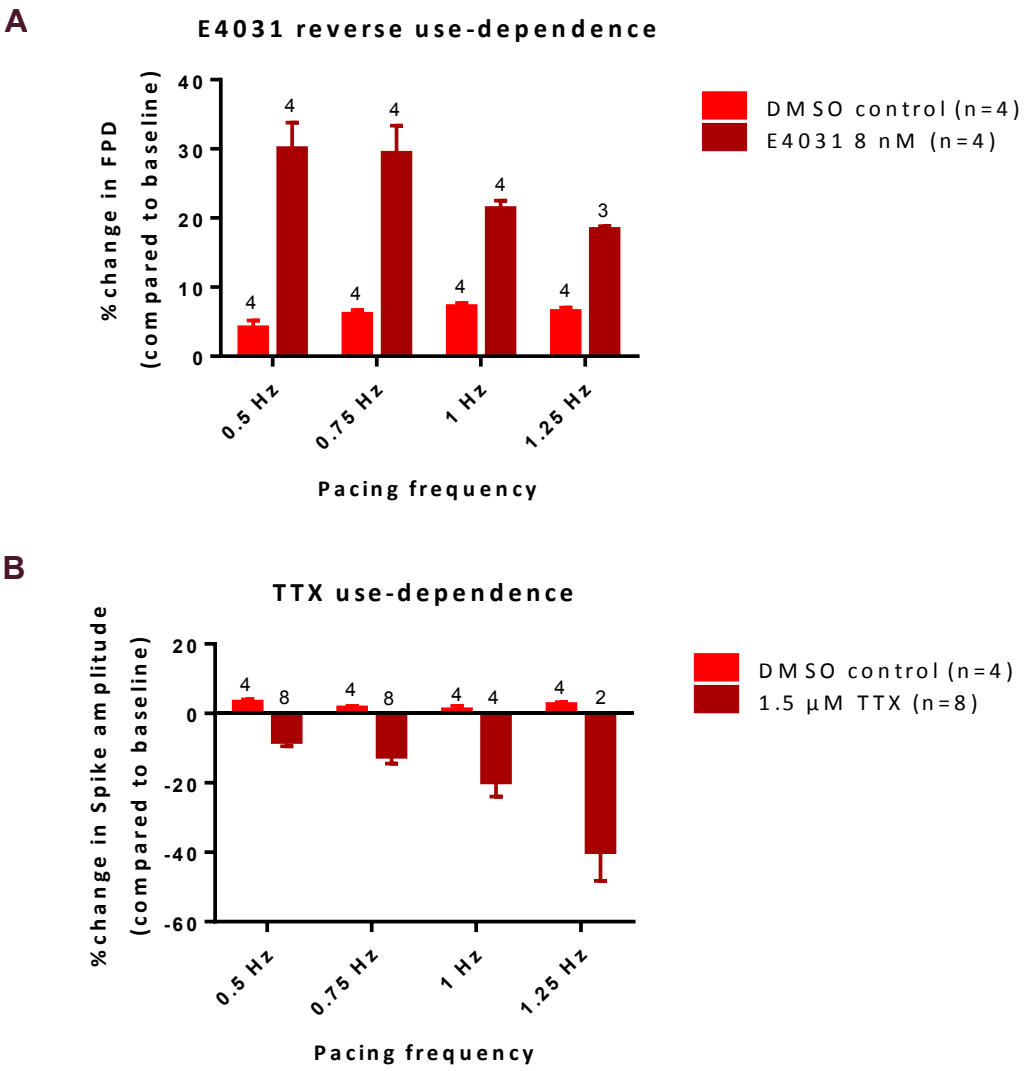

Figure 2.3. Effects of 8 nM E4031 and 1.5 µM TTX at different beat rates. Error bars depict standard error of the mean, number on top of bars depict number of wells successfully paced. (A) Reverse use-dependent effects of hERG channel blocker E4031 on the field potential duration of Pluricyte® Cardiomyocytes paced at 0.5-1.25 Hz. (B) Use-dependence of sodium channel blocker TTX on the spike amplitude of Pluricyte® Cardiomyocytes paced at 0.5-1.25 Hz. Note that TTX treatment also results in loss of capture in a considerate amount of wells, due to lower opening probability of the sodium channels.

3. References

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