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Rate-dependent effects of state-specific sodium channel blockers in cardiac tissue: Insights from idealized models

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ABSTRACT

A common side effect of pharmaceutical drugs is an increased propensity for cardiac arrhythmias. Many drugs bind to cardiac ion-channels in a state-specific manner, which alters the ionic conductances in complicated ways, making it difficult to identify the mechanisms underlying pro-arrhythmic drug effects. To better understand the fundamental mechanisms underlying the diverse effects of state-dependent sodium (Na^+) channel blockers on cellular excitability, we consider two canonical motifs of drug-ion-channel interactions and compare the effects of Na^+ channel blockers on the rate-dependence of peak upstroke velocity, conduction velocity, and vulnerable window size. In the literature, both motifs are referred to as “guarded receptor,” but here we distinguish between state-specific binding that does not alter channel gating (referred to here as “guarded receptor”) and state-specific binding that blocks certain gating transitions (“gate immobilization”). For each drug binding motif, we consider drugs that bind to the inactivated state and drugs that bind to the non-inactivated state of the Na^+ channel. Exploiting the idealized nature of the canonical binding motifs, we identify the fundamental mechanisms underlying the effects on excitability of the various binding interactions. Specifically, we derive the voltage-dependence of the drug binding time constants and the equilibrium fractions of channels bound to drug, and we then derive a formula that incorporates these time constants and equilibrium fractions to elucidate the fundamental mechanisms. In the case of charged drug, we find that drugs that bind to inactivated channels exhibit greater rate-dependence than drugs that bind to non-inactivated channels. For neutral drugs, the effects of guarded receptor interactions are rate-independent, and we describe a novel mechanism for reverse rate-dependence resulting from neutral drug binding to non-inactivated channels via the gate immobilization motif.

1. Introduction

Drug induced cardiac arrhythmias are a well-documented and often unforeseen side effect of pharmaceutical drugs (Echt et al., 1991; Waldo et al., 1996; Sesti et al., 2000). Drugs that increase the propensity for cardiac arrhythmias typically bind to cardiac ion-channels and alter the corresponding ionic conductance. For many of these drugs, the binding affinity is highly dependent on the conformational state of the channel (i.e., the drug exhibits state-specific binding), and drugs can thereby fundamentally change the kinetics of the ion-channels (Hondeghe and Katzung, 1984; Liu et al., 2003; Yang et al., 2020; Hille, 1977; Huang et al., 2017).

Over 45 years ago, Hille (Hille, 1977) and Hondeghe and Katzung (Hondeghe and Katzung, 1977) hypothesized that drugs such as

lidocaine bind to Na^+ channels in a state-specific manner. More recently, the biophysical basis of state-specific binding is starting to be elucidated for particular drug-ion channel combinations. Studies of Na^+ channel protein structure and site-directed mutagenesis analysis have identified several drug binding sites on various Na^+ channels (Ahuja, 2015; Xu et al., 2019; Xiao et al., 2008; McCormack et al., 2013; Nguyen et al., 2019; Nguyen and Yarov-Yarovoy, 2022). In a few instances, the structural mechanisms underlying state-specific binding, such as binding site occlusion in particular conformational states (Ahuja, 2015) or drug-induced restriction of conformational change (Xu et al., 2019; Xiao et al., 2008). Despite the recent advances in understanding the molecular physiology driving state-specific binding, the link between drug-induced changes in ion-channel kinetics and the resultant effects on cardiac rhythms is not yet well characterized. A better understanding of

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the mechanisms underlying pro-arrhythmic effects of state-specific channel blockers would aid in the screening and design of effective and safe drugs.

Mathematical modeling of drug-ion channel interactions has been helpful in identifying the properties of state-specific drugs (Moreno et al., 2011; Moreno et al., 2013; Weirich and Antoni, 1989; Starmer, 1991). Complicated models of high dimensionality have been used to capture the biophysics of ion channel kinetics with binding of particular state-specific drugs (Yang et al., 2020; Moreno et al., 2011; Moreno et al., 2013; Liu and Rasmusson, 1997; Zhou et al., 2012) (e.g., the Moreno et al. (Moreno et al., 2011) models of the Na⁺ channel's interaction with flecainide or lidocaine consists of 26 states). Such models assume that a drug can bind to channels in multiple conformational states with varying affinities (i.e. the modulated receptor hypothesis (Hille, 1977)). However, the complexity of these models can make it difficult to identify the fundamental mechanisms that lead to pro-arrhythmic effects. On the other hand, idealized models of drug block that capture the essential elements of drug-Na⁺ channel interactions consist of as few as 3 or 4 variables (Weirich and Antoni, 1989; Starmer, 1991; Docken et al., 2021). Therefore, idealized models are both less computationally expensive and more amenable to mathematical analysis, which can reveal fundamental mechanisms causing pro-arrhythmic effects of drug block.

Starmer et al. proposed mathematical models for two canonical binding motifs for strongly state-specific drugs: the guarded receptor motif and what we refer to as the gate immobilization motif (Starmer et al., 1984; Starmer et al., 1987). Starmer et al. used “guarded receptor” to refer to both motifs, but here, we distinguish between state-specific binding drugs that have no effect on channel gating kinetics, “guarded receptor,” and those that immobilize certain gating transitions, “gate immobilization.” The guarded receptor and gate immobilization motifs have been used to characterize how state-specific binding modulates effective binding rates (Weirich and Antoni, 1989; Starmer et al., 1984; Starmer et al., 1987; Karoly et al., 2010) and to examine the effects of particular drug binding models on pro-arrhythmic indicators (Starmer et al., 1991; Aguilar-Shardonofsky et al., 2012; Starmer et al., 2003; Weirich and Antoni, 1990). However, to our knowledge, no framework has been developed that mechanistically connects effects on pro-arrhythmic indicators, such as peak upstroke velocity, conduction velocity, and vulnerable window size (Moreno et al., 2011; Starmer et al., 1991; Starmer et al., 2003; King et al., 2013; Lu et al., 2010), to how various drug binding schemes interact with ion channel dynamics. To begin to develop such a framework, we systematically examine the effect of Na⁺ channel binding drugs whose binding is inactivation dependent, as this is a common feature for state-specific Na⁺ channel blockers (e.g., lidocaine and flecainide) (Liu et al., 2003; Starmer, 1991; Bean et al., 1983; Ragsdale et al., 1996; Liu et al., 2002).

In this study, we introduce four canonical drug binding schemes and use mass action kinetics to derive mathematical models for these binding schemes. We show that each of the models can be reformulated into a low dimensional Hodgkin-Huxley-type model in the case of sufficiently slow drug binding kinetics. Following conventional terminology in cardiac electrophysiology literature, we will refer to the formulations derived directly from mass action kinetics as “Markov models” and the reformulated models as “Hodgkin-Huxley-type models”. We incorporate the four drug binding schemes into a modified ten Tusscher et al. model of ventricular myocytes (ten Tusscher et al., 2004; ten Tusscher and Panfilov, 2006) and assess how peak upstroke velocity, conduction velocity, and vulnerable window depend on basic cycle length (BCL) and drug binding rates for the four drug binding schemes in the context of charged and neutral drug. Exploiting the relatively simple structure of the Hodgkin-Huxley-type models, we identify the fundamental mechanisms underlying the rate-dependent effects of the four drug binding schemes.

2. Models

The fast Na⁺ current is well modeled by ohmic resistance, i.e., the product of the Na⁺ conductance, ($\bar{g}_{Na}f_{open}$), and the driving force, ($V - E_{Na}$)

$$I_{Na} = \bar{g}_{Na}f_{open}(V - E_{Na}), \quad (1)$$

where f_{open} is the fraction of Na⁺ channels in the open (conducting) state, $\bar{g}_{Na}$ is the maximal Na⁺ conductance (i.e., the conductance when all Na⁺ channels are open), V is the transmembrane potential, and E_{Na} is the Na⁺ reversal potential (Hodgkin and Huxley, 1952). According to Hodgkin-Huxley dynamics, each Na⁺ channel is typically assumed to have three activation gates and a single inactivation gate that act independently, and all gates must be in the open state in order for the channel to be open (Hodgkin and Huxley, 1952). Therefore, the fraction of open channels is

$$f_{open} = m^3h, \quad (2)$$

where the variable m represents the fraction of open activation gates, and the variable h represents the fraction of open inactivation gates. The dynamics of the gating variables m and h are described by first order kinetics,

$$\frac{dz}{dt} = \alpha_z(V)(1 - z) - \beta_z(V)z = \frac{1}{\tau_z(V)}(z_{\infty}(V) - z), \quad (3)$$

where $z = m$ or h , $\alpha_z(V)$ and $\beta_z(V)$ are the voltage-dependent opening and closing rate constants for corresponding gates, and $\tau_z(V) = \frac{1}{\alpha_z(V) + \beta_z(V)}$ and $z_{\infty}(V) = \frac{\alpha_z(V)}{\alpha_z(V) + \beta_z(V)}$ are the time constant and steady state fraction of open gates for gate z .

Here, we modify Hodgkin-Huxley dynamics to include the binding of state-specific Na⁺ channel blockers. Specifically, we maintain the assumption that activation and inactivation processes occur independently, but we extend the model of inactivation kinetics to include drug binding. We consider both the guarded receptor and gate immobilization drug-binding motifs with either inactivated or non-inactivated state binding, resulting in a total of four drug binding schemes.

2.1. Markov models

Each of the four drug binding schemes that we consider is illustrated as a conformational state diagram in Fig. 1B. The guarded receptor and gate immobilization motifs are shown in the top and bottom rows, respectively. Inactivated state binding and non-inactivated state binding are shown in the left and right columns, respectively. In the diagrams, I_O represents the conformational states in which the inactivation gate is open (i.e., includes all non-inactivated states) and no drug is bound to the channel, and I_C represents the conformational states in which the inactivation gate is closed and no drug is bound to the channel. The non-inactivated and inactivated conformational states in which drug is bound are represented by I_O^* and I_C^* , respectively. The rate constants for drug binding and unbinding are $[D]k_{on}$ and k_{off} , where $[D]$ is the concentration of drug.

The guarded receptor motif (Fig. 1B, top row) assumes that drug binding has no effect on the kinetics of the inactivation gate, but drug binding and unbinding can only occur when a channel is in specific conformational states (Starmer et al., 1984). That is, when a channel is not in a conformational state that allows drug binding, the inactivation gate “guards” access to the drug binding site. The gate immobilization motif is fundamentally different from the guarded receptor motif in that drug binding alters gating kinetics (Fig. 1B, bottom row). As in the guarded receptor motif, drug binding is state-specific, but unlike in the guarded receptor motif, once a drug binds, the inactivation gate cannot change conformations until the drug unbinds (Starmer et al., 1987). In

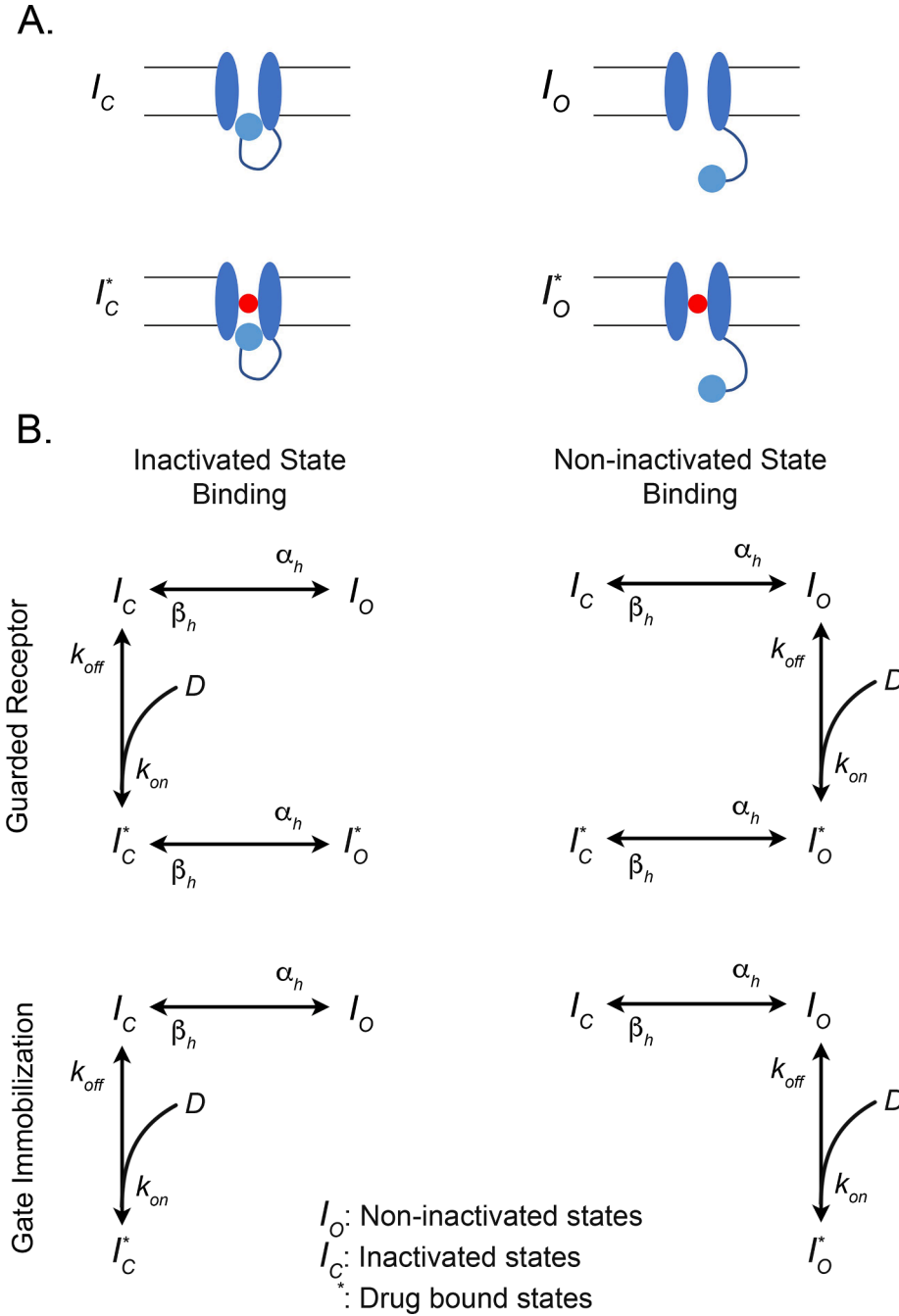


Fig. 1. Drug binding schemes. (A) Schematics of various conformational states of the Na^+ channel: inactivated non-drug bound (I_C), non-inactivated non-drug bound (I_O), inactivated drug bound (I_C^*), and non-inactivated drug bound (I_O^*). (B) Conformational state diagrams illustrating the four drug binding models being considered: the guarded receptor model with inactivated state binding, GRI (top left), the guarded receptor model with non-inactivated state binding, GRN (top right), the gate immobilization model with inactivated state binding, GII (bottom left), and the gate immobilization model with non-inactivated state binding, GIN (bottom right).

this way, the gate immobilization motif is similar to competitive enzyme inhibitors, where the inhibitor blocks the enzyme from binding to the substrate (Keener, 2009).

For each of the four drug binding models, we use the law of mass action to derive the equations governing the evolution of the state of Na^+ channels. We refer to these models as “Markov models” as is the convention in cardiac electrophysiology literature. The variables I_O , I_C , I_O^* , and I_C^* are defined as the fraction of Na^+ channels in the corresponding states. We assume that the drug bound states are non-conducting (i.e., the drug fully blocks the channel). Thus, the fraction of open Na^+ channels in each drug binding model is

$$f_{\text{open}} = m^3 I_O,$$

where m is described by the standard Hodgkin-Huxley activation variable model (Eq. (3)). The drug concentration $[D]$ is assumed to be high

compared to the concentration of Na^+ channels so that $[D]$ can be approximated as a constant. By applying the algebraic constraint $I_O + I_C + I_O^* + I_C^* = 1$, we obtain the following systems of two or three ordinary differential equations for each drug binding scheme.

GRI model: Guarded receptor model with inactivation state binding (Fig. 1B, top left)

$$\frac{dI_O}{dt} = \alpha_h(V)I_C - \beta_h(V)I_O$$

$$\frac{dI_C}{dt} = \beta_h(V)I_O + k_{\text{off}}I_C^* - (\alpha_h(V) + [D]k_{\text{on}})I_C$$

$$\frac{dI_C^*}{dt} = \beta_h(V)(1 - I_O - I_C - I_C^*) + [D]k_{\text{on}}I_C - (k_{\text{off}} + \alpha_h(V))I_C^*, \quad (4)$$

$$\text{and } I_O^* = 1 - I_O - I_C - I_C^*.$$

GRN model: Guarded receptor model with non-inactivation state binding (Fig. 1B, top right)

$$\begin{aligned}\frac{dI_O}{dt} &= \alpha_h(V)I_C + k_{off}(1 - I_O - I_C - I_C^*) - (\beta_h(V) + [D]k_{on})I_O \\ \frac{dI_C}{dt} &= \beta_h(V)I_O - \alpha_h(V)I_C \\ \frac{dI_C^*}{dt} &= \beta_h(V)(1 - I_O - I_C - I_C^*) - \alpha_h(V)I_C^*,\end{aligned}\quad (5)$$

$$\text{and } I_O^* = 1 - I_O - I_C - I_C^*.$$

GII model: Gate immobilization model with inactivated state binding (Fig. 1B, bottom left):

$$\begin{aligned}\frac{dI_O}{dt} &= \alpha_h(V)(1 - I_O - I_C^*) - \beta_h(V)I_O \\ \frac{dI_C^*}{dt} &= [D]k_{on}(1 - I_C^* - I_O) - k_{off}I_C^*,\end{aligned}\quad (6)$$

$$\text{and } I_C = 1 - I_O - I_C^*.$$

GIN model: Gate immobilization model with non-inactivated state binding (Fig. 1B, bottom right):

$$\begin{aligned}\frac{dI_O}{dt} &= \alpha_h(V)(1 - I_O - I_O^*) + k_{off}I_O^* - (\beta_h(V) + [D]k_{on})I_O \\ \frac{dI_O^*}{dt} &= [D]k_{on}I_O - k_{off}I_O^*,\end{aligned}\quad (7)$$

$$\text{and } I_C = 1 - I_O - I_O^*.$$

2.2. Channel blocker model reformulation and reduction

Although the Markov models are only 2- or 3-dimensional, the current formulation does not lend itself to mathematical analysis. However, when drug binding occurs on a time-scale much slower than gating kinetics, $k_{off} + [D]k_{on} \ll \alpha_h(V) + \beta_h(V)$ (e.g., in the case of lidocaine and potentially flecainide (Moreno et al., 2011)), the drug binding models can be transformed into models that are amenable to both mathematical analysis and biophysical interpretation (Starmer et al., 1984; Starmer et al., 1987). Using singular perturbation theory to exploit the difference in time-scales between inactivation and drug binding (see Supplemental Information S1 for details) (Keener, 2009), the Markov models are recast into a new formulation in which the Na^+ channel conductance is

$$\bar{g}_{Na}f_{open} = \bar{g}_{Na}m^3I_O = \bar{g}_{Na}m^3h(1 - b). \quad (8)$$

Note that the fraction of unblocked channels $(1 - b)$ appears as an additional Hodgkin-Huxley-like gating term, and therefore we refer to these models as the “Hodgkin-Huxley-type models.” In this new formulation, the inactivation variable dynamics are independent of drug binding dynamics and revert to the standard Hodgkin-Huxley form for all of the drug binding models considered,

$$\frac{dh}{dt} = \frac{1}{\tau_h(V)}(h_\infty(V) - h),$$

where $\tau_h(V) = \frac{1}{\alpha_h(V) + \beta_h(V)}$ and $h_\infty(V) = \frac{\alpha_h(V)}{\alpha_h(V) + \beta_h(V)}$. Dynamics for drug binding variable, b , take on a modified Hodgkin-Huxley form,

$$\frac{db}{dt} = \frac{1}{\tau_{b,X}(V, h)}(b_{\infty,X}(V, h) - b), \quad (9)$$

where $\tau_{b,X}$ and $b_{\infty,X}$ are the time constant and quasi-steady state of b , and the index X identifies the drug binding scheme (i.e., GRI, GRN, GII, or GIN). As is the case for standard Hodgkin-Huxley gating variables, the

evolution of b is described by first-order kinetics, but the rate constants for b explicitly depend on the level of inactivation, h . Note that the different kinetics of the four drug binding models generate different dependence on h , as indicated by the $\tau_{b,X}$ and $b_{\infty,X}$ formulae provided in Table 1.

The Hodgkin-Huxley-type formulation was first proposed by Starmer et al. (Starmer et al., 1984; Starmer et al., 1987), but to our knowledge, no detailed derivation of the equations was provided, therefore we include our derivations in the Supplemental Information S1. Our derivations imply that the Hodgkin-Huxley-type formulations are only strictly valid for sufficiently slow drug binding kinetics, but we will show in Section 3.2 that the models are often surprisingly accurate in parameter regimes where the reduction is not rigorously justified.

There are a few items to note about the differences between the Hodgkin-Huxley-type guarded receptor and gate immobilization models. 1) The essential feature of the guarded receptor models is that rate of change of the fraction of blocked channels is scaled by the fraction of channels available for drug binding (i.e., $1 - h$ and h for inactivated and non-inactivated state binding, respectively), whereas in the gate immobilization models, both the time constant of drug binding and the steady state fraction of channels bound to drug are modulated by the fraction of channels available for binding. 2) In the guarded receptor binding schemes, h directly corresponds to the fraction of channels that are not inactivated (i.e., $h = I_O + I_O^*$), whereas in the gate immobilization binding schemes, h is the fraction of non-drug-bound channels that are not inactivated (i.e., $h = \frac{I_O}{I_O + I_C}$). 3) The Hodgkin-Huxley-type reformulation of the guarded receptor models reduces the dimension from 3 to 2, whereas the dimension of the gate immobilization models remains at 2. However, as mentioned above, the partial separation of h and b dynamics provided by this formulation will prove helpful in our analysis.

2.3. Simulation parameters

For all simulations presented, the parameters of the Na^+ current model are from the 37 °C parameterization of our previously published model (Docken et al., 2021) and are reported in Appendix A1. The rate constants of drug binding, k_{on} and k_{off} , have the functional forms used by Moreno et al. (Moreno et al., 2011) to model flecainide and lidocaine. Specifically, k_{on} is a constant, and for charged drug, $k_{off} = k_{on}K_{D0}e^{-0.7VF/RT}$ with $F/RT = 0.0374 \text{ mV}^{-1}$. In our consideration of neutral drug in Section 3.6, $k_{off} = k_{on}K_{D0}$ is a constant. Unless otherwise specified, $K_{D0} = 10 \text{ } \mu\text{M}$ (similar to the $11.2 \text{ } \mu\text{M}$ used for flecainide in Moreno et al. (Moreno et al., 2011) and $[D] = 20 \text{ } \mu\text{M}$. We consider values of k_{on} that span several orders of magnitude: 10^1 – $10^7 \text{ M}^{-1}\text{ms}^{-1}$. This range of values for k_{on} includes the values that Moreno et al. (Moreno et al., 2011) used to model flecainide ($k_{on} =$

Table 1

Effective affinity and time constant of drug binding. Formulae for $\tau_{b,X}$ and $b_{\infty,X}$ from Equation (9) for the various drug binding models given in terms of the time constant and steady state fraction of channels bound to drug in the non-state-specific binding case: $\tau_b(V) = \frac{1}{[D]k_{on} + k_{off}(V)}$ and $b_\infty(V) = \frac{[D]k_{on}}{[D]k_{on} + k_{off}(V)} =$

	Inactivated State Binding (I)	Non-inactivated State Binding (N)
Guarded Receptor (GR)	$\tau_{b,GRI}(V, h) = \frac{\tau_b(V)}{1 - h}$ $b_{\infty,GRI}(V, h) = b_\infty(V)$	$\tau_{b,GRN}(V, h) = \frac{\tau_b(V)}{h}$ $b_{\infty,GRN}(V, h) = b_\infty(V)$
Gate Immobilization (GI)	$\tau_{b,GII}(V, h) = \frac{\tau_b(V)}{1 - hb_\infty(V)}$ $b_{\infty,GII}(V, h) = \frac{b_\infty(V)(1 - h)}{1 - hb_\infty(V)}$	$\tau_{b,GIN}(V) = \frac{\tau_b(V)}{1 - (1 - h)b_\infty(V)}$ $b_{\infty,GIN}(V) = \frac{hb_\infty(V)}{1 - (1 - h)b_\infty(V)}$

5500 $M^{-1}ms^{-1}$) and lidocaine ($k_{on} = 500 M^{-1}ms^{-1}$). We will usually describe drug binding rates in terms of the time-constants of drug binding at a transmembrane potential of $-20 mV$, $\tau_b(-20 mV) = ([D]k_{on} + k_{off}(-20 mV))^{-1}$, which provides context for how the rate of drug binding compares to the rate of inactivation ($\tau_h(-20 mV) = 0.27 ms$) near the point of the peak upstroke velocity. The k_{on} values of $10 M^{-1}ms^{-1}$ and $10^7 M^{-1}ms^{-1}$ correspond to drug binding time-constants, $\tau_b(-20 mV)$, of $2.7 \times 10^3 ms$ and $2.7 \times 10^{-3} ms$, respectively, for charged drug.

3. Results

3.1. Comparison of the effects of different drug binding models on action potential upstroke velocity

To examine how the drug binding schemes and binding rates influence single-cell excitability, we incorporate the Markov models of the four drug binding schemes (Eqs. (4)–(7)) into a modified ten Tusscher model for human ventricular cells (ten Tusscher et al., 2004; ten Tusscher and Panfilov, 2006) (see Appendices A1 and A2 for details) and measure maximum upstroke velocity ($\dot{V}_{max}$) of action potentials as a function of pacing frequency. The model cells are paced at basic cycle lengths (BCL) from 300 ms to 1000 ms, i.e., 60–200 beats per minute. We will refer to this range of BCLs as the “physiological range” of BCLs. For each BCL, the model cell is paced 500 times before recording $\dot{V}_{max}$. The amplitude and duration of stimuli were $-80 pA/pF$ and 0.6 ms, respectively. In Fig. 2, $\dot{V}_{max}$ is plotted as a function of BCL for each drug binding model and for the three drug binding rates: “slow” ($\tau_b(-20 mV) = 2700 ms$), “intermediate” ($\tau_b(-20 mV) = 27 ms$), and “fast” ($\tau_b(-20 mV) = 0.27 ms$). In the absence of drug (thick black lines), $\dot{V}_{max}$ in our model varies little (276–294 mV/ms) over the physiological range of BCLs. For action potentials elicited from rest or following extremely long BCL, $\dot{V}_{max}$ is 326 mV/ms (black circle to the right of each graph).

For the slowest drug binding rate considered ($\tau_b(-20 mV) =$

2700 ms, Fig. 2A), the four drug binding models lead to different levels of drug block (i.e., reduction in $\dot{V}_{max}$) and rate-dependencies. For resting level block and long BCL, $\dot{V}_{max}$ in the GII model (solid faded red line) is close to the no drug case, whereas $\dot{V}_{max}$ is reduced by approximately 20% in the three other drug binding models. In inactivated state binding schemes (GRI and GII), the level of block increases markedly as BCL decreases. The reduction of $\dot{V}_{max}$ in the GII model (solid faded red line) increases from 1% for long BCL to 8% and 33% for BCLs of 1000 ms and 300 ms, whereas the level of block in the GRI model (solid faded blue line) increases from 20% for long BCL to 51% for BCL = 1000 ms and 60% for BCL = 300 ms. Therefore, GRI exhibits greater rate-dependence over the entire possible range of BCLs and a greater overall level of block, but the GII scheme displays greater rate-dependence over the physiological range of BCLs. The rate-dependence of $\dot{V}_{max}$ is substantially weaker in the non-inactivated state binding models (GRN and GIN) than in the inactivated state binding models (GRI and GII). In fact, the reduction of $\dot{V}_{max}$ for the GRN (dashed faded blue line) remains close to 20% for all BCL, which indicates that drug-binding is not rate-dependent in the GRN scheme. The GIN scheme (dashed faded red line) interestingly exhibits slight reverse rate-dependence of peak upstroke velocity, with the reduction of $\dot{V}_{max}$ slowly decreasing from 20% to 15% as BCL decreases to 300 ms.

Fig. 2B illustrates the levels of block for an “intermediate” drug binding time-constant ($\tau_b(-20 mV) = 27 ms$) that is longer than that of the inactivation process but shorter than typical action potential durations (APDs) and diastolic intervals (DIs). Several striking changes arise from increasing the rate of drug binding (i.e., compare Fig. 2A to B). The level of block for the GII model and the GRI model is substantially lower than that for the case with slower drug binding. The drug binding in the GII scheme now has almost no effect on $\dot{V}_{max}$ (solid faded red line), and the maximal block for the GRI model has decreased from approximately 60% to 30%, with a corresponding decrease in the overall rate-dependence (solid faded blue line). The level of drug block in the GRN scheme for intermediate drug binding (Fig. 2B dashed faded blue line) is virtually unchanged from that for the slow drug binding (Fig. 2A),

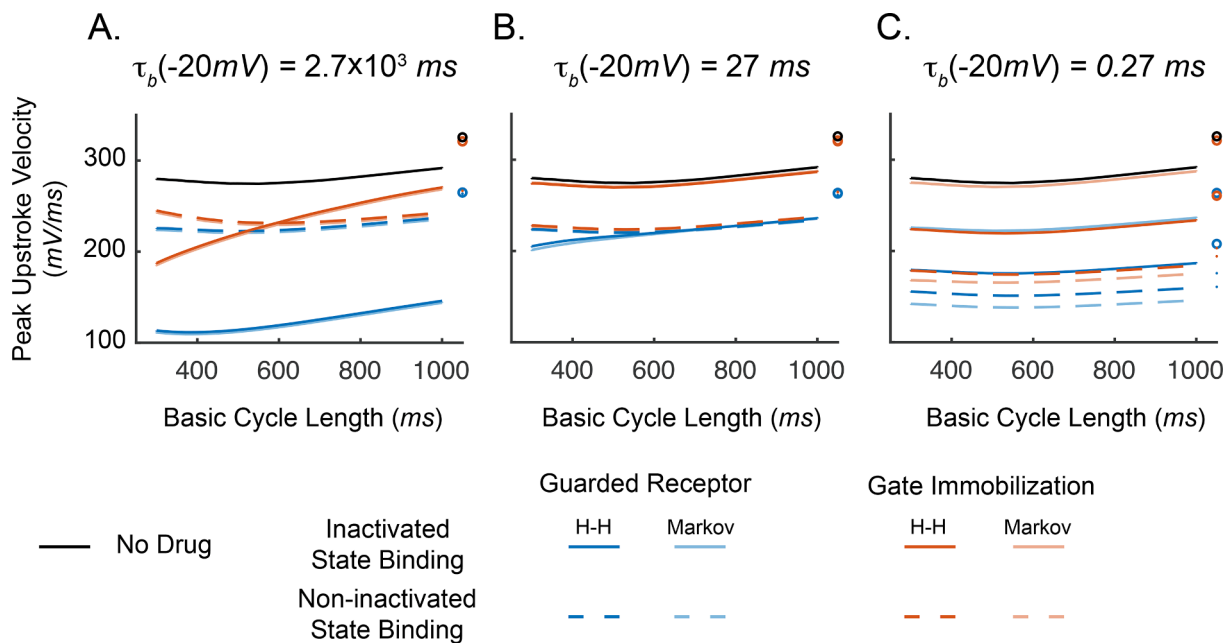


Fig. 2. Peak upstroke velocity vs. BCL. $\dot{V}_{max}$ as a function of basic cycle length (BCL) is shown for no drug (black), the GRI model (solid blue), the GRN model (dashed blue), the GII model (solid red), and the GIN model (dashed red) and for drug binding time-constants of $\tau_b(-20 mV) = 2700 ms$ “slow” (A), $\tau_b(-20 mV) = 27 ms$ “intermediate” (B), and $\tau_b(-20 mV) = 0.27 ms$ “fast” (C). Results for Markov models of drug binding dynamics are shown as faded lines, and results for Hodgkin-Huxley-type models are shown as dark lines. Circles (dots) to the right of each plot represent the $\dot{V}_{max}$ in inactivated (non-inactivated) state binding in each model for extremely long BCLs. In (A), Hodgkin-Huxley-type models artificially shifted up 2 mV/ms for visibility.

differing by only 1–2 mV/ms. Moreover, the reverse rate-dependence in the GIN scheme (dashed red line) is eliminated for the faster drug binding, and $\dot{V}_{max}$ is almost identical to that of the GRN scheme.

Fig. 2C shows the effects of further decreasing the time-constant of drug binding so that it is now similar to the time-constant of inactivation ($\tau_b(-20\text{ mV}) = 0.27\text{ ms}$). As was the case with drug binding at the intermediate time-scale, the effects of drug binding for the GII model (solid faded red line) are negligible. For the GRI model (solid faded blue line), the increase from “intermediate” to “fast” drug binding has little effect on the overall level of block, but it eliminates the weak rate-dependent effects seen for “intermediate” drug binding. On the other hand, for the non-inactivated state binding schemes, the increase in drug binding rate from “intermediate” to “fast” causes a substantial increase in the overall level of block. Specifically, the 20% reduction in $\dot{V}_{max}$ for “intermediate” drug binding rates increases to 50% in the GRN (dashed faded blue line) and 40% in the GIN models (dashed faded red line) for “fast” drug binding.

Interestingly, the effects of the models with non-inactivated state binding follow a different trend from the inactivated state binding models as the drug binding time-constant changes. Specifically, the level of block in the case of inactivate state binding decreases as drug binding rate increases, whereas the level of block in the case of non-inactivate state binding increases as drug binding rate increases. Fig. 3 explicitly illustrates these trends in $\dot{V}_{max}$ as a function of binding rate, using a BCL of 1000 ms. For models with non-inactivated state binding (dashed faded lines), the level of block increases from 19% to 61% in the guarded receptor (GRN) model and from 17% to 38% in the gate immobilization (GIN) model as the time-constant of drug binding is decreased. Whereas for models with inactivated state binding (solid faded lines), the level of block decreases from 51% to 19% in the guarded receptor (GRI) model and from 8% to 2% in the gate immobilization (GII) model as the time-constant of drug binding is decreased.

3.2. Assessment of the Hodgkin-Huxley-type drug binding models

Figs. 2 and 3 provide a comparison of results using the Markov models of the four drug binding schemes (faded lines) and the “reduced” Hodgkin-Huxley-type models (dark lines). Recall that the derivation of the Hodgkin-Huxley-type models assumes that the time-constant of drug binding is much larger than the time-constant of inactivation ($\tau_b \gg \tau_h$). As expected, for sufficiently slow drug binding rates, there is excellent agreement between the Hodgkin-Huxley-type and Markov models for all drug binding schemes (i.e., negligible difference between Markov and Hodgkin-Huxley-type models for $\tau_b(-20\text{ mV}) > 20\text{ ms}$; Fig. 2A and B and Fig. 3). Surprisingly, the Hodgkin-Huxley-type and the Markov models for non-inactivated state binding schemes (Fig. 2C and Fig. 3 dashed lines) maintain relatively good agreement, even when $\tau_b < \tau_h$. The largest disagreement for the non-inactivated binding schemes occurs when the drug binding rate is similar to the rate of inactivation ($\tau_b \sim \tau_h$), with Hodgkin-Huxley-type models under-predicting the reduction in $\dot{V}_{max}$ due to drug binding by no more than 5%. On the other hand, $\dot{V}_{max}$ in the Hodgkin-Huxley-type models deviates substantially from that in the Markov models for inactivated state binding (Fig. 3 solid lines) for $\tau_b < 5\text{ ms}$ (i.e., $\tau_b \lesssim \tau_h$). In fact, while fast drug binding in the Markov models reduces $\dot{V}_{max}$ by only 19% and 2% in the GRI and GII schemes, respectively, the Hodgkin-Huxley-type models over-predict the reduction in $\dot{V}_{max}$ by as much as an additional 40–50%.

The mechanisms underlying the disparities and similarities in peak upstroke velocity between the Markov models and the Hodgkin-Huxley-type models described above are discussed in detail in Supplemental Information S2.

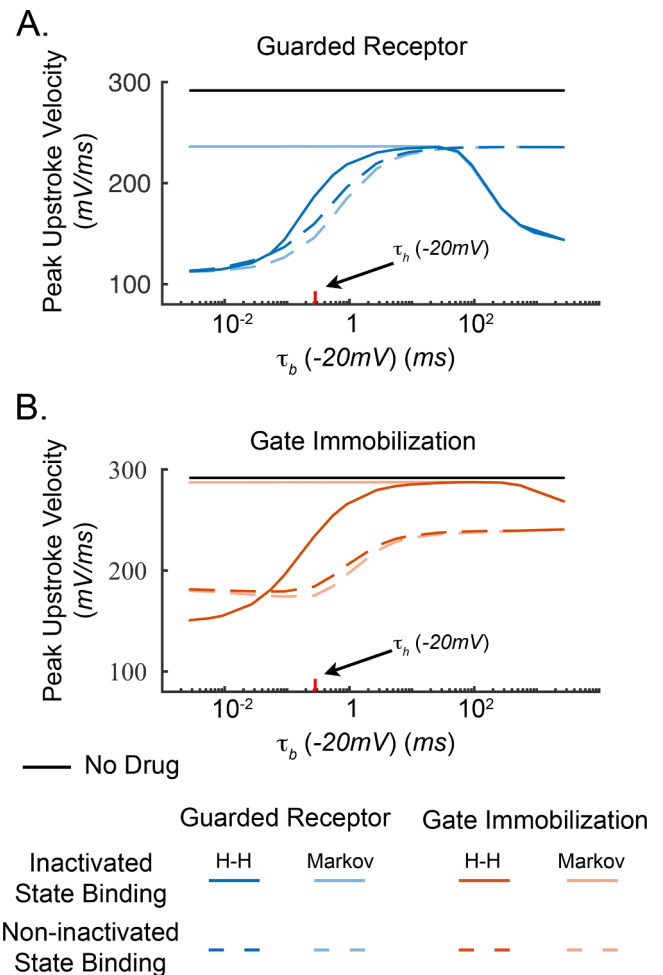


Fig. 3. Peak upstroke velocity vs. drug binding rate. $\dot{V}_{max}$ dependence in the modified ten Tusscher et al. model on drug binding rate for a BCL of 1000 ms: guarded receptor (top panel) and gate immobilization (lower panel) models with inactivated (solid lines) and non-inactivated (dashed lines) state binding, and Markov (faded lines) and Hodgkin-Huxley-type (dark lines) models of drug binding schemes. The shortest drug binding time-constant is two orders of magnitude faster than the time-constant used in Fig. 2C, and the longest time-constant corresponds to the time-constant used in Fig. 2A.

3.3. Effects of drug binding on upstroke velocity are captured by a one-dimensional map

When drug binding is sufficiently slow ($\tau_b \gg \tau_h, \tau_V$ (τ_V is the time constant of transmembrane potential), as in Fig. 2A and B, i.e., “slow” and “intermediate” drug binding rates), the dynamics of the modified ten Tusscher et al. model can be reduced to the consideration of a one-dimensional (1D) map (recursion equation) (Docken et al., 2021; Starmer et al., 1987; Weirich and Antoni, 1990). Specifically, the 1D map provides the fraction of Na^+ channels bound to drug during the $n + 1^{\text{th}}$ action potential upstroke as a function of the fraction of channels bound to drug during the n^{th} upstroke, thus capturing the beat-to-beat variation in the level of block during the action potential upstroke.

Key modeling assumptions in the derivation of the 1D map for the fraction of channels blocked (b) rely on several characteristics of the dynamics throughout each action potential (AP) cycle. Each AP cycle consists of phases of fast-transitions of V (i.e., the upstroke and rapid depolarization of the AP) and phases in which the changes in V are slow and relatively small (i.e., the AP plateau and diastolic interval (DI)). Therefore, the membrane potential, V , during a train of action potentials can be well approximated by a square wave (Weirich and Antoni, 1989;

Docken et al., 2021; Weirich and Antoni, 1990), setting $V = V_{AP} = 20$ mV during the plateau phase and $V = V_{DI} = -80$ mV during the DI (Fig. 4A). Because Na^+ channel inactivation is relatively fast, the inactivation variable can be taken to be $h = h_\infty(V)$ during the DI and AP plateau phases (Fig. 4B). Due to the brevity of the fast-transition phases and the relatively long time-constant of drug binding, the fraction of blocked channels, b , can be assumed to be constant during these rapid transitions in V . On the other hand, during the slow-phases, b has the potential to change dramatically (Fig. 4C-F). Finally, for all drug binding models and model parameter sets considered, while drug binding can have a substantial effect on $\dot{V}_{max}$, it has a negligible effect on the duration of the action potential (APD) and the transmembrane potential during the plateau and DI. Accordingly, for any given BCL, the APD and DI are taken from the steady-state restitution curve of the drug-free modified ten Tusscher model (see Fig S3.1 for restitution curves).

Under the assumptions and approximations outlined above, the equations governing the full dynamics of the fraction of drug bound Na^+ channels, b , reduce to a single piecewise-defined linear ordinary differential equation

$$\frac{db}{dt} = \frac{1}{\tau_{b,X}(V)} (b_{\infty,X}(V) - b)$$

$$V = \begin{cases} V_{AP}, & 0 \leq \text{mod}(t, BCL) < APD \\ V_{DI}, & APD \leq \text{mod}(t, BCL) < BCL \end{cases} \quad (10)$$

where X is *GRI*, *GRN*, *GII*, and *GIN* for the guarded receptor with inactivated state binding, guarded receptor with non-inactivated state binding, gate immobilization with inactivated state binding, and gate immobilization with non-inactivated state binding models, respectively. $\tau_{b,X}(V)$ and $b_{\infty,X}(V)$ are the effective time-constants and steady-state functions (affinity) for drug binding as modified by the level of inactivation in the four different models, as listed in Table 1 with $h = h_\infty(V)$ (see derivations in Supplemental Information S1).

The linear nature of Eq. (10) allows explicit solutions to be computed for b during the action potential and during the diastolic interval (plotted in Fig. 4C-F), and the beat-to-beat evolution of b is obtained by

appropriately piecing these solutions together. If b_n is defined to be the fraction of channels blocked during the upstroke of the n^{th} AP, then the fraction of channels blocked at the end of the n^{th} AP (i.e., after a time APD) is

$$b_{n+\frac{1}{2}} = b_{\infty,X}(V_{AP}) - (b_{\infty,X}(V_{AP}) - b_n) e^{-\frac{APD}{\tau_{b,X}(V_{AP})}}, \# \quad (11)$$

and the fraction of channels bound to drug during the upstroke of the $n+1^{\text{th}}$ AP (i.e., after a further time DI) is

$$b_{n+1} = b_{\infty,X}(V_{DI}) - \left(b_{\infty,X}(V_{DI}) - b_{n+\frac{1}{2}} \right) e^{-\frac{DI}{\tau_{b,X}(V_{DI})}}, \# \quad (12)$$

The composition of Eqs. (11) and (12) yields the 1D map describing the beat-to-beat variation of the level of block (b) at the beginning of each action potential

$$b_{n+1} = F_X(b_n, BCL),$$

where the subscript X indicates the dependence of map on the different drug binding models (*GRI*, *GRN*, *GII*, and *GIN*) through $\tau_{b,X}(V)$ and $b_{\infty,X}(V)$. Again, note that the APD and DI are determined by the BCL via the APD restitution curve.

The steady-state level of block during the upstroke of the AP as a function of BCL is given by the fixed point of the map

$$b_X^*(BCL) = F_X(b_X^*(BCL); BCL),$$

which is

$$b_X^*(BCL) = w b_{\infty,X}(V_{DI}) + (1-w) b_{\infty,X}(V_{AP}), \quad (13)$$

where

$$w = \frac{1-d}{1-ad},$$

and

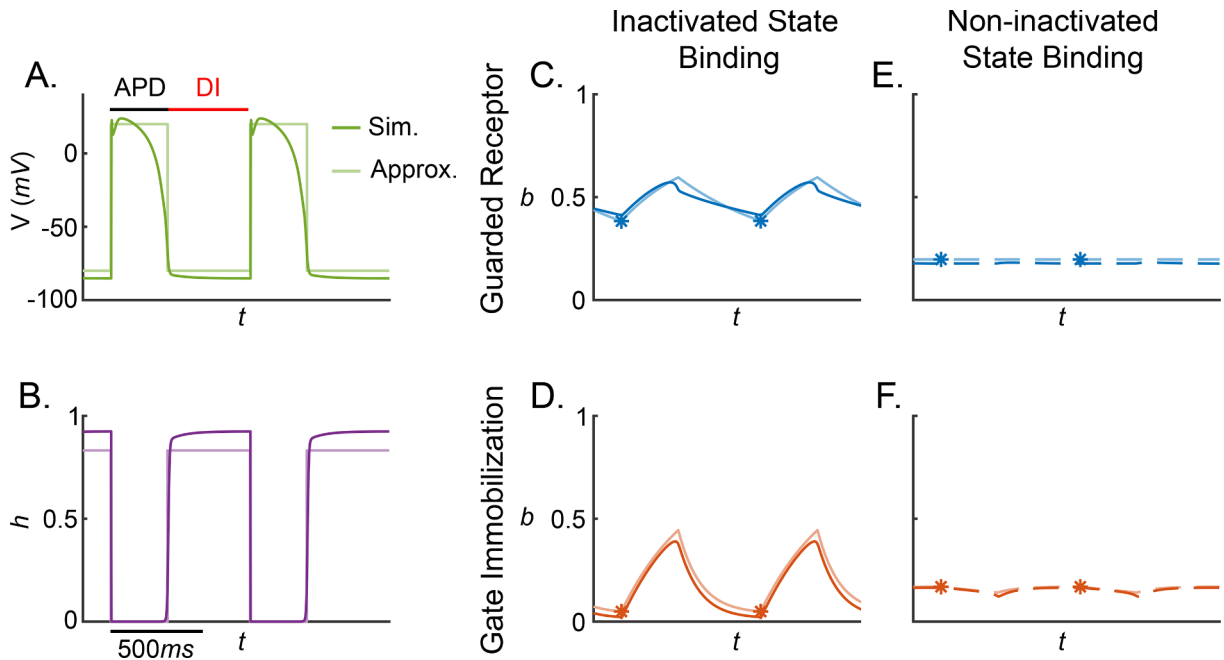


Fig. 4. Square wave approximation of action potential cycle. (A) Action potentials for the modified ten Tusscher model (dark green) and a square wave approximation of V (faded green) during pacing with a BCL of 750 ms. The dynamics of h (B) and b (C-F) resulting from the modified ten Tusscher simulation (dark lines) and square wave approximation of transmembrane potential (faded lines) in the *GRI* model (C), *GII* model (D), *GRN* model (E), and *GIN* model (F) state binding with $\tau_b(-20 \text{ mV}) = 270$ ms are shown.

$$a = e^{-\frac{APD}{\tau_{b,X}(V_{AP})}}, d = e^{-\frac{DI}{\tau_{b,X}(V_{DI})}}.$$

It is straightforward to show that the map is a linear contraction map and therefore the fixed point, $b_X^*(BCL)$, is always unique and stable.

To assess the applicability of the 1D map, we compare the fraction of channels not bound to drug at the upstroke as predicted by the map ($1 - b_X^*(BCL)$) to the steady-state peak upstroke velocity of the modified ten Tusscher et al. model in the presence of drug ($\dot{V}_{max,drug}$). To do this, we assume that $\dot{V}_{max,drug}$ can be approximated by the product of $1 - b_X^*(BCL)$ and peak upstroke velocity when no drug is present, $\dot{V}_{max,nodrug}$. That is, $1 - b_X^*(BCL)$ is equal to the normalized peak upstroke velocity

$$\frac{\dot{V}_{max,drug}}{\dot{V}_{max,nodrug}} \cong (1 - b_X^*(BCL)).$$

Fig. 5 depicts $1 - b_X^*(BCL)$ from the 1D map (faded lines) and peak upstroke velocities from the ventricular cell model simulations (Fig. 2A and B) normalized by the no drug case, $\frac{\dot{V}_{max,drug}}{\dot{V}_{max,nodrug}}$, (dark lines) for each drug binding model. For the GII, GIN, and GRN binding schemes, $1 - b_X^*(BCL)$ and the normalized peak upstroke velocities are almost indistinguishable at both slow (Fig. 5A) and intermediate (Fig. 5B) drug binding rates. The agreement between the 1D map and the full cell model is also good for the GRI binding scheme, which has a maximum deviation of 19% at a BCL of 1000 ms in the case of slow binding. (We note that the agreement could be made even better by optimizing the choice of V_{AP} and V_{DI}).

3.4. Mechanisms underlying the effects of drug binding schemes on peak upstroke velocity

The excellent agreement between $1 - b_X^*(BCL)$ and normalized peak upstroke velocity demonstrates that the 1D map captures the essential features of the drug binding models when binding is slow relative to the rate of Na^+ channel inactivation. This suggests that the effects of the various drug binding schemes on $\dot{V}_{max}$ described in Section 3.1 for

“sufficiently slow” drug binding can be explained by analyzing the expression for $b_X^*(BCL)$ (Eq. (13)).

For all drug binding models, the fraction of channels blocked during the upstroke is the result of the competition between AP and DI drug binding dynamics, and the expression for $b_X^*(BCL)$ concisely captures this competition in a single equation. During the AP and DI, the fraction of blocked Na^+ channels, b , exponentially approaches the target values of $b_{\infty,X}(V_{AP})$ and $b_{\infty,X}(V_{DI})$, respectively (as indicated by Eqs. (11)–(12) and seen in Fig. 4C–F). The magnitudes of change during these phases are determined not only by $b_{\infty,X}(V_{AP})$ and $b_{\infty,X}(V_{DI})$ but also by the duration of the phases normalized by the corresponding effective drug binding time constants, $APD/\tau_{b,X}(V_{AP})$ and $DI/\tau_{b,X}(V_{DI})$. Therefore, $b_X^*(BCL)$ takes the form of an appropriately weighted sum of the steady state fractions of channels bound to drug (i.e., $b_{\infty,X}(V_{AP})$ and $b_{\infty,X}(V_{DI})$), where the factors determining the weighting, a and d , are set by the normalized duration of the phases.

In this section, we utilize the equation for $b_X^*(BCL)$ (Eq. (13)) to explain the key observations of the full modified ten Tusscher et al. model with drug binding in Fig. 2A and B: 1) the resting level of block for each binding scheme, 2) the greater rate-dependence of inactivated compared to non-inactivated state binding among the gate immobilization schemes, 3) the reverse rate-dependence of the GIN scheme, and 4) the greater rate-dependence of inactivated compared to non-inactivated state binding among the guarded receptor schemes. For each of these observation, we first provide an explanation based on the terms of Eq. (13) (i.e., $b_{\infty,X}(V_{AP})$, $b_{\infty,X}(V_{DI})$, $APD/\tau_{b,X}(V_{AP})$ and $DI/\tau_{b,X}(V_{DI})$), highlighting the disparities in these terms for the various binding schemes, as illustrated in Fig. 6. We then describe the biophysical mechanisms underlying the disparities in steady state fraction of channels bound to drug, $b_{\infty,X}(V)$, and time constant of drug binding, $\tau_{b,X}(V)$, between the binding schemes. Thus, we provide the biophysical mechanisms for the drug-induced changes in $\dot{V}_{max}$ for the four drug binding schemes.

3.4.1. Resting level block is lower in GII than other three binding schemes

At rest or for long BCL, the GII model exhibits negligible levels of

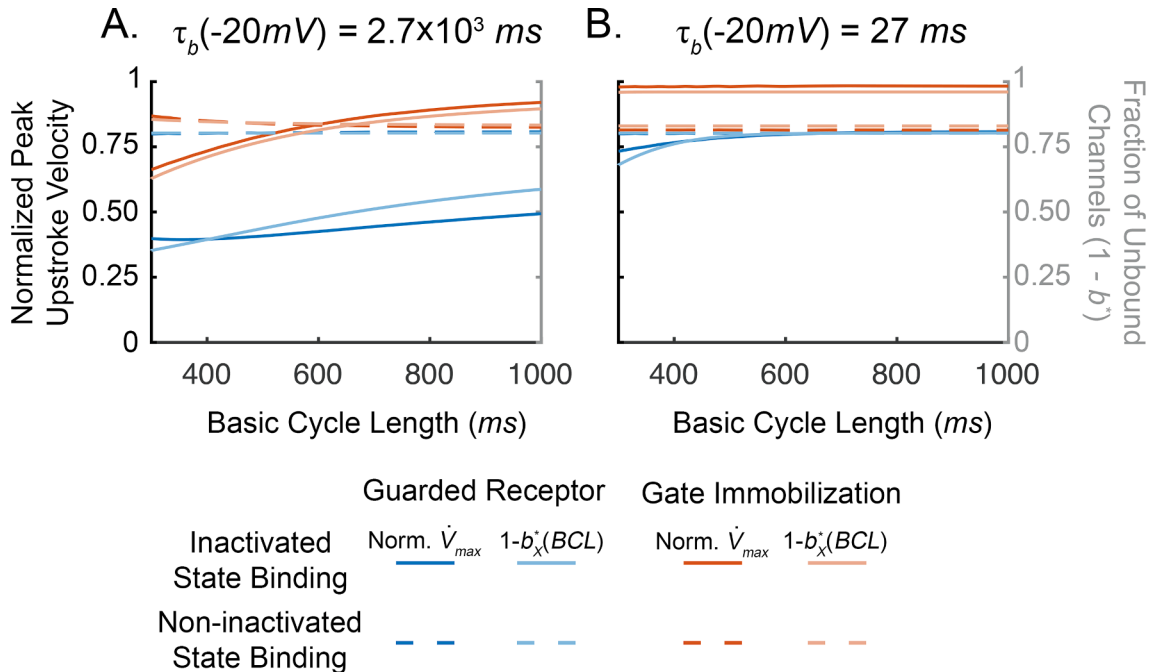


Fig. 5. 1D map captures modified ten Tusscher et al. model dynamics. The steady-state fraction of channels not bound to drug during the upstroke, $1 - b_X^*(BCL)$, (faded lines) as determined by the 1D map, Eq. (13), and peak upstroke velocity in the presence of drug normalized to peak upstroke velocity in the no drug case (darker lines) are plotted as functions of BCL. Drug binding time-constants at -20 mV of 2.7×10^3 ms (A) and 27 ms (B) correspond to Fig. 2A and B.

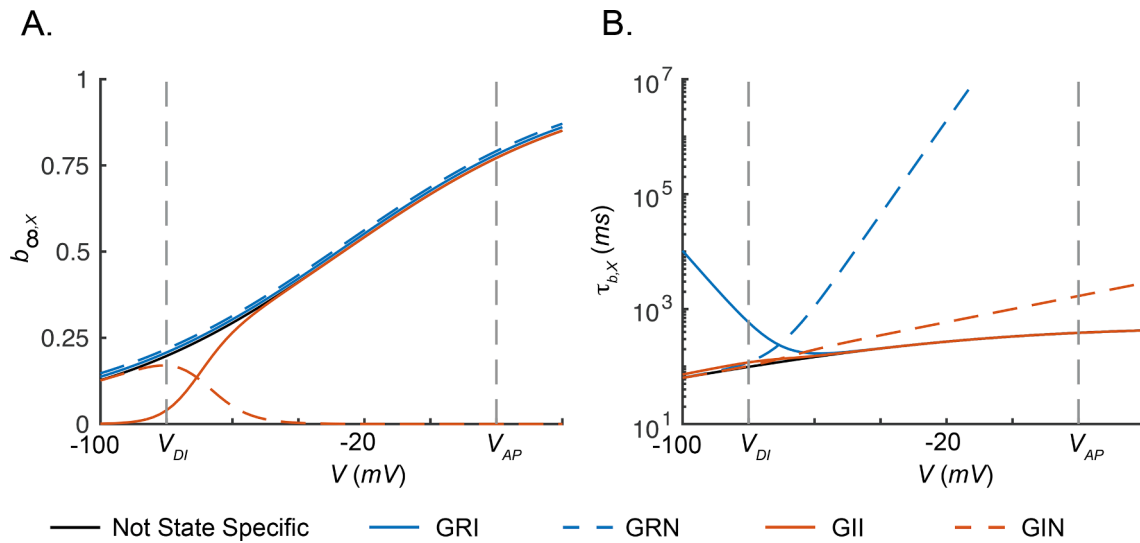


Fig. 6. Effective affinity and time constant of drug binding. Steady-state fraction, $b_{\infty,X}$ (A) and time-constant, $\tau_{b,X}$ (B) of drug binding as functions of transmembrane potential for the GRI (solid blue), GRN (dashed blue), GII (solid red), and GIN (dashed red) models, and non-state-specific binding (black line). For these plots, $\tau_b(-20 \text{ mV}) = 270 \text{ ms}$. Note, $b_{\infty,X}$ lines for GRI and GRN are artificially shifted upward by .01 and .02, respectively, for visibility.

block ($b_X^*(BCL)$), whereas the other three models exhibit 20% block. Note that resting level block is equivalent to $b_{\infty,X}(V_{DI})$, which is 0.04 for the GII model and 0.2 for all other models (Fig. 6A). To see that $b_X^*(BCL) \rightarrow b_{\infty,X}(V_{DI})$ for long BCL in Eq. (13), note that in the limit of DI approaching infinity (i.e., at rest or during slow BCL), $d \rightarrow 0$, leading to $w \rightarrow 1$, and as a result $b_X^*(BCL) \rightarrow b_{\infty,X}(V_{DI})$.

- **Biophysical mechanism of differences in $b_{\infty,X}(V_{DI})$:** The guarded receptor models assume that drug binding does not alter the rate constants of inactivation, and as a result, the fraction of channels bound to drug, not bound to drug, inactivated, and non-inactivated all eventually equilibrate to the same levels as in the case of non-state-specific binding. Hence, $b_{\infty,GRI}(V) = b_{\infty,GRN}(V) = b_{\infty}(V)$ (see Table 1), and because we set $b_{\infty}(V_{DI}) \sim 0.2$, resting level block for the GRI and GRN models is 20%. In the gate immobilization schemes, the steady state fraction of channels bound to drug is altered from that of non-state-specific binding because drug can only be bound to a particular conformational state (Fig. 1B). In the GIN model, because most Na^+ channels are non-inactivated and available for binding at the diastolic potential, the steady-state fraction of channels bound to drug turns out to be approximately the same as in the non-state-specific binding case. That is, $b_{\infty,GIN}(V_{DI}) \sim b_{\infty}(V_{DI}) \sim 0.2$. However, for the GII model, most Na^+ channels are unavailable for binding at the diastolic potential, and hence $b_{\infty,GII}(V_{DI}) \sim 0$.

3.4.2. Greater rate-dependence of GII than GIN

Despite both having the gate immobilization binding motif the change in the level of block from BCL of 1000 to 300 ms is 5 times greater in the GII model than GIN. The greater rate-dependence in the gate immobilization model with inactivated state binding compared to non-inactivated state binding is due to the differences in the steady state fraction of channels bound to drug, $b_{\infty,X}(V)$, for the two drug binding models, as given in Table 1 and seen in Fig. 6A. That is, the fraction of channels bound to drug during the upstroke, $b_X^*(BCL)$, will only be rate-dependent if there is a difference between $b_{\infty,X}(V_{AP})$ and $b_{\infty,X}(V_{DI})$, and the level of rate-dependence is conditional on the magnitude of $b_{\infty,X}(V_{AP}) - b_{\infty,X}(V_{DI})$. This is because, for long BCL, which result in a long DI, $b_X^*(BCL) \approx b_{\infty,X}(V_{DI})$ as explained above. However, as DI goes to 0 due to shortening BCL, $b_X^*(BCL) \rightarrow b_{\infty,X}(V_{AP})$, because $d \rightarrow 1$ and the weightings of $b_{\infty,X}(V_{DI})$ and $b_{\infty,X}(V_{AP})$ go to 0 and 1, respectively

(assuming APD, and therefore a , stays constant, which is relatively true in our modified ten Tusscher model; see Supplemental Information S3). Fig. 6A shows that the gate immobilization model with inactivated state binding has a large disparity between $b_{\infty,GII}(V_{AP}) = 0.77$ and $b_{\infty,GII}(V_{DI}) = 0.04$, and this explains the high degree of rate-dependence seen with this drug binding model. On the other hand, there is only a small disparity between $b_{\infty,GIN}(V_{AP}) = 4.5 \times 10^{-7}$ and $b_{\infty,GIN}(V_{DI}) = 0.17$, which leads to only minor rate-dependence.

- **Biophysical mechanism of disparity in $b_{\infty,X}(V_{AP})$:** The difference in steady state fraction of channels bound to drug at depolarized potentials between the GII and GIN models is similar to the mechanism for the disparity in $b_{\infty,X}(V_{DI})$. As stated above, $b_{\infty,GIN}(V_{DI}) \sim b_{\infty}(V_{DI})$ and $b_{\infty,GII}(V_{DI}) \sim 0$ because most Na^+ channels are non-inactivated at the diastolic potential. On the other hand, at V_{AP} , Na^+ channels are almost 100% inactivated, causing low and high availabilities of channels for drug binding in the GIN and GII models, respectively. Hence, $b_{\infty,GIN}(V_{AP}) \sim 0$ and $b_{\infty,GII}(V_{AP}) \sim b_{\infty}(V_{AP})$.

3.4.3. Reverse rate-dependency of GIN

The GIN model exhibits a lower level of block at a BCL of 300 ms than at 1000 ms. The reverse rate-dependence of the gate immobilization model with non-inactivated state binding results from the steady state fraction of channels bound to drug, $b_{\infty,GIN}(V)$, decreasing as V increases over the range of physiological transmembrane potentials, which causes $b_{\infty,GIN}(V_{AP}) < b_{\infty,GIN}(V_{DI})$. That is, because $b_{GIN}^*(BCL) \rightarrow b_{\infty,GIN}(V_{AP})$ as BCL is shortened (as explained above), $b_{GIN}^*(BCL)$ decreases as BCL is shortened. Because $b_{\infty,X}(V)$ increases as V increases in all other drug binding models considered, reverse rate-dependence cannot occur in these models.

- **Biophysical mechanism of reverse voltage-dependence of $b_{\infty,GIN}(V)$:** As explained above, $b_{\infty,GIN}(V)$ decreases as V increases through the physiological range because inactivation increases as V increases, causing a decrease in the availability of Na^+ channels for drug binding.

3.4.4. High rate-dependence of GRI but not GRN

Despite both having the guarded receptor binding motif, the GRN binding scheme does not exhibit rate-dependence whereas the GRI binding scheme does.

In the guarded receptor model with inactivated state binding, the disparity in steady state fraction of channels bound to drug is similar to that of the gate immobilization model with inactivated state binding ($b_{\infty, \text{GRI}}(V_{\text{AP}}) = 0.77$ and $b_{\infty, \text{GRI}}(V_{\text{DI}}) = 0.20$). This leads to the large rate-dependence seen in Figs. 2 and 5.

Interestingly, for the guarded receptor model with non-inactivated state binding, $b_{\infty, \text{GRN}}(V_{\text{AP}})$ and $b_{\infty, \text{GRN}}(V_{\text{DI}})$ are the same as $b_{\infty, \text{GRI}}(V_{\text{AP}})$ and $b_{\infty, \text{GRI}}(V_{\text{DI}})$, which might make one expect similarly strong rate-dependence in the GRN model as observed in the GRI model. However, Figs. 2 and 5 show minimal rate-dependence for the guarded receptor model with non-inactivated state binding. This is due to the time constant of drug binding at depolarized and resting potentials (i.e., $\tau_{b, \text{GRN}}(V_{\text{AP}})$ and $\tau_{b, \text{GRN}}(V_{\text{DI}})$). As shown in Fig. 6B, the time constant of drug binding at depolarized potentials is extremely slow in the guarded receptor model with non-inactivated state binding, so $\tau_{b, \text{GRN}}(V_{\text{DI}}) \ll \tau_{b, \text{GRN}}(V_{\text{AP}})$ (1.2×10^2 ms and 2.9×10^9 ms, respectively). As a result, $\text{DI}/\tau_{b, \text{GRN}}(V_{\text{DI}}) \gg \text{APD}/\tau_{b, \text{GRN}}(V_{\text{AP}})$, and therefore $d \ll a$, for all DI and APD considered here. Consequently, by Eq. (13), $b^*(\text{BCL}) \sim b_{\infty, \text{GRN}}(V_{\text{DI}})$ for all BCL considered here. The relatively slow rate of drug binding during the AP explains why b increases negligibly during the AP in Fig. 4E and $b \sim b_{\infty, \text{GRN}}(V_{\text{DI}})$ throughout the AP and DI. It should be noted that $\tau_{b, \text{X}}(V_{\text{AP}})$ and $\tau_{b, \text{X}}(V_{\text{DI}})$ are comparable in the three other models, and therefore are not the principal factors determining rate-dependency.

- **Biophysical mechanism of large $\tau_{b, \text{GRN}}(V_{\text{AP}})$:** In the guarded receptor models, drug can only bind to and unbind from Na^+ channels in particular conformational states, leading to slow drug binding kinetics when few Na^+ channels are in the required conformational states. Specifically, in the non-inactivated state binding scheme, drug can only bind and unbind from the non-inactivated channels, causing a scaling of the time constant of drug binding by the fraction of channels in a non-inactivated state, i.e., $\tau_{b, \text{GRN}}(V) = \frac{\tau_b(V)}{h_{\infty}(V)}$. At resting potentials ($V \leq V_{\text{DI}}$), when most Na^+ channels are non-inactivated and available for binding, $\tau_{b, \text{GRN}}(V) \sim \tau_b(V)$, whereas at depolarized potentials ($V \sim V_{\text{AP}}$), when most Na^+ channels are inactivated and unavailable for binding, $\tau_{b, \text{GRN}}(V) \gg \tau_b(V)$. Similarly, the fraction of inactivated channels scales the time constant of drug binding in the inactivated state binding model, i.e., $\tau_{b, \text{GRI}}(V) = \frac{\tau_b(V)}{1-h_{\infty}(V)}$. Therefore, at depolarized potential, $\tau_{b, \text{GRI}}(V) \sim \tau_b(V)$, whereas at hyperpolarized potentials $\tau_{b, \text{GRI}}(V) \gg \tau_b(V)$. However, in the modified ten Tusscher model used in this study, a non-negligible fraction of Na^+ channels are inactivated at resting potentials, and therefore $\tau_{b, \text{GRI}}(V)$ is not particularly large at V_{DI} .

Note: In the gate immobilization models, the time constant of drug binding is never multiple orders of magnitude larger than in the non-state-specific case. This is because even when few channels are available for drug binding, the time constant is set by the off-rate (e.g., $\tau_{b, \text{GII}}(V) = \frac{\tau_b(V)}{1-h_{\infty}(V)b_{\infty}(V)} = \frac{1}{(1-h_{\infty}(V))D|k_{\text{on}}+k_{\text{off}}(V)} \leq \frac{1}{k_{\text{off}}(V)}$), and the off-rate is the same as in the non-state-specific case because bound drug does not become trapped.

3.5. Effects of drug binding scheme on conduction velocity and the vulnerable window

Thus far, we have used the peak upstroke velocity in a single isolated cell to assess the effects of various drug binding schemes, but most serious arrhythmias are inherently spatial phenomena, i.e., they involve disruption of action potentials propagating through cardiac tissue. Two characteristics of the propagated action potential commonly used to assess propensity for arrhythmias are conduction velocity and the vulnerable window (i.e., interval of vulnerability to unidirectional block and retrograde conduction) (Moreno et al., 2011; Starmer et al., 1991; Starmer et al., 2003; King et al., 2013; Lu et al., 2010). Therefore, we directly examine the effects of drug binding scheme (using the Hodgkin-

Huxley-type formulation) on conduction velocity and the size of the vulnerable window by expanding our simulations from single cells to a one-dimensional cable of electrically coupled ventricular cells.

Conduction velocity is measured by pacing the proximal end of a 1.1 cm long cable 500 times at a BCL of 1000 ms and then decreasing the BCL to 300 ms in increments of 50 ms with 10 stimuli applied at each BCL. Conduction velocity is recorded for the last two stimuli at each BCL at a point 0.6 cm distal to the first cell. (See Appendix A2 for full details of model and methods.) Fig. 7A shows that the rate-dependent effects of different binding schemes on conduction velocity largely reflect the effects on peak upstroke velocity (compare Fig. 7A to Fig. 2A). The two non-inactivated state binding models (GRN and GIN; dashed blue and red lines) exhibit moderate levels of block and weak rate-dependence, to degrees similar to that observed for upstroke velocity. The gate immobilization model with inactivated state binding (GII; solid red line) has the least effect on conduction velocity at long BCL but exhibits the strongest rate-dependence; the percent reductions in conduction velocity are similar to those observed for upstroke velocity at all BCL. As was the case for upstroke velocity, the guarded receptor with inactivated state binding scheme (GRI; solid blue line), exhibits the largest drug effect and displays minor rate-dependence over the physiological range of BCLs. Interestingly, the percent reduction of conduction velocity in the GRI model is considerably greater than the percent reduction of peak upstroke velocity in a single cell (90% compared to 51% at a BCL of 1000 ms). Note that the curves in Fig. 7A for the GII and GRI binding schemes terminate at 350 ms and 500 ms, respectively, because for BCLs below these values, not all stimuli delivered to the tissue evoked propagating action potentials.

When a stimulus is applied in the wake of a propagated action potential, three qualitatively different responses can occur. If a stimulus is applied sufficiently early in the recovery process, no new action potential will be initiated (Fig. 7C, left panel). If a stimulus is applied sufficiently long after the action potential, two counter propagating action potentials are initiated (Fig. 7C, right panel). However, if the stimulus is applied during an intermediate time interval behind the propagating action potential, i.e., during the “vulnerable window”, a single retrograde propagating action potential is initiated (Fig. 7C, middle panel). A single retrograde action potential such as this can initiate sustained reentry in cardiac tissue (Moreno et al., 2011; Alessie et al., 1973). Thus, the size of the vulnerable window provides a measure of arrhythmia susceptibility in response to spontaneous stimuli.

To calculate the size of the vulnerable window, we pace a one-dimensional cable, 5.1 cm in length, 500 times at a fixed BCL (400, 675, or 1000 ms) at the proximal end, and then systematically vary the time of a “spontaneous” point source stimulus (-400 pA/pF in amplitude, 2 ms in duration) at a point 2.6 cm distal from the proximal end of the cable and monitor for unidirectional block. In Fig. 7B, the size of the vulnerable window (i.e., interval in which stimuli produce unidirectional block) is plotted, in terms of the fraction of the BCL, for the drug free case and for the four drug binding schemes. The effects of the drugs on the size of the vulnerable window mirror those for upstroke velocity and conduction velocity. In the no drug case (black), the fraction of the BCL composed of the vulnerable window is very small, increasing from 0.4% at a BCL of 1000 ms to 1.3% at a BCL of 400 ms. The non-inactivated state binding models (GRI and GRN) slightly increase the size of the vulnerable window compared to the no drug case, and rate-dependent effects remain small, as was the case for peak upstroke and conduction velocity. Specifically, the vulnerable window ranges from 0.7% to 3% for the GRN model (dashed blue line) and 0.6% to 2% for the GIN model (dashed red line). The GII model (solid red line), which had the largest rate-dependent effects on peak upstroke velocity and conduction velocity, has little effect on the size of the vulnerable window for long BCL (0.5% and 1.3% at BCLs of 1000 ms and 675 ms), but the vulnerable window is substantially increased for short BCL (8.5% at a BCL of 400 ms). As is the case for peak upstroke velocity and conduction velocity, the GRI model (solid blue line) has by far the largest effect on

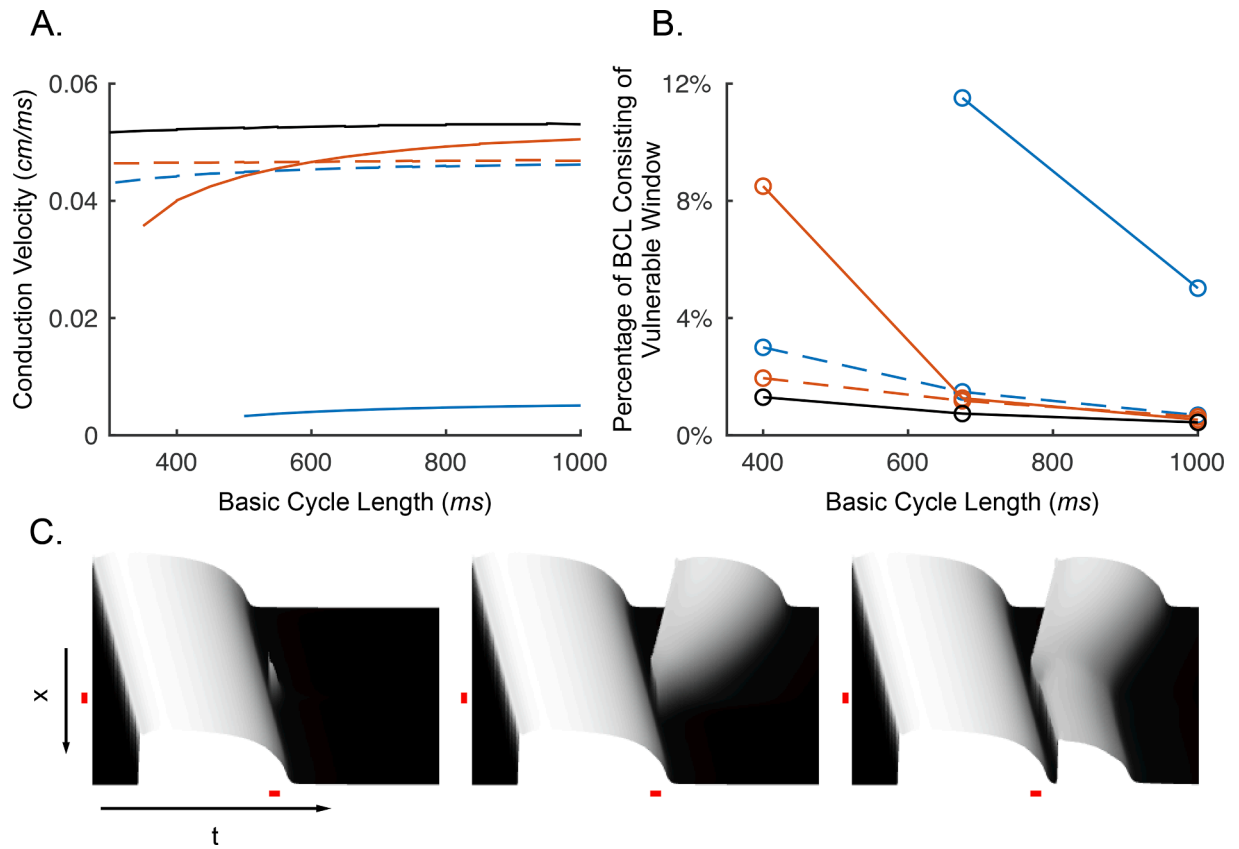


Fig. 7. Drug effects on propagation. Rate-dependent effects of drug binding models on conduction velocity and the vulnerable window in a one-dimensional cable of cardiac tissue. (A) Conduction velocity as a function of BCL; no drug (black), guarded receptor with inactivated (solid blue) and non-inactivated (dashed blue) state binding, gate immobilization with inactivated (solid red) and non-inactivated (dashed red) state binding. (B) Fraction of the BCL comprising the vulnerable window for the various drug binding models as a function of BCL. (C) Illustration of the vulnerable window. Transmembrane potential (V) plotted as a function of space (x) and time (t). Greyscale indicates the magnitude of V with lighter color indicating higher potentials. Spatial and temporal locations of a secondary stimulus are indicated by red dashes.

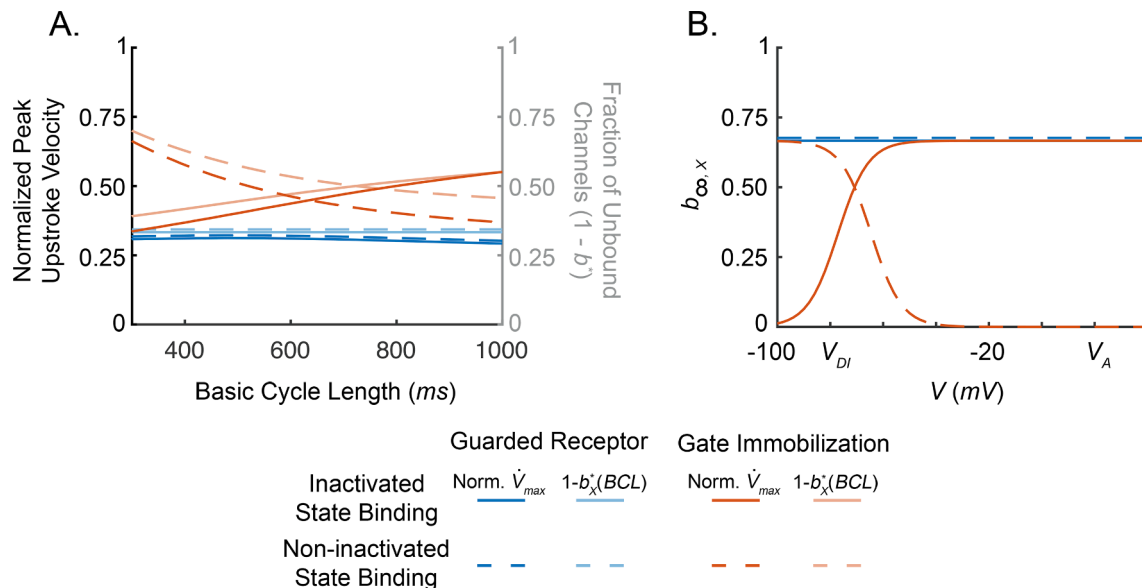


Fig. 8. Neutral drug effects. Rate-dependent effects of drug binding models for neutral drugs (i.e., k_{off} is independent of V). (A) The steady-state fraction of channels not bound to drug during the upstroke, $1 - b^*(BCL)$, (faded lines) as determined by Eq. (13), and peak upstroke velocity in the presence of drug normalized to peak upstroke velocity in the no drug case (darker lines) are plotted as functions of BCL. As in Fig. 2A, $k_{on} = 10 M^{-1}ms^{-1}$, which corresponds to a drug binding time-constant of $3.3 \times 10^3 ms$. (B) Steady-state fraction of channels bound to drug as a function of transmembrane potential (i.e., $b_{\infty, X}(V)$). Note that in both A and B, the data for the guarded receptor model with non-inactivated state binding is artificially shifted up 0.01 for visibility.

the size of vulnerable window at longer BCL (i.e. the vulnerable window was extended to 5.0% and 12% of the BCL at BCLs of 1000 ms and 675 ms, respectively), and action potentials did not propagate at a BCL of 400 ms.

3.6. Effects of neutral drugs and binding scheme on level of block

So far, we have considered only the effects of charged drugs for which the unbinding rate constant (k_{off}) is voltage-dependent. In this section, we consider the effects of neutral drugs (uncharged drugs) for which both k_{on} and k_{off} are independent of voltage. In the case of neutral drug for fixed drug concentration $[D]$, $b_{\infty} = \frac{k_{on}[D]}{k_{on}[D] + k_{off}}$ is constant, and therefore there can be no rate-dependent effects for drugs that lack state-specific binding. However, in the case of state-specific drug binding, rate-dependent effects can arise through the voltage-dependence of the channel gating. That is, $b_{\infty,X}(V)$ can inherit voltage-dependence from $h_{\infty}(V)$.

Fig. 8 displays the rate-dependent block in the GRI, GRN, GII, and GIN models for neutral drugs with $k_{on} = 10 \mu M^{-1}ms^{-1}$, $k_{off} = k_{on}K_{D0} = 100 ms^{-1}$, and $[D] = 20 \mu M$, which corresponds to $b_{\infty} = 2/3$ and a dissociation constant of $K_D = k_{off}/k_{on} = K_{D0} = 10 \mu M$ (where K_{D0} is the K_D at $V = 0$ from the charged drug case). While there are similarities between the effects of neutral drugs and those of charged drugs, there are several fundamental differences as well. Similar to the analysis in Section 3.4 for charged drug, the rate-dependent effects of neutral drugs can be explained by considering the voltage-dependence of the steady state fractions of channels bound to drug, $b_{\infty,X}$, for the four binding schemes (Fig. 8B).

First, the overall level of block in the case of the neutral drug is considerably larger than that of the charged drug for all drug binding models considered. This difference can be attributed to our choice of k_{off} for the neutral drug. Specifically, we set k_{off} such that the dissociation constant, K_D , of the neutral drug matches the K_D for the charged drug at $V = 0$, resulting in a constant b_{∞} of $2/3$. On the other hand, b_{∞} for the charged drug oscillates between 0.20 near the resting potential and 0.75 during the action potential, which leads to an average b_{∞} that is much less than $2/3$ for all physiological BCLs. The APD would have to be 90% of the BCL for the overall level of block to be similar in the neutral and charged drug cases with our choice of parameters. (Note, however, that the b_{∞} for the neutral drug could be adjusted so that the overall level of block would be similar to that for the charged drug.)

Second, as in the case of charged drug, the GRN model with neutral drug displays little to no rate-dependency of block; however, the high rate-dependency that was present in the GRI model with charged drug is abolished in the neutral drug case. The lack of rate-dependency for both guarded receptor models with neutral drugs is a consequence of the voltage-independence of b_{∞} for neutral drugs and the overall assumption that drug binding does not alter inactivation kinetics in guarded receptor models. Thus, the steady-state fraction of channels bound to drug ($b_{\infty,GRI}$ and $b_{\infty,GRN}$) are constant for the GRI and GRN models (Fig. 8B, Table 1). As a result, neither guarded receptor model exhibits rate-dependent effects.

Third, both gate immobilization models still exhibit rate-dependent block with neutral drug. In fact, the trend and magnitude of the rate-dependency of block in the GII model is similar in the charged and neutral drug cases, however the reverse rate-dependence observed for the GIN model with charged drug is substantially enhanced with neutral drug. As described in section 3.4.4, the GIN model exhibits reverse rate-dependence because the steady state fraction of channels bound to drug is largest at diastolic potentials where most channels are non-inactivated, and therefore $b_{\infty,GIN}(V_{DI}) \sim b_{\infty}(V_{DI})$ is greater than $b_{\infty,GIN}(V_{AP}) \sim 0$. Furthermore, the level of the reverse rate-dependency is primarily determined by the difference between these two quantities. As mentioned above, b_{∞} for the neutral drug is constant at $2/3$, whereas $b_{\infty}(V_{DI})$ for the charged drug is 0.20, thus the reverse rate-

dependence in the neutral drug case is enhanced compared to the charged drug case.

On the other hand, Na^+ channels in the GII model are available for drug binding at depolarized potentials (i.e., $b_{\infty,GII}(V_{AP}) \sim b_{\infty}(V_{AP})$ and $b_{\infty,GII}(V_{DI}) \sim 0$). Thus, because $b_{\infty}(V_{AP})$ is similar for the charged drug case and neutral drug case (0.77 and 0.67, respectively), the rate-dependence is also similar for charged and neutral drugs that bind according to the GII scheme.

4. Discussion

Most drugs bind to ion channels in a state-specific manner, and therefore models of drug-channel interactions must include state-specificity in order to accurately replicate the effects of such drugs. Two common motifs for state-specific drug-channel interactions are the guarded receptor and gate immobilization motifs. Here, we systematically compare and contrast the guarded receptor and gate immobilization motifs in the context of drugs that interact with the inactivation gate of cardiac Na^+ channels. Specifically, we assess the impact of guarded receptor and gate immobilization models with inactivated state or non-inactivated state binding on peak upstroke velocity for a wide range of pacing frequencies and drug binding rates. In doing so, we connect the rate-dependent effects on upstroke velocity (Fig. 2A and B) to the distinct drug binding kinetics of the various binding schemes (Fig. 1B), and we elucidate the biophysical mechanisms by which differences in drug-ion channel interactions at the molecular level give rise to differences in the rate-dependence of peak upstroke velocity at the cellular level.

In the guarded receptor motif, drug binding does not alter channel gating kinetics, but drug binding/unbinding is restricted to a particular subset of channel conformations (e.g., conformations with the inactivation gate open). Similarly, in the gate immobilization motif, drug can only bind when the channel is in a particular subset of conformations, but once bound, the drug locks the channel in that subset of conformations until the drug unbinds. Starmer et al. (Starmer et al., 1984; Starmer et al., 1987) referred to both of these binding motifs as the “guarded receptor” hypothesis. However, the two binding motifs have distinct features, and our results show that they can have drastically different effects on the upstroke velocity of the cardiac AP. Therefore, we refer to the guarded receptor and gate immobilization binding motifs using distinct names.

The drug binding models utilized in this study are of the minimal dimension that can include the guarded receptor or gate immobilization motifs. These models are capable of recapitulating the binding dynamics of real drugs. For example, in Docken et al. (Docken et al., 2021), we compared the dynamics of a detailed high dimensional model of lidocaine's interaction with the Na^+ channel (Moreno et al., 2011) to a minimal dimensional model with the gate immobilization motif (as discussed here). Our gate immobilization model replicated multiple voltage-clamp data sets and predicted similar effects of lidocaine on action potential upstroke velocity and conduction velocity as the original high dimensional model. Furthermore, the minimal models are more mathematically tractable than high-dimensional models, making it easier to identify the fundamental mechanisms that lead to particular drug effects. Our analysis finds that the mechanisms underlying the rate-dependent effects of the drug binding models can be explained in terms of the oscillations in fraction of channels bound to drug over the course of the duty cycle, which result from different drug binding kinetics at resting and depolarized potentials (and therefore the oscillations in drug binding and rate-dependency of effects) are fully defined by the steady state fraction of channels bound to drug, $b_{\infty,X}(V)$, and the drug binding time constant, $\tau_{b,X}(V)$ in the various drug binding models.

Our study is limited to the consideration of specific drug concentration, binding rate constants, and electrophysiological model, specif-

ically the modified ten Tusscher et al. human ventricular model. However, based on the insight gained from our analysis, we can predict the impact of altered conditions on the rate-dependency of drug effects. That is, based on the understanding of the determinants of the fraction of channels bound to drug during the upstroke (gained through the formula for b^* ; Eq. (13)), we can determine the global sensitivity of drug effects to changes in drug binding parameters (i.e., $[D]$, k_{on} , and k_{off}) and electrophysiological characteristics (e.g., restitution properties or Na^+ channel inactivation kinetics). We now summarize the most salient response properties observed for each drug binding motif and subsequently explain how variations in cellular electrophysiology or drug binding rates would diminish or augment rate-dependent response properties.

Of the drug binding models considered here, the gate immobilization model with inactivated state binding (GII) exhibits both the lowest resting (tonic) level of block and the greatest rate-dependence over the range of physiological BCLs. Na^+ channels exhibit minimal inactivation at the resting membrane potential (i.e., $h_{\infty}(V_{DI}) \approx 1$), and therefore few channels are bound to drug in the GII model at the diastolic potential ($b_{\infty,GII}(V_{DI}) \approx 0$), resulting in the low level of tonic block. On the other hand, the majority of channels bind to drug at depolarized potentials in the GII model ($b_{\infty,GII}(V_{AP}) > 0.5$). The disparity between the steady-state fraction of channels bound to drug ($b_{\infty,GII}$) at resting and depolarized potentials along with APD restitution properties endows the GII model with the capability of high rate-dependence. A rightward shift in the Na^+ channel steady state inactivation curve would reduce resting inactivation further (i.e., $h_{\infty}(V_{DI}) \rightarrow 1$), thus decreasing $b_{\infty,GII}(V_{DI})$, leading to decreased tonic block and increased rate-dependence, while a leftward shift in the inactivation curve would have opposite effects. Note that changes in tonic block and rate-dependence are both more sensitive to a leftward shift as a result of the curvature of $h_{\infty}(V)$ near V_{DI} . Even higher degrees of rate-dependency would be seen if shorter DIs were feasible due to either higher stimulus amplitudes or altered cellular electrophysiology.

The most noteworthy response property seen in simulations with the gate immobilization model with non-inactivated state binding (GIN) is reverse rate-dependence. In the GIN model, drug tends to bind at diastolic potentials when Na^+ channels are not inactivated ($h_{\infty}(V_{DI}) \approx 1$ corresponding to $b_{\infty,GIN}(V_{DI}) \approx b_{\infty}(V_{DI})$) and unbinds during the AP when channels are inactivated ($h_{\infty}(V_{AP}) \approx 0$ corresponding to $b_{\infty,GIN}(V_{AP}) \approx 0$). Therefore, as BCL decreases, DI decreases faster than the AP causing fewer channels to be bound to drug on average. The reverse rate-dependence is weak in our simulations with charged drug because $b_{\infty,GIN}(V_{DI})$ is relatively small due to the voltage dependence of binding, and thus the disparity between $b_{\infty,GIN}(V_{DI})$ and $b_{\infty,GIN}(V_{AP})$ is small. However, if $K_D(V) = \frac{k_{off}(V)}{k_{on}}$ is decreased, $[D]$ is increased, or voltage-dependence of drug binding decreased (as was the case with neutral drug in Section 3.6), $b_{\infty,GIN}(V_{DI})$ would increase, and the resulting the reverse rate-dependence in the GIN model would be more noticeable. Furthermore, a rightward shift in the inactivation curve would also slightly increase $b_{\infty,GIN}(V_{DI})$, leading to greater reverse rate-dependence, while a leftward shift would decrease $b_{\infty,GIN}(V_{DI})$ and decrease the reverse rate-dependence.

For the guarded receptor model with non-inactivated state binding (GRN), the most salient feature is the lack of rate-dependent effects. The vast majority of Na^+ channels are inactivated during the action potential plateau ($h_{\infty}(V_{AP}) \approx 0$), and therefore they are unavailable for drug binding. As a result, the effective time constant of drug binding at depolarized potentials is multiple orders of magnitude longer than the APD ($\tau_{b,GRN}(V_{AP}) = \frac{\tau_b(V_{AP})}{h_{\infty}(V_{AP})} \gg \text{APD}$), and the fraction of channels bound to drug remains close to $b_{\infty,GRN}(V_{DI})$ throughout the cycle. Note that $h_{\infty}(V_{AP})$ is insensitive to shifts in the inactivation curve, because Na^+ channel inactivation is nearly complete during the action potential. Thus, the effective time constant of drug binding in the GRN model

would remain larger than APD (so long as drug binding kinetics are relatively slow), and the lack of rate-dependent effects is robust to changes in the drug binding rates or cellular electrophysiology.

The guarded receptor model with inactivated state binding (GRI) exhibits significant rate-dependent effects and the largest level of block of the drug binding models considered here. The rate-dependent effects are due to the large disparity between $b_{\infty,GRI}(V_{DI})$ and $b_{\infty,GRI}(V_{AP})$. The high degree of drug block is due to the large time constant of drug binding at the resting transmembrane potential, $\tau_{b,GRI}(V_{DI}) = \frac{\tau_b(V_{DI})}{1-h_{\infty}(V_{DI})}$, which causes drug unbinding during the DI to be much slower than in the other models. A rightward shift in the steady-state inactivation curve ($h_{\infty}(V)$) would further increase the time constant of drug binding at resting potentials. In fact, if the time constant is increased sufficiently, drug unbinding during the diastolic interval would be negligible, causing the fraction of channels bound to drug to remain near $b_{\infty,GRI}(V_{AP})$ throughout the cycle, and no rate-dependent effects would be seen over the physiological range of BCLs.

In this study, we primarily focus on the case of charged drugs for which binding affinity to available channels (b_{∞}) increases with transmembrane potential, but we also consider the effects of neutral drugs, for which b_{∞} is voltage-independent. Neutral drugs that bind via the guarded receptor motif exhibit no rate-dependent effects, and this lack of rate-dependence is robust to changes in binding rates or electrophysiology. However, rate-dependent effects can still be seen in the gate immobilization models with neutral drugs, because the steady-state fractions of channels bound to drug inherit voltage-dependence through their dependence on the level of inactivation, $h_{\infty}(V)$. Furthermore, due to h_{∞} driving rate-dependence in both the neutral and charged drug cases, the sensitivities to shifts in $h_{\infty}(V)$ and changes in drug binding rates and concentration in the neutral drug case are similar to those discussed for the charged drugs.

“Markov models” of guarded receptor and gate immobilization drug binding models can be directly derived from the corresponding binding schemes using mass action kinetics. However, Starmer et al. provided “Hodgkin-Huxley-type” mathematical models of these binding schemes that implicitly assume drug binding occurs on a much slower time-scale than that of the relevant gating kinetics (Starmer, 1991; Starmer et al., 1984; Starmer et al., 1987; Starmer et al., 1991; Starmer et al., 2003). We found Hodgkin-Huxley-type models to be accurate for drug binding models when drug binding is relatively slow (τ_b is approximately an order of magnitude larger than gating kinetics), and surprisingly, Hodgkin-Huxley-type models of non-inactivated state binding were accurate for all τ_b examined. (To our knowledge, no derivation of these Hodgkin-Huxley-type models from the Markov models has been published, therefore we provide such a derivation in Supplemental Information S1.) It should be noted that compared to Markov models, Hodgkin-Huxley-type models of the guarded receptor model only decrease the dimensionality of the system of equations describing drug channel interactions by one, and Hodgkin-Huxley-type models of the gate immobilization model do not reduce the dimensionality of the system at all. Therefore, utilizing Hodgkin-Huxley-type instead of the Markov models does not greatly improve computational efficiency. On the other hand, compared to the Markov models, the Hodgkin-Huxley-type models more clearly separate the drug binding process from the inactivation process, allowing for easier identification of the fundamental mechanisms underlying the effects of drugs.

In this study, we limited our consideration to Na^+ channel blockers that only interact with the inactivation gate, but our methods could be applied to Na^+ channel blockers that bind to other conformational states (e.g., the fully open state (Moreno et al., 2011; Ragsdale et al., 1996). The analysis could also be extended to drugs that block other ion-channels (e.g., Ca^{++} or K^+ channel blockers (Hondeghem and Katzung, 1984; Yang et al., 2020; Lee and Tsien, 1983; Starmer et al., 1989; Hockerman et al., 2000; Shabbir et al., 2011; DeMarco et al., 2021), including mutant channels linked to diseased states (Sesti et al., 2000;

Rivolta et al., 2001; Curran et al., 1995; Bezzina et al., 2001), and could aid in the design of drugs with targeted rate-dependent effects.

CRediT authorship contribution statement

Steffen S. Docken: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Colleen E. Clancy:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Timothy J. Lewis:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

https://github.com/ClancyLabUCD/state_spec_Na_channel_blocker_modeling.

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Appendices

A1. Na^+ channel model

In this study, we use a Na^+ current model we previously developed and fit to experimental data (Docken et al., 2021). This Na^+ current model is a Hodgkin-Huxley-type model, but the rate constant functions are simpler than most Hodgkin-Huxley models. Specifically, the Na^+ current model is

$$I_{\text{Na}} = \bar{g}_{\text{Na}} m^3 h (V - E_{\text{Na}})$$

$$\frac{dm}{dt} = \alpha_m(V)(1 - m) - \beta_m(V)m$$

$$\frac{dh}{dt} = \alpha_h(V)(1 - h) - \beta_h(V)h, \quad (\text{A1.1})$$

where the rate constants are

$$\alpha_m(V) = 45.43e^{V/13.78}$$

$$\beta_m(V) = 0.6628e^{V/-23.25}$$

$$\alpha_h(V) = 6.169 \times 10^{-5} e^{V/-9.328}$$

$$\beta_h(V) = 14.15e^{V/14.91} \quad (\text{A1.2})$$

A2. Cell and tissue models

We modified the ten Tusscher et al. model (ten Tusscher et al., 2004; ten Tusscher and Panfilov, 2006) by replacing the ten Tusscher et al. Na^+ current model with Na^+ current model in Appendix A1 augmented with the drug- Na^+ current interaction models described in the Models section of the main text. The maximal conductance per capacitance of the Na^+ channel model is set to $G_{\text{Na}} = \frac{\bar{g}_{\text{Na}}}{C_m} = 17.25 \text{ nS/pF}$ (where C_m is the membrane capacitance), which produces of a peak upstroke velocity of 300 mV/ms at a BCL of 1000 ms . Spatial effects of the drug binding models were evaluated by simulating the modified ten Tusscher et al. model in a one-dimensional cable, which is done by adding a diffusion term to the differential equation for transmembrane potential

$$\frac{\partial V}{\partial t} = -\frac{I_{\text{ion}} + I_{\text{applied}}}{C_m} + D \frac{\partial^2 V}{\partial x^2} \quad (\text{A2.1})$$

where $D = 0.00154 \text{ cm}^2/\text{ms}$ (ten Tusscher et al., 2004). Propagating waves were initiated in a one-dimensional cable by periodically raising the proximal 0.1 cm of tissue to 0 mV .

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtbi.2023.111595>.

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