

# A Study of Properties of the $\text{Ca}^{2+}$ -Dependent Delayed Afterdepolarizations in a Mathematical Model for Human Ventricular Myocytes.

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## Abstract

*Delayed afterdepolarizations (DADs), observed in cardiac myocytes can be arrhythmogenic. These DADs may arise in diseased conditions or mutations that induce numerous changes in the expression levels of exchangers, ion channels, or ionic pumps. It is difficult to reproduce these changes in experiments; therefore, we carry out a detailed in silico study of these modifications in the human ventricular myocyte model due to ten Tusscher and Panfilov (TP06). We find three types of DADs. Furthermore, by using parameter-sensitivity analysis, we show that the  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchanger and the SERCA pump uptake rate are the critical parameters for triggering DADs. We also show that the  $\text{Na}^+$ - $\text{Ca}^{2+}$  conductance increases the DAD amplitude, whereas the SERCA uptake rate increases the frequency of the DADs. We obtain a phase diagram for the TP06 model and present regions of parameter space which show various types of DADs.*

## 1. Introduction

Arrhythmogenic afterdepolarizations occur in the late phases of the cardiac action potential (AP). These afterdepolarizations are categorized into two types, depending upon the phase of the AP. The afterdepolarizations that occur during the plateau phase of the AP are called early afterdepolarizations (EADs); the ones that occur during the diastolic interval (DI) are called delayed afterdepolarizations (DADs). Experiments on mammalian hearts [1], such as *in vitro* studies in ferret [2] and cat [3] myocytes, suggest that DADs are triggered by  $\text{Ca}^{2+}$ -induced- $\text{Ca}^{2+}$ -release (CICR), i.e., the release of  $\text{Ca}^{2+}$ -ions from ryanodine receptors (RyR) by the action of  $\text{Ca}^{2+}$  alone. During the diastolic interval, when L-type  $\text{Ca}^{2+}$  channels are closed, these CICR events lead to DAD, which are observed during the following heart conditions: a hypertrophied failing heart [4], the post-acidotic incidence of cardiac arrhythmias [5], and exercise-induced catecholaminergic polymorphic ventricular tachycardia (CPVT)[6]. These conditions are associated primarily with  $\text{Ca}^{2+}$ -overload and RyR mutations.

One well-known mechanism for DADs is the leak of  $\text{Ca}^{2+}$  from these mutated RyRs. The  $\text{Ca}^{2+}$  leak from RyRs triggers other RyRs to open and generate spontaneous  $\text{Ca}^{2+}$  release, which is known as a  $\text{Ca}^{2+}$ -spark. Once a  $\text{Ca}^{2+}$ -spark occurs, the  $\text{Ca}^{2+}$ -concentration rises in the intracellular spaces. The  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchanger (NCX) senses the increased  $\text{Ca}^{2+}$  in the intracellular space and acts to evacuate the excess  $\text{Ca}^{2+}$ . The Electrogenic nature of the NCX (exchanges 1  $\text{Ca}^{2+}$  ion for 3  $\text{Na}^+$  ions), depolarises the myocyte membrane. Given the strength of the  $\text{Ca}^{2+}$ -spark, the amplitude of the resultant DAD may depend on multiple parameters. DADs are conventionally divided into two types: a suprathreshold DAD, if it reaches the AP-excitation threshold, and subthreshold DADs, which do not reach the AP-excitation threshold. DADs are one of the known precursors of cardiac disturbances at the whole heart level, so it is vital to understand these DADs in cardiac-myocyte parameter space. We carry out such a study by using the TP06 model for human ventricular myocytes. Among the available models in the literature, only a few mathematical models can yield DADs as discussed in Ref. [7], which has classified the models capable of inducing DADs and methods to identify them. We organize our study as follows: Section 2 describes our Model and Methods. In Section 3 we give Results and Conclusions.

## 2. Model and Methods

### 2.1. Model description

We use the TP06 human ventricular myocyte model, for which we show the  $\text{Ca}^{2+}$ -subsystem via a schematic diagram in Fig. 1. The myocyte volume is divided into three compartments: SR is the sarcoplasmic reticulum volume, SS is the subspace volume, and CYTO is the cytoplasmic volume of the myocyte. The ordinary differential equation (ODE) for the membrane potential is

$$\frac{dV}{dt} = -\frac{I_{stim} + I_{ion}}{C_m}, \quad (1)$$

where  $V$  is the potential difference across the myocyte membrane,  $t$  is the time and  $C_m$  is the membrane capaci-

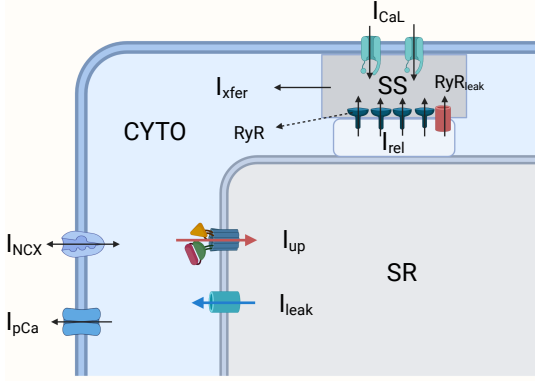


Figure 1. Schematic diagram of the TP06 model's  $\text{Ca}^{2+}$  subsystem. Here the myocyte volume is divided into three compartments: the sarcoplasmic reticulum (SR), the subspace (SS), and the cytoplasm (CYTO). RyRs are the ryanodine receptors,  $I_{rel}$  is the RyRs  $\text{Ca}^{2+}$ -release rate. SS is the volume where  $\text{Ca}^{2+}$ -induced- $\text{Ca}^{2+}$ -release occurs.  $I_{up}$  is the SERCA pump uptake from the CYTO to the SR.  $I_{leak}$  is  $\text{Ca}^{2+}$ -leak from the SR to the CYTO,  $\text{RyR}_{leak}$  is  $\text{Ca}^{2+}$ -leak from the SR volume to the SS volume and  $I_{xfer}$  is diffusion flux of  $\text{Ca}^{2+}$ -ions from the SS to the CYTO.  $I_{pCa}$ ,  $I_{NCX}$  and  $I_{CaL}$  are transmembrane currents. Figure created with [BioRender.com](https://www.biorender.com).

tance. The TP06 model has 12 transmembrane ionic currents, and  $I_{ion}$  is the sum of all these currents:

$$I_{ion} = I_{Na} + I_{CaL} + I_{K1} + I_{Kr} + I_{Ks} + I_{to} + I_{pK} + I_{bCa} + I_{NaCa} + I_{NaK} + I_{bNa} + I_{pCa}. \quad (2)$$

These currents are as follows: the fast  $\text{Na}^+$  current ( $I_{Na}$ ); L-type  $\text{Ca}^{2+}$  ( $I_{CaL}$ ); inward rectifier ( $I_{K1}$ ); rapid-delayed rectifier ( $I_{Kr}$ ); slow-delayed rectifier ( $I_{Ks}$ ); transient-outward ( $I_{to}$ ); plateau  $\text{K}^+$  ( $I_{pK}$ ); background  $\text{Ca}^{2+}$  ( $I_{bCa}$ );  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchanger ( $I_{NaCa}$ );  $\text{Na}^+$ - $\text{K}^+$  ATPase exchanger ( $I_{NaK}$ );  $\text{Na}^+$  background ( $I_{bNa}$ ); plateau  $\text{Ca}^{2+}$  current ( $I_{pCa}$ ). The conductances for these currents are  $G_{Na}$ ,  $G_{CaL}$ ,  $G_{to}$ ,  $G_{pK}$ ,  $G_{bCa}$ ,  $K_{NaCa}$ ,  $P_{NaK}$ ,  $G_{bNa}$  and  $G_{pCa}$ , respectively.

The detailed ODEs for the currents and  $\text{Ca}^{2+}$ -concentrations of the model are given in the Ref.[8]. In the equations for  $\text{Ca}^{2+}$  concentrations, there are equations for  $I_{rel}$  and  $I_{up}$ , which are the molar flow rates of  $\text{Ca}^{2+}$ -ions from the SR volume to the SS volume and CYTO to SR, respectively. We have modified the  $I_{rel}$  in this model by adding a small leak rate  $0.00018\text{ms}^{-1}$  as follows:

$$I_{rel} = (V_{rel} \cdot O + 0.00018)([\text{Ca}^{2+}]_{SR} - [\text{Ca}^{2+}]_{SS})$$

$$I_{up} = \frac{V_{maxup}}{1 + \frac{K_{up}^2}{\text{Ca}_i^2}}, \quad (3)$$

where the control value of  $V_{rel} = 0.102 \text{ms}^{-1}$  and  $O$  is the probability of the RyR being open,  $[\text{Ca}^{2+}]_{SR}$  and  $[\text{Ca}^{2+}]_{SS}$  are molar concentrations in spaces SR and SS respectively.  $V_{maxup} = 0.006375 \text{mM/ms}$  is the control parameter for the SERCA uptake rate,  $K_{up} = 0.00025 \text{mM}$  and  $\text{Ca}_i$  is the  $\text{Ca}^{2+}$ -concentration in the CYTO. We use the numerical scheme from Ref. ([9]) to integrate Eq. (1) with the time step  $\delta t = 0.02 \text{ms}$ . In our simulations, the frequency of stimulation of  $I_{stim}$  is 1 Hz. All the results we show are recorded and analyzed after 500 APs.

## 2.2. Method for sensitivity analysis

We use sensitivity analysis to find out which parameters among all conductances or fluxes (e.g.,  $G_{Na}$  and  $V_{maxup}$ ) affect DADs the most. For calculating sensitivity values, we use the algorithm mentioned in Ref.[10]. We choose 500 randomly sampled scale factors for each one of these parameters; e.g., the scale factor for  $G_{Na}$  follows from  $G_{Na} = S_{GNa} \times G_{NaC}$ , where  $G_{NaC}$  is the control value for  $G_{Na}$  and  $S_{GNa}$  is the scale factor for  $G_{Na}$  [11]. We use similar notations for other conductances and fluxes. These scale factors are chosen from a log-normal distribution which we obtain from a Gaussian distribution with standard deviation  $\sigma = 0.1$  and mean  $\mu = 0$ . For each set of parameters, we generate 500 APs, by stimulation, and record the last 10 APs. From the recorded APs we calculate the outputs and their  $Z$ -scores, i.e.,  $(x - \mu_x)/\sigma_x$ . For every output  $x$ ,  $\mu_x$  is the mean and  $\sigma_x$  is the standard deviation; we consider two outputs, namely, the amplitude and frequency of the DADs ( $\text{DAD}_{amp}$  and  $\text{DAD}_{freq}$ ). To observe DADs in our sensitivity simulations, we use  $S_{GCaL} = 2$  and  $S_{Vmaxup} = 3$ .

## 3. Results and Conclusion

### 3.1. Role of leak current

In Fig. 2 we present two channel plots without and with RyR-leak modifications; Figs. 2(a) and 2(b) shows plots of the AP; Figs. 2(c) and 2(d) show plots for NCX ( $I_{NaCa}$ ); Figs. 2(e) and 2(f) shows plots of  $I_{rel}$ . In Figures 2(c) and 2(d) elucidates the role of NCX ( $I_{NaCa}$ ) in depolarising the membrane once  $\text{Ca}^{2+}$ -sparks occur. Sharp peaks of  $I_{rel}$  in Fig. 2(f) are  $\text{Ca}^{2+}$ -sparks; note that the  $\text{Ca}^{2+}$ -spark at about 600 ms is associated with a DAD in Fig. 2(b).

### 3.2. Sensitivity analysis

The three types of DADs we have studied are shown in Fig. 3: (a) subthreshold DADs [Fig. 3(d)]; (b) multi-blip DADs [Fig. 3(e)]; (c) suprathreshold DADs [Fig. 3(f)].

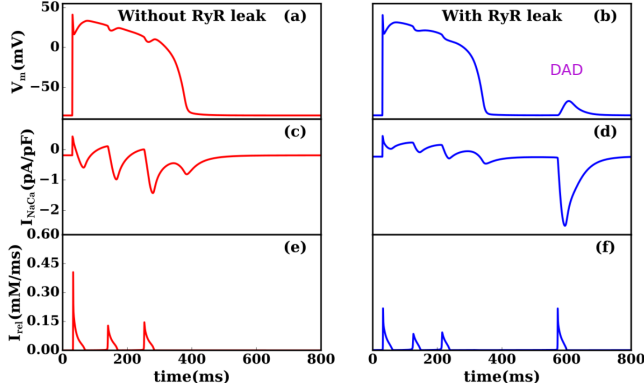


Figure 2. Two channel plots without and with RyR-leak modifications. We use scale factors ( $S_{G_{CaL}} = 2, S_{V_{maxup}} = 3$ ) for both the channels. (a)-(b): Show plots of membrane potential. (c)-(d): Show plots for NCX ( $I_{NaCa}$ ) role in depolarising membrane when  $Ca^{2+}$ -sparks occurred. (e)-(f): Show  $Ca^{2+}$  release  $I_{rel}$  from RyR.

To determine in which parameter ranges these DADs occur, we have carried out a sensitivity analysis (Sec. 2.2) to determine the parameters that affect the frequency and amplitude of these DADs crucially.

Results from our sensitivity analysis indicate that  $K_{NaCa}$ ,  $G_{K1}$ ,  $V_{maxup}$  affect the amplitude of DADs sensitively. For the DAD frequency  $V_{maxup}$  and  $K_{NaCa}$  are the most sensitive parameters as we show Table[1]. Even though in the TP06 model  $V_{rel}$  determines the amplitude of the RyR release  $I_{rel}$ , our sensitivity results show that  $V_{rel}$  has a negligible effect on  $DAD_{amp}$  and  $DAD_{freq}$ .

$DAD_{amp}$		$DAD_{freq}$	
Rel. sens.	Biomarkers	Rel. sens.	Biomarkers
0.6508	$K_{NaCa}$	0.7497	$V_{maxup}$
0.1865	$G_{CaL}$	0.2342	$G_{bCa}$
-0.4713	$V_{maxup}$	-0.2302	$G_{Kr}$
-0.6697	$G_{K1}$	-0.3848	$K_{NaCa}$

Table 1. Relative sensitivities (Rel. sens.) of the most sensitive biomarkers for the amplitude and frequency of DADs, i.e.,  $DAD_{amp}$  and  $DAD_{freq}$  respectively. Positive Rel. sens. indicate that increase in biomarkers increases the corresponding observables ( $DAD_{amp}$  and  $DAD_{freq}$ ), whereas negative rel. sens. Indicates an increase in biomarkers will decrease the observable value.

### 3.3. Phase diagrams

In Fig. 3(a), we show how we can increase  $G_{CaL}$  and  $G_{Kr}$  to obtain  $Ca^{2+}$ -overload without changing the AP duration. This  $Ca^{2+}$ -overload is required for triggering DADs in the TP06 model. By enhancing  $Ca^{2+}$ -overload we ob-

serve three types of DADs in the TP06 model [see Figs. 3(d)-(f)]. In our future detailed study [12] we will show various phase diagrams.

In Fig. 3(b) we show an illustrative phase diagram in the  $S_{K_{NaCa}}, S_{V_{maxup}}$  parameter space, where cyan, blue, magenta and red regions yield the APs shown in Figs. 3(c), 3(d), 3(e) and 3(f), respectively.

We will present other phase diagram in our future detailed study.

### 3.4. Conclusions

It is important to understand the parameter-dependence of DADs, as they are known to be arrhythmogenic. We have carried out such a study for the TP06 mathematical model for human ventricular myocytes. Our study re-emphasizes the role of the RyR-leak in forming such DADs. Our sensitivity analysis in Table[1] helps us to identify the principal parameters that affect  $DAD_{amp}$  and  $DAD_{freq}$  in the TP06 model (Table [1]). Phase diagrams, such as the one we give in Fig. 3(b), help us to identify the parameter regions in which we obtain three types of DADs.

### 3.5. Limitations of our study

In the model we have studied, the distribution of the  $Na^+$ - $Ca^{2+}$  exchanger is limited only to the cytoplasm; however, in more realistic models, e.g., in Ref.[13], the  $Na^+$ - $Ca^{2+}$  exchanger is distributed in other compartments also. We will report our study of such models in elsewhere. The model We have studied is a common-pool model which lacks the description of spatial details inside myocyte.

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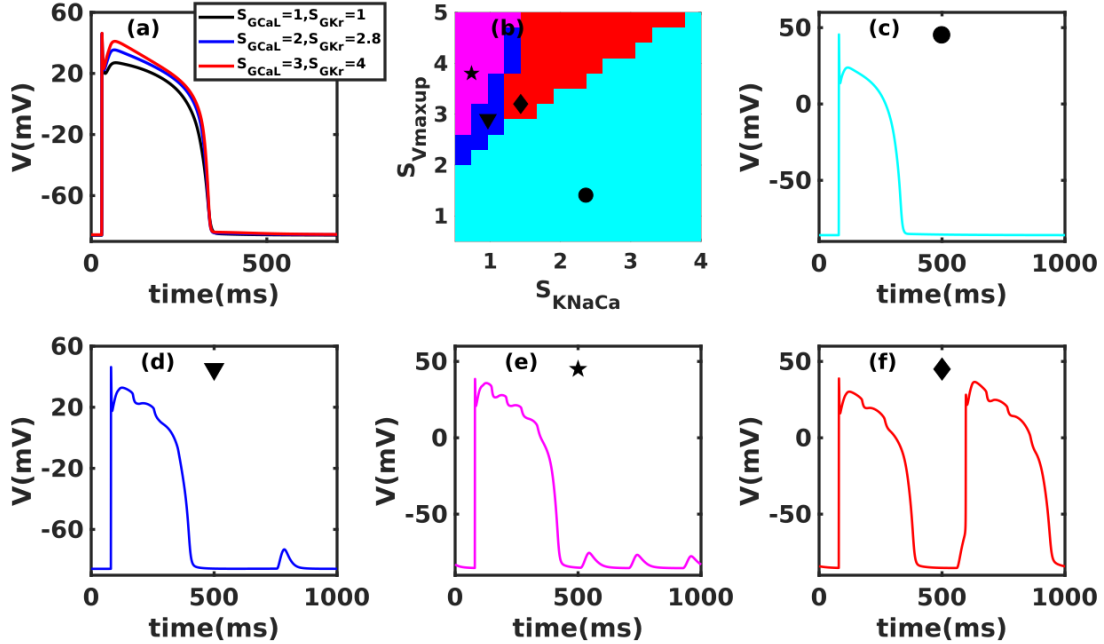


Figure 3. (a): AP plots for different values of  $S_{GCaL}$  and  $S_{GKr}$  (b): Phase diagram in the  $S_{KNaCa}$  and  $S_{Vmaxup}$  parameter space for various types of DADs at  $S_{GCaL} = 2$ . Where cyan, blue, magenta and red regions yield the APs shown in (c): Normal action potential. (d): Subthreshold DAD. (e): Multi-blip DAD. (f): Suprathreshold DAD.

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