The dual effect of ephaptic coupling on cardiac conduction with heterogeneous expression of connexin 43

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Abstract

Decreased and heterogeneous expression of connexin 43 (Cx43) are common features in animal heart failure models. Ephaptic coupling, which relies on the presence of junctional cleft space between the ends of adjacent cells, has been suggested to play a more active role in mediating intercellular electrical communication when gap junctions are reduced. To better understand the interplay of Cx43 expression and ephaptic coupling on cardiac conduction during heart failure, we performed numerical simulations on our model when Cx43 expression is reduced and heterogeneous. Under severely reduced Cx43 expression, we identified three new phenomena in the presence of ephaptic coupling: alternating conduction, in which ephaptic and gap junction-mediated mechanisms alternate; instability of planar fronts; and small amplitude action potential (SAP), which has a smaller potential amplitude than the normal action potential. In the presence of heterogeneous Cx43 expression, ephaptic coupling can either prevent or promote con-

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duction block (CB) depending on the Cx43 knockout (Cx43KO) content. When Cx43KO content is relatively high, ephaptic coupling reduces the probabilities of CB. However, ephaptic coupling promotes CB when Cx43KO and wild type cells are mixed in roughly equal proportion, which can be attributed to an increase in current-to-load mismatch.

Keywords: Heterogeneous connexin 43, Bidomain model, Alternating conduction, Small amplitude action potential, Instability of planar fronts, Conduction block.

1 1. Introduction

2 Cardiac cells communicate electrically to coordinate the muscular contraction
3 of the heart to pump blood. Gap junctions are low-resistance pathways that medi-
4 ate the electrical impulses between myocytes. Gap junctional coupling is one of
5 the key factors that guarantees the velocity and reliability of cardiac conduction
6 Shaw and Rudy (1997); Vaidya et al. (2001); Beauchamp et al. (2004, 2012).

7 Ventricular myocytes are connected electrically through gap junction channels
8 formed mainly by connexin 43 (Cx43) and, to a smaller extent, Cx45 Davis et al.
9 (1995). Growing experimental and theoretical evidence suggests that heterogene-
10 ity of Cx43 can lead to altered patterns of conduction, re-entrant arrhythmias and
11 sudden death, Danik et al. (2004); Gutstein et al. (2001); Jongsma and Wilders
12 (2000); Kanno and Saffitz (2001); Prudat and Kucera (2014). For example, in
13 Danik et al. (2004), authors investigated susceptibility to arrhythmias in a ge-
14 netically engineered murine model to express progressively decreasing levels of
15 Cx43. Lethal tachyarrhythmias were initiated if cardiac Cx43 abundance was de-
16 creased in a heterogeneous fashion to 18% of the control level. In Gutstein et al.

17 (2001), authors designed a murine model of heterogeneous gap junction channel
18 expression, which results in conduction defects as well as markedly depressed
19 contractile function. Additionally, in Prudat and Kucera (2014), authors showed
20 that velocity and stability of conduction behave in a nonlinear manner when car-
21 diomyocytes expressing different amounts of Cx43 are combined. In particular,
22 conduction became very heterogeneous and was susceptible to block in the co-
23 culturing of Cx43 knockout (Cx43KO) and wild type (WT) cells, while block was
24 never observed in monogenotypic preparations.

25 It is widely accepted that gap junctional coupling is the primary mechanism re-
26 sponsible for electrical communication between cardiac cells Rohr (2004). How-
27 ever, recent experimental observation has raised the question of whether conduc-
28 tion can be sustained in the absence of gap junctions Danik et al. (2004); Yao et al.
29 (2003). For instance, in Yao et al. (2003), intercellular conductance measured by
30 dual whole cell voltage clamp in pairs of adult rat ventricular myocytes with ge-
31 netic Cx43 ablation was dramatically reduced from 588 nS to 10 nS. However,
32 impulse propagation was reduced by only 50% in tissue composed of the same
33 cells.

34 One possible explanation of this intriguing observation is ephaptic coupling,
35 first proposed in Sperelakis and Mann (1977) and further elaborated in Sperelakis
36 and McConnell (2002). In it, neighboring cells are electrically coupled through a
37 narrow cleft space that is resistively connected to the extracellular space. It is fur-
38 ther assumed that Na^+ channels are preferentially localized to the cleft space, as
39 shown by experiments in Cohen (1996); Maier et al. (2002); Kucera et al. (2002);
40 Sperelakis and McConnell (2002). When an action potential reaches one cell,
41 the Na^+ current flows into the cell from extracellular bath via the cleft, and the

42 potential in the cleft space drops. Once the cleft potential becomes sufficiently
43 negative, it depolarizes the membrane of the adjacent cell to the threshold, and
44 that cell then produces an action potential. Several theoretical studies investigated
45 the possible role of ephaptic coupling in cardiac signal propagation Kucera et al.
46 (2002); Mori et al. (2008); Veeraraghavan et al. (2014b); Hand and Peskin (2010);
47 Copene and Keener (2008). In Kucera et al. (2002); Hand and Peskin (2010),
48 the authors assessed the hypothesis that conduction could be modulated by the
49 preferential localization of Na^+ channels in the cleft space, which causes a large
50 negative potential in the cleft and influences conduction in two opposite ways, de-
51 pending on the strength of gap junctional coupling. In particular, for normal and
52 moderately reduced gap junctional coupling, localization of Na^+ channels to the
53 cleft space forces Na^+ current to go through the large resistance between cleft and
54 extracellular space, which slowed down the conduction. In contrast, for greatly
55 reduced gap junctional coupling, the negative cleft potential induced by Na^+ cur-
56 rent in the prejunctional membrane lead to a suprathreshold depolarization of the
57 postjunctional membrane, which facilitated and accelerated conduction. In Mori
58 et al. (2008), the authors identified a mode of conduction in which the ephaptic
59 and gap-junction-mediated mechanisms alternate. Authors of Copene and Keener
60 (2008) explored the feasibility of ephaptic mechanism in the absence of gap junc-
61 tions, which is highly parameter dependent.

62 Decreased and heterogeneous expression of Cx43 are common features in ani-
63 mal heart failure models Wang and Gerdes (1999); HUANG et al. (1999); Yoshida
64 et al. (2011). Emerging evidence suggests a more active role for ephaptic cou-
65 pling in mediating intercellular electrical communication when gap junctions are
66 reduced in a homogeneous maner Yao et al. (2003); Kucera et al. (2002); Hand

67 and Peskin (2010); Lin and Keener (2014). However, the interplay between het-
 68 erogeneous Cx43 expression and ephaptic coupling on cardiac conduction has not
 69 been investigated yet. In this manuscript, we aim to explore the effects of ephap-
 70 tic coupling on cardiac impulse propagation via numerical simulations when Cx43
 71 expression is reduced and heterogeneous.

72 2. Materials and Methods

73 2.1. Mathematical model

74 2.1.1. Bidomain model with ephaptic coupling

75 A two dimensional (2D) bidomain model (Fig. 1A) of electrical conduction
 76 was modified to incorporate ephaptic coupling Hand and Peskin (2010); Copene
 77 and Keener (2008), which relies on the presence of a junctional cleft space be-
 78 tween the ends of adjacent cells. Each cell is assumed to be cylindrical in shape
 79 with radius r and length L . The cells are connected by gap junctions to form
 80 an $M \times N$ rectangular lattice. At each lattice point (i, j) , we associated intracel-
 81 lular potential $\phi_i^{(i,j)}$ and extracellular potential $\phi_e^{(i,j)}$. The cleft lies in between
 82 cells (i, j) and $(i, j + 1)$. We then defined a cleft potential $\phi_c^{(i,j+\frac{1}{2})}$ at location
 83 $(i, j + \frac{1}{2})$. We called the space, which lies in the extracellular space and stays ad-
 84 jacent to the cleft, the extracellular-cleft space. The potential in the extracellular-
 85 cleft space is denoted by $\phi_{ec}^{(i,j+\frac{1}{2})}$. Note that Fig. 1A shows the lattice view of the
 86 model, whereas Fig. 1B shows the connection between lattices. In particular, Fig.
 87 1B shows a circuit diagram for two adjacent cells that are ephaptically coupled
 88 through a common cleft space in the presence of end-to-end gap junctions (GJ_{end}),
 89 side-to-side gap junctions (GJ_{side}) and resistive connections between extracellular
 90 spaces (R_{ee}) are not shown here. Cleft space was modelled as a single narrow

91 compartment with resistive connections (R_c) to the extracellular space; Resistive
 92 connections between extracellular space and the extracellular-cleft space are de-
 93 noted by R_{ec} . Intracellular and extracellular spaces of each cell are separated by
 94 the cell side membrane; intracellular and cleft spaces are separated by the cell end
 95 membrane. Both side and end membranes allow the free flow of ionic and capac-
 96 itive currents. Ephaptic coupling is maintained by the active end membranes that
 97 are connected by a shared cleft potential. In order to save on computational cost,
 98 we treated intracellular and extracellular spaces of each cell to be isopotential.

99 As can be seen from Fig. 1, four different compartments, namely intracellu-
 100 lar, extracellular, cleft, and extracellular-cleft spaces, were modelled separately.
 101 Current conservation in each compartment provides the following equations, with
 102 parameters listed in Table 1.

- 103 • Intracellular space ($\phi_i^{(i,j)}$; $1 \leq i \leq M, 2 \leq j \leq N-1$) :

$$\begin{aligned}
 & GJ_{\text{end}}(\phi_i^{(i,j)} - \phi_i^{(i,j-1)}) + GJ_{\text{end}}(\phi_i^{(i,j)} - \phi_i^{(i,j+1)}) \\
 & + GJ_{\text{side}}(\phi_i^{(i,j)} - \phi_i^{(i-1,j)}) + GJ_{\text{side}}(\phi_i^{(i,j)} - \phi_i^{(i+1,j)}) \\
 & + A_{\text{end}}C_m \frac{\partial(\phi_i^{(i,j)} - \phi_c^{(i,j-\frac{1}{2})})}{\partial t} + A_{\text{end}}C_m \frac{\partial(\phi_i^{(i,j)} - \phi_c^{(i,j+\frac{1}{2})})}{\partial t} + I_{\text{end}} \\
 & + A_{\text{side}}C_m \frac{\partial(\phi_i^{(i,j)} - \phi_e^{(i,j)})}{\partial t} + I_{\text{side}} = 0,
 \end{aligned} \tag{2.1}$$

104 where $A_{\text{end}} = \pi r^2$ and $A_{\text{side}} = 2\pi rL$ denote the cross-sectional and side
 105 areas of a cell, respectively; $GJ_{\text{end}} = A_{\text{end}}g_{\text{end}}$ and $GJ_{\text{side}} = \frac{A_{\text{side}}}{2}g_{\text{side}}$ de-
 106 note end-to-end and side-to-side gap junctional conductance, respectively;
 107 $\phi_i^{(i,j)}$, $\phi_i^{(i,j-1)}$ and $\phi_i^{(i,j+1)}$ denote the intracellular potentials of cells (i, j) ,
 108 $(i, j-1)$ and $(i, j+1)$, respectively; $\phi_i^{(i-1,j)}$ and $\phi_i^{(i+1,j)}$ denote the intra-
 109 cellular potentials of cells $(i-1, j)$ and $(i+1, j)$, respectively; $\phi_c^{(i,j-\frac{1}{2})}$ and

110 $\phi_c^{(i,j+\frac{1}{2})}$ denote cleft potentials at locations $(i, j - \frac{1}{2})$ and $(i, j + \frac{1}{2})$, respec-
 111 tively; C_m represents the membrane capacitance per area; I_{end} and I_{side} de-
 112 note the outward ionic current of the end and side membranes, respectively;
 113 $\phi_e^{(i,j)}$ represents the extracellular potential of cell (i, j) .

114 Note that for $j = 1$, terms $GJ_{\text{end}}(\phi_i^{(i,j)} - \phi_i^{(i,j-1)})$ and $A_{\text{end}}C_m \frac{\partial(\phi_i^{(i,j)} - \phi_c^{(i,j-\frac{1}{2})})}{\partial t}$
 115 are dropped from Eq. (2.1). Similarly, for $j = N$, terms $GJ_{\text{end}}(\phi_i^{(i,j)} -$
 116 $\phi_i^{(i,j+1)})$ and $A_{\text{end}}C_m \frac{\partial(\phi_i^{(i,j)} - \phi_c^{(i,j+\frac{1}{2})})}{\partial t}$ are dropped from Eq. (2.1). In order to
 117 minimize the edge effects, we imposed periodic boundary condition in the
 118 x direction (see Fig. 1A): we identified $\phi_i^{(i-1,j)}$ with $\phi_i^{(M,j)}$ for $i = 1$, and
 119 we identified $\phi_i^{(i+1,j)}$ with $\phi_i^{(1,j)}$ for $i = M$.

120 • Extracellular space ($\phi_e^{(i,j)}$; $1 \leq i \leq M$, $2 \leq j \leq N - 1$) :

$$\begin{aligned}
 & \frac{\phi_e^{(i,j)} - \phi_{ec}^{(i,j-\frac{1}{2})}}{R_{ec}} + \frac{\phi_e^{(i,j)} - \phi_{ec}^{(i,j+\frac{1}{2})}}{R_{ec}} \\
 & + \frac{\phi_e^{(i,j)} - \phi_e^{(i-1,j)}}{R_{ee}} + \frac{\phi_e^{(i,j)} - \phi_e^{(i+1,j)}}{R_{ee}} \\
 & - A_{\text{side}}C_m \frac{\partial(\phi_i^{(i,j)} - \phi_e^{(i,j)})}{\partial t} - I_{\text{side}} = 0,
 \end{aligned} \tag{2.2}$$

121 where $\phi_{ec}^{(i,j-\frac{1}{2})}$ and $\phi_{ec}^{(i,j+\frac{1}{2})}$ denotes the extracellular-cleft potentials at lo-
 122 cation $(i, j - \frac{1}{2})$ and $(i, j + \frac{1}{2})$, respectively; R_{ec} represents the resistance
 123 between extracellular space and the extracellular-cleft space; $\phi_e^{(i-1,j)}$ and
 124 $\phi_e^{(i+1,j)}$ denote the extracellular potentials of cells $(i - 1, j)$ and $(i + 1, j)$,
 125 respectively; R_{ee} represents the resistance between extracellular spaces in
 126 the x direction (see Fig. 1A).

127 Note that for $j = 1$, term $\frac{\phi_e^{(i,j)} - \phi_{ec}^{(i,j-\frac{1}{2})}}{R_{ec}}$ is dropped from Eq. (2.2). Similarly,
 128 for $j = N$, $\frac{\phi_e^{(i,j)} - \phi_{ec}^{(i,j+\frac{1}{2})}}{R_{ec}}$ is dropped from Eq. (2.2). In order to minimize

the edge effects, we imposed periodic boundary condition in the x direction (see Fig. 1A): we identified $\phi_e^{(i-1,j)}$ with $\phi_e^{(M,j)}$ for $i = 1$, and we identified $\phi_e^{(i+1,j)}$ with $\phi_e^{(1,j)}$ for $i = M$.

- Cleft space ($\phi_c^{(i,j+\frac{1}{2})}$; $1 \leq i \leq M, 1 \leq j \leq N-1$):

$$\begin{aligned} & -A_{\text{end}}C_m \frac{\partial(\phi_i^{(i,j)} - \phi_c^{(i,j+\frac{1}{2})})}{\partial t} - A_{\text{end}}C_m \frac{\partial(\phi_i^{(i,j+1)} - \phi_c^{(i,j+\frac{1}{2})})}{\partial t} - I_{\text{end}} \\ & + \frac{\phi_c^{(i,j+\frac{1}{2})} - \phi_{ec}^{(i,j+\frac{1}{2})}}{R_c} = 0, \end{aligned} \quad (2.3)$$

where R_c represents the resistance between cleft and extracellular space.

- Extracellular-cleft space ($\phi_{ec}^{(i,j+\frac{1}{2})}$; $1 \leq i \leq M, 1 \leq j \leq N-1$):

$$-\frac{\phi_e^{(i,j)} - \phi_{ec}^{(i,j+\frac{1}{2})}}{R_{ec}} - \frac{\phi_e^{(i,j+1)} - \phi_{ec}^{(i,j+\frac{1}{2})}}{R_{ec}} - \frac{\phi_c^{(i,j+\frac{1}{2})} - \phi_{ec}^{(i,j+\frac{1}{2})}}{R_c} = 0. \quad (2.4)$$

2.1.2. Modeling Cx43 expression

Since we aim to study the effect of distribution pattern of Cx43KO and WT cells on conduction features in the presence of ephaptic coupling, we followed Prudat and Kucera (2014) to model heterogeneous Cx43 expression, where genotypes of cells were allocated stochastically according to the predefined Cx43KO content (p). In other words, under each p , we stochastically assigned cell genotypes to be Cx43KO or WT. Here, $p = 0$ implies that the Cx43KO content is 0%, i.e. all cells are WT. In contrast, $p = 1$ implies that Cx43KO content is 100%, i.e. all cells are knockout.

Conductance of gap junctions was set proportionally to the area of contacts between adjacent cells using proportionality constants g_{end} and g_{side} (Table 1) for end-to-end and side-to-side contacts, respectively. For connections between

147 WT cells, g_{end} and g_{side} were scaled by a factor k_{WT} (Table 1). For connections
 148 between Cx43KO cells, g_{end} and g_{side} were scaled by a factor k_{Cx43KO} (Table 1).
 149 The value of k_{Cx43KO} (Table 1) was chosen to keep the longitudinal conduction
 150 velocity (CV_L) in the absence of ephaptic coupling (which can be modelled by a
 151 sufficiently large cleft width) the same as CV_L for Cx43KO strands reported in
 152 Prudat and Kucera (2014), where ephaptic coupling was not considered.

153 As reported in previous studies, the residual low connexin expressions be-
 154 tween Cx43KO and WT cells are mediated by Cx43/Cx45 Beauchamp et al.
 155 (2012); McCain et al. (2012). For connections between WT and Cx43KO cells,
 156 g_{end} and g_{side} were also scaled by the factor k_{Cx43KO} based on the assumption that
 157 the coupling between cells is essentially determined by the cell expressing the
 158 least amount of connexins Beauchamp et al. (2012). In Beauchamp et al. (2012),
 159 the authors experimentally measures the intercellular conductance between pairs
 160 of ventricular myocytes with different Cx43 expression. They found that abla-
 161 tion of Cx43 in one cell of a pair caused a marked decrease in mean intercellular
 162 conductance, from 68.3 ± 9.6 nS to 5.2 ± 1.7 nS.

163 2.1.3. Membrane currents

164 The excitable dynamics of normal cardiac tissue was described by Luo-Rudy
 165 dynamic guinea pig ventricular model 2007 (LRd 2007) Livshitz and Rudy (2007).
 166 Two different distributions were used for Na^+ channels: (1) uniform distribution,
 167 in which Na^+ channels are evenly distributed along the cell membrane; (2) local-
 168 ized distribution, in which all Na^+ channels are localized to the cleft spaces at the
 169 two cell ends, while the total conductance of Na^+ channels is fixed to its normal
 170 value. In this study, Na^+ channels are assumed to be localized to the cleft, unless
 171 indicated otherwise. The other ionic channels of LRd 2007 model are uniformly

distributed along the cell membrane.

Table 1: Parameters that enter our model

Symbol	Parameter	Value/Expression	Reference
r	Radius of cell	1.1×10^{-3} cm	Hand and Peskin (2010)
L	Length of cell	10^{-2} cm	Hand and Peskin (2010)
g_{end}	End-to-end gap junctional conductance per area	6.66×10^2 mS/cm ²	Hand and Peskin (2010)
α	Ratio between g_{end} and g_{side}	6	Hand et al. (2009)
g_{side}	Side-to-side gap junctional conductance per area	$\frac{g_{\text{end}}}{\alpha}$	Derived
C_m	Membrane capacitance per area	$1 \mu\text{F}/\text{cm}^2$	Hand and Peskin (2010)
d_{cleft}	Cleft width	$2 - 115$ nm	Hand and Peskin (2010)
σ_{ext}	Extracellular conductivity	6.67 mS/cm	Hand and Peskin (2010)
R_c	Resistance between cleft and extracellular space	$\frac{1}{8\pi\sigma_{\text{ext}}d_{\text{cleft}}}$	Hand and Peskin (2010)
AR_x	Anisotropy ratio of resistance in x direction	1.33	Keener and Sneyd (2010)
AR_y	Anisotropy ratio of resistance in y direction	0.35	Keener and Sneyd (2010)
R_{ec}	Resistance between extracellular space and extracellular cleft	$\frac{1}{2\text{AR}_y A_{\text{end}} g_{\text{end}}}$	Keener and Sneyd (2010)
R_{ee}	Resistance between extracellular spaces in x direction	$\frac{2}{\text{AR}_x A_{\text{side}} g_{\text{side}}}$	Keener and Sneyd (2010)
k_{WT}	Scaling factor for g_{end} and g_{side} between WT cells	1	Prudat and Kucera (2014)
k_{Cx43KO}	Scaling factor for g_{end} and g_{side} between Cx43KO cells / WT and Cx43KO cells	0.005	Derived
ϕ_i	Intracellular potential		
ϕ_e	Extracellular potential		
ϕ_c	Cleft potential		
ϕ	Side transmembrane potential	$\phi_i - \phi_e$	

173 2.2. Numerical simulation

174 Simulations were run in a lattice consisting of $M \times N$ cells (Fig. 1A), where
 175 $M = 2, 3, 6, 10$, and 16 , and $N = 75$. Initially, all potentials and gating variables
 176 were held at resting states. Conduction was initiated by applying 0.5 ms excitatory
 177 current pulses of amplitude $0.15 \mu\text{A}$ into all cells at the left hand side of the lattice.

178 The resulting model consists of a large number of differential algebraic equa-
 179 tions. We used a splitting method by updating the potential and gating variables of
 180 ion channels separately to solve the system. When updating the potential, gating
 181 variables are fixed; the linear parts of the system (linear in potential) were treated
 182 using backward Euler method; the ionic currents and dynamics were linearized
 183 and treated using backward Euler method afterwards. The system was solved via
 184 a direct method (backslash operator in Matlab).

185 We defined the wavefront of a normal travelling action potential as the spatial
 186 location where the side transmembrane potential (ϕ) crosses -30 mV with $\frac{\partial \phi}{\partial t} > 0$.
 187 This value was set to distinguish small amplitude action potential (SAP) (Sec.
 188 3.2.3) from normal action potential, since SAP does not reach -30 mV. Activation
 189 was monitored by identifying the earliest activation time (EAT) at every column
 190 of the $M \times N$ lattice (Fig. 1A). CV_L was computed by linear regression of EAT
 191 over 20% to 80% of lattice length to exclude the boundary effect. Conduction
 192 block (CB) was defined as the failure of wavefront to reach the right hand side of
 193 the lattice.

194 3. Results

195 3.1. The effect of ephaptic coupling on cardiac conduction.

196 To explore the effects of ephaptic coupling on impulse propagation, we identified several possible values of cleft width (d_{cleft}), from 2 nm to 115 nm (see 197 Table 1). Note that smaller d_{cleft} corresponds to larger R_c , which therefore implies 198 stronger ephaptic coupling.

200 In Figs. 2A and 2B, CV_L is plotted as a function of d_{cleft} for $p = 1$ (A, Cx43KO 201 cells) and $p = 0$ (B, WT cells), respectively. As observed, Fig. 2A displays a 202 biphasic behavior of CV_L : with increasing d_{cleft} , CV_L exhibits a marked increase 203 to 13.1 cm/s (at $d_{\text{cleft}} = 8$ nm) followed by a decrease to 2.5 cm/s with further 204 cleft widening. In contrast, when gap junctional coupling is strong, CV_L shows a 205 monotone increase as d_{cleft} increases (see Fig. 2B). Note that in Fig. 2, the most 206 notable differences in qualitative behaviors of CV_L are seen for $d_{\text{cleft}} < 20$ nm.

207 To further investigate the effects of ephaptic coupling on conduction, we chose 208 $d_{\text{cleft}} = 2$ nm, 3.5 nm, 8 nm, 8.5 nm, and 115 nm as the representative values in 209 our numerical simulations.

210 3.2. Conduction features when Cx43KO content is 100% ($p = 1$).

211 To further explore spatiotemporal excitation patterns that occur in the near absence of gap junctions, we performed numerical simulations in lattices consisting 212 of 100% Cx43KO cells. The following three novel conduction features were observed in the presence of ephaptic coupling: alternating conduction (Sec. 3.2.1), 213 instability of planar fronts (Sec. 3.2.2) and SAP (Sec. 3.2.3). However, when 214 gap junction-mediated mechanism dominates (d_{cleft} is sufficiently large), none of 215 these features can be observed. Note that alternating conduction and SAP are one- 216

dimensional phenomena, which do not depend on lattice width. In contrast, the instability of planar fronts can only be observed in a 2D simulation.

3.2.1. Alternating conduction

Fig. 3 shows EAT as a function of N for $d_{\text{cleft}} = 8.5$ nm (A), 8.6 nm (B), 8.7 nm (C), 8.9 nm (D), which demonstrates the presence of periodic transitions between fast and slow conduction under severely reduced gap junctions. For instance, at $d_{\text{cleft}} = 8.9$ nm, action potential propagates fast between columns N and $N + 1$ when N is even, but slowly between columns N and $N + 1$ when N is odd. Note that for $9 \text{ nm} \leq d_{\text{cleft}} \leq 9.6 \text{ nm}$, the EAT pattern is the same as that for $d_{\text{cleft}} = 8.9 \text{ nm}$.

Fig. 4 shows the end transmembrane potential ($\phi_{ic} = \phi_i - \phi_c$) traces of the left (ϕ_{icL}) and right sides (ϕ_{icR}) of cells at location (3, 24), (3, 25), (3, 26) and (3, 27), where $d_{\text{cleft}} = 5$ nm (A), 8.9 nm (B) and 10 nm (C). As indicated in Fig. 4B, ϕ_{ic} traces of the fast conduction phase (cell (3, 24) to cell (3, 25), and cell (3, 26) to cell (3, 27) in Fig. 3D) are similar to the potential traces ($\phi_{icR}^{(3, 24)}$, $\phi_{icL}^{(3, 25)}$, $\phi_{icR}^{(3, 26)}$ and $\phi_{icL}^{(3, 27)}$) seen when $d_{\text{cleft}} = 5$ nm, at which the propagation is driven primarily by ephaptic coupling. However, ϕ_{ic} profiles of the slow conduction phase (cell (3, 25) to cell (3, 26) in Fig. 3D) are similar to the potential traces ($\phi_{icR}^{(3, 25)}$ and $\phi_{icL}^{(3, 26)}$) seen when $d_{\text{cleft}} = 10$ nm, at which the conduction can be attributed to the residual gap junctional coupling. Alternating conduction shown in Fig. 3 thus lies in an intermediate regime, in which the two aforementioned mechanisms play an equal role, but spatially alternate in being the dominant mode of conduction. Alternating conduction was reported in Mori et al. (2008), where the authors only demonstrated the pattern similar to the one shown in Fig. 3D. However, patterns seen in Figs. 3A - 3C have never been reported previously.

243 Note that two upstrokes are observed in $\phi_{icR}^{(3,25)}$ and $\phi_{icL}^{(3,26)}$ traces of Fig. 4B
 244 and in multiple traces of Fig. 4C. For $\phi_{icR}^{(3,25)}$ trace in Fig. 4B, the first upstroke
 245 corresponds to the depolarization of the right end membrane of cell (3, 25), which
 246 results in a drop in the cleft potential shared by cells (3, 25) and (3, 26). The drop
 247 in the aforementioned cleft potential gives rise to a passive increase in $\phi_{icL}^{(3,26)}$
 248 (first upstroke in $\phi_{icL}^{(3,26)}$ trace), which is not large enough to activate the left end
 249 membrane of cell (3, 26). Similarly, the depolarization of left end membrane of
 250 cell (3, 26) (second upstroke in $\phi_{icL}^{(3,26)}$ trace) can lead to a passive increase in
 251 $\phi_{icR}^{(3,25)}$ (second upstroke in $\phi_{icR}^{(3,25)}$ trace). Similar explanation can be applied to
 252 traces in Fig. 4C. However, in Fig. 4A ($d_{\text{cleft}} = 5$ nm), cells are more tightly
 253 connected, which gives rise to stronger ephaptic coupling, and therefore, only one
 254 upstroke is seen on ϕ_{ic} traces.

255 We point out that the differences in upstroke timing in the ϕ_{ic} traces of Fig. 4
 256 are primarily a result of ephaptic coupling (for example $\phi_{icR}^{(3,24)}$ and $\phi_{icL}^{(3,25)}$ traces)
 257 and different action potential arrival times in the cleft spaces (for example $\phi_{icL}^{(3,25)}$
 258 and $\phi_{icR}^{(3,25)}$ traces). The upstroke timing differences here should thus not be con-
 259 fused with the junctional and intracellular delays discussed in Rohr et al. (1998);
 260 Shaw and Rudy (1997), where ephaptic coupling were not considered.

261 3.2.2. *Instability of planar fronts*

262 Fig. 5 shows a propagation of an unusual action potential at $d_{\text{cleft}} = 9.8$ nm,
 263 in which instability of planar fronts occurs. This effect is only observed for
 264 $9.7 \text{ nm} \leq d_{\text{cleft}} \leq 9.8 \text{ nm}$. Figs. 5A - 5D show a sequence of snapshots demon-
 265 strating propagation when an external current activates all cells at the left hand
 266 side of the lattice. The snapshots plot ϕ at time (t) = 89 ms (A), 112 ms (B),
 267 139 ms (C) and 173 ms (D). In order to make the wavefront easier to observe, we

rotated the figures by 180° . As indicated in Fig. 5, there is an irregular change in the shape of the wavefront (denoted by black arrows), which proceeds in a zigzag fashion. Such wavefronts propagate to neighboring cells in a spatially heterogeneous manner, rather than evenly along the lattice.

This zigzag wavefront pattern is a result of instability of the planar fronts, reminiscent of stability of front solutions studies in Mori and Matano (2015). Inevitable numerical deviations from the planar front, which will decay and remain small in case of a stable planar front, and grows when the planar front is unstable. Such numerical deviations, in biophysical terms, correspond to external noise; the planar front thus destabilizes in the presence of noise.

We speculate that the instability also results from the presence of two modes of conduction, the ephaptic and the gap junction mediated. Unlike alternating conduction (Sec. 3.2.1) in which the mode of conduction depends only on the y -coordinate (Fig. 1A), here, the mode of conduction depends on the x -direction as well. A checkerboard pattern of ephaptic and gap junction mediated alternation gives rise to the instability of planar fronts.

3.2.3. *Small amplitude action potentials (SAP)*

Fig. 6 demonstrates a novel type of action potential propagation at $d_{\text{cleft}} = 3.5$ nm, which has a much lower potential amplitude than the normal conduction, with the maximum value of $\phi = -40$ mV. Figs. 6A - 6D show a sequence of snapshots demonstrating SAP when an external current activates all cells at the left hand side of the lattice. The snapshots illustrate ϕ at time (t) = 13 ms (A), 51 ms (B), 80 ms (C) and 126 ms (D), respectively.

Note that propagation patterns depend highly on the values of d_{cleft} . For instance, SAP occurs for $3.5 \text{ nm} \leq d_{\text{cleft}} \leq 3.7 \text{ nm}$, while CB occurs for $3.2 \text{ nm} \leq$

293 $d_{\text{cleft}} \leq 3.4$ nm. For $3.8 \text{ nm} \leq d_{\text{cleft}} \leq 5$ nm, one observes a wave that has char-
 294 acteristics in between normal conduction and SAP (see Fig. 7), reminiscent of
 295 stacked fronts observed in Iida et al. (2011). Finally, for $2 \text{ nm} \leq d_{\text{cleft}} \leq 3.1$ nm
 296 and $d_{\text{cleft}} > 5$ nm, normal conduction is seen. However, when $2 \text{ nm} \leq d_{\text{cleft}} \leq 3.1$
 297 nm, conduction is extremely slow with $CV_L = 0.7$ cm/s.

298 At $d_{\text{cleft}} = 3.5$ nm, R_c is very high. In this case, the cleft potential drops low
 299 enough to excite the end membrane. However, the resulting inward Na^+ current
 300 is not sufficient to fully activate the side membrane, and therefore SAP occurs.
 301 In other words, when SAP occurs, Na^+ channels residing on the end membrane
 302 are fully activated, as indicated by the activation gating variable (m_{end}) of Na^+
 303 channels that resides on the end membrane of cell (3, 25) (Fig. 8A). Nevertheless,
 304 the side membrane is not fully excited, as indicated by ϕ profile of that cell (Fig.
 305 8B). SAP may thus be seen as a cardiac propagation jumping from cleft to cleft.

306 When SAP occurs, L-type Ca^{2+} channels are not fully activated. Figs. 9A and
 307 9B show the activation gating variable (d) of Ca^{2+} channels that reside on the end
 308 (d_{end} , A) and side (d_{side} , B) membranes of cell (3, 25); Fig. 9C exhibits ϕ profile
 309 of cell (3, 25), where $d_{\text{cleft}} = 3.5$ nm (blue), 3.8 nm (red), and 115 nm (green).
 310 As observed in Figs. 9A and 9B, gating variable d is not active when SAP occurs
 311 ($d_{\text{cleft}} = 3.5$ nm). This is in contrast to normal conduction ($d_{\text{cleft}} = 115$ nm), in
 312 which Ca^{2+} channels are fully activated. SAP is thus a wave in which an electrical
 313 pulse propagates without a large rise in Ca^{2+} concentration. We may therefore
 314 regard SAP as an electromechanically dissociated wave.

315 As shown in Fig. 9, for $d_{\text{cleft}} = 3.8$ nm, the behaviors of d_{end} , d_{side} and ϕ
 316 lie in an intermediate regime. Unlike SAP, the resulting influx of Na^+ current is
 317 sufficient to activate Ca^{2+} channels residing on the side membrane, indicated by

318 d_{side} in Fig. 9B. In other words, the steep depolarization of side membrane results
 319 from Na^+ current, whereas the shallow depolarization results from L-type Ca^{2+}
 320 current (Fig. 9C). An intermediate wave thus occurs.

321 3.3. Conduction block (CB) in mixed genotype lattices.

322 In this section, we aim to investigate the possible impacts of interplay between
 323 heterogeneous Cx43 expression and ephaptic coupling on cardiac propagation,
 324 specially looking at the occurrence of CB. We performed numerical simulations
 325 in an $M \times N$ lattice with a random distribution of WT and Cx43 cells according to
 326 the predefined Cx43KO content ($0 \leq p \leq 1$), while incorporating different values
 327 of d_{cleft} . As stated in Sec. 2.2, $M = 2, 3, 6, 10$ and 16 , $N = 75$.

328 For each lattice, numerical simulations were implemented 100 iterations for
 329 every p , d_{cleft} and Na^+ channel distribution, thus taking into account different
 330 realizations of cellular architecture.

331 3.3.1. Ephaptic coupling can suppress CB when $p \geq 0.7$.

332 Tables 2 and 3 show the number of CB observed for different p and d_{cleft} in
 333 2×75 and 3×75 lattices, respectively. Note that there is no CB for monogeno-
 334 typic preparations ($p = 0$ or 1). However, co-culturing of Cx43KO and WT cells
 335 results in increased probabilities of CB, especially for $0.2 \leq p \leq 0.6$. This is
 336 consistent with the results of Prudat and Kucera (2014), where CB was studied
 337 without ephaptic coupling (corresponding to $d_{\text{cleft}} = 115$ nm in our simulations).
 338 Tables 2 and 3 indicate that the proportion of CB in the above range is independent
 339 of the value of d_{cleft} and thus of ephaptic coupling.

340 The number of CB in Tables 2 and 3, however, shows a dependency on d_{cleft}
 341 when $0.7 \leq p \leq 0.9$. The proportion of CB for $d_{\text{cleft}} = 8$ nm is lower than that for

$d_{\text{cleft}} = 115$ nm. Indeed, in Table 2, for $0.7 \leq p \leq 0.8$, the differences in the number of CB at $d_{\text{cleft}} = 8$ nm and $d_{\text{cleft}} = 115$ nm are statistically significant according to two-proportion z -test ($z = 3.6$ and 2.5 , respectively). For $p = 0.9$, statistically significant difference ($z = 2.3$) can be detected if more than 800 numerical experiments are performed (data is not shown in Table 2). In Table 3, for $p = 0.7$, we observed statistically significant difference ($z = 3.3$) in the occurrence of CB between $d_{\text{cleft}} = 8$ nm and 115 nm. At $p = 0.8$, statistically significant difference ($z = 4.8$) can be observed when the number of numerical experiments is increased to 400 (data is not shown in Table 3). Recall that the maximum CV_L was achieved at $d_{\text{cleft}} = 8$ nm (see Fig. 2A) in the near absence of gap junctional coupling; and at $d_{\text{cleft}} = 115$ nm, propagation is mainly supported by gap junctional coupling (see Section 3.1). When $0.7 \leq p \leq 0.9$, ephaptic coupling can thus reduce the number of CB, which points to an advantage of ephaptic coupling.

Recall from Sec. 3.2.3 that SAP occurs at $d_{\text{cleft}} = 3.5$ nm when $p = 1$. At $p = 0.9$ and $d_{\text{cleft}} = 3.5$ nm in Tables 2 and 3, SAP is seen when there is no CB.

3.3.2. Ephaptic coupling can enhance CB when $0.4 \leq p \leq 0.6$.

Tables 4 - 6 show the number of CB for different p and d_{cleft} in 6×75 , 10×75 and 16×75 lattices, respectively. Note that CB was never observed in monogenic preparations ($p = 0$ or 1). Nevertheless, similarly to simulations discussed in the previous section, conduction is susceptible to CB in preparations with mixed WT and Cx43KO cells, especially for $0.4 \leq p \leq 0.6$. This is seen for all ephaptic coupling strengths and lattice widths.

It can be seen from Tables 2 - 6 that the number of CB generally decreases as the lattice becomes wider. This is most likely because the presence of a larger number of alternative pathways for propagation in wider lattices permits the wave-

front to bypass sites of low excitability. Exceptions to this rule are seen when $p = 0.9$, $d_{\text{cleft}} = 2$ nm and 3.5 nm. When $d_{\text{cleft}} = 2$ nm, the number of CB increases in wider lattices. When $d_{\text{cleft}} = 3.5$ nm, the number of CB displays an irregular dependency on the width of the lattice. The mechanism behind the phenomena is still unclear, but the unexplained variations are statistically significant.

The alleviatory effect of ephaptic coupling on CB in narrower lattices shown in Tables 2 and 3 (discussed in the previous section) is also present in wider lattices (Tables 4 - 6, $0.7 \leq p \leq 0.9$). The magnitude of this effect, seems to be smaller, however.

A new feature observed in wider lattices but not seen in narrower lattices is that the number of CB shows a dependency on d_{cleft} when $0.4 \leq p \leq 0.6$. As seen in Tables 4 - 6 the proportion of CB is *lower* at $d_{\text{cleft}} = 115$ nm than at smaller d_{cleft} . This implies that ephaptic coupling does not prevent, but rather, facilitates CB in this parameter range. Indeed, in Table 6, for $0.4 \leq p \leq 0.6$, the differences in the number of CB at $d_{\text{cleft}} = 115$ nm and smaller d_{cleft} are statistically significant according to the two-proportion z -test. Additionally, in Table 5, when $0.4 \leq p \leq 0.6$, the statistically significant differences in the occurrence of CB between $d_{\text{cleft}} = 115$ nm and $d_{\text{cleft}} = 8$ nm or 8.5 nm can be detected if 400 numerical experiments are performed (data is not shown in Table 5). In Table 4, for $p = 0.6$, a statistically significant difference between $d_{\text{cleft}} = 115$ nm and 8 nm can be seen if the number of numerical experiments is increased to 400 (data is not shown in Table 4). Ephaptic coupling can thus increase the possibility of CB in the co-cultures, which points to a disadvantage of ephaptic coupling. We see that this effect is more pronounced in wider lattices.

When $p = 0.9$, SAP still occurs at $d_{\text{cleft}} = 3.5$ nm in Tables 4 and 5, but the

number of occurrences of CB is 2 and 1, respectively.

We have checked that the effect of suppressing (Sec. 3.3.1) or promoting CB (Sec. 3.3.2) is only seen when Na^+ channels are localized to the cleft; indeed, when Na^+ channel is uniformly distributed, the number of CB is independent of d_{cleft} values and is almost the same as the numbers in Tables 2 - 6 for $d_{\text{cleft}} = 115$ nm (data is not shown). This implies that ephaptic coupling does not modulate CB when Na^+ channel distribution is uniform.

Table 2: Number of CB for a 2×75 lattice. For $0.7 \leq p \leq 0.8$, the differences in the number of CB at $d_{\text{cleft}} = 8$ nm and $d_{\text{cleft}} = 115$ nm are statistically significant according to the two-proportions z -test ($z = 3.6$ and 2.5 , respectively). For $p = 0.9$, statistically significant difference ($z = 2.3$) can be detected if more than 800 numerical experiments are performed (data not shown).

$d_{\text{cleft}} \text{ (nm)} \backslash p$	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
2	0	93	100	100	100	100	100	100	100	74	0
3.5	0	92	100	100	100	100	100	100	100	78	SAP
8	0	92	100	100	100	100	95	55	16	3	0
8.5	0	92	100	100	100	100	95	55	18	4	0
115	0	92	100	100	100	100	99	79	31	5	0

Table 3: Number of CB for a 3×75 lattice. For $p = 0.7$, according to the two-proportion z -test, we observed statistically significant difference ($z = 3.3$) in the occurrence of CB between $d_{\text{cleft}} = 8$ nm and 115 nm. At $p = 0.8$, statistically significant difference ($z = 4.8$) can be observed when the number of numerical experiments is increased to 400 (data not shown).

$d_{\text{cleft}} \text{ (nm)} \backslash p$	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
2	0	48	99	100	100	100	100	100	100	80	0
3.5	0	35	94	100	100	100	100	100	100	83	SAP
8	0	35	93	100	100	100	98	46	3	0	0
8.5	0	35	93	100	100	100	97	51	5	0	0
115	0	35	94	100	100	100	99	69	10	1	0

Table 4: Number of CB for a 6×75 lattice, For $p = 0.6$, there are statistically significant differences in the occurrence of CB between $d_{\text{cleft}} = 115$ nm and $d_{\text{cleft}} = 2$ nm, 3.5 nm or 8 nm. In particular, the significant differences between $d_{\text{cleft}} = 115$ nm and $d_{\text{cleft}} = 8$ nm can be detected if 400 numerical experiments are performed (data not shown).

$d_{\text{cleft}} \text{ (nm)} \backslash p$	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
2	0	15	97	100	100	100	100	100	100	79	0
3.5	0	0	25	91	100	100	100	100	98	93	SAP
8	0	0	24	88	100	100	81	23	1	0	0
8.5	0	0	24	88	100	99	74	14	0	0	0
115	0	0	24	87	99	100	72	18	2	0	0

Table 5: Number of CB for a 10×75 lattice. When $0.4 \leq p \leq 0.6$, there are statistically significant differences in the number of CB at $d_{\text{cleft}} = 115$ and smaller d_{cleft} . In particular, the statistically significant differences between $d_{\text{cleft}} = 115$ nm and $d_{\text{cleft}} = 8$ nm or 8.5 nm can be detected if 400 numerical experiments are performed (data not shown).

$d_{\text{cleft}} \text{ (nm)} \backslash p$	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
2	0	4	89	100	100	100	100	100	100	92	0
3.5	0	0	3	74	100	100	100	100	52	54	SAP
8	0	0	1	41	97	99	64	0	0	0	0
8.5	0	0	1	40	96	96	40	0	0	0	0
115	0	0	1	39	92	86	31	0	0	0	0

Table 6: Number of CB for a 16×75 lattice. When $0.4 \leq p \leq 0.6$, the number of CB at $d_{\text{cleft}} = 115$ nm is significantly less than at smaller d_{cleft} .

$d_{\text{cleft}} \text{ (nm)} \backslash p$	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
2	0	2	89	100	100	100	100	100	100	99	0
3.5	0	0	1	56	100	100	100	98	37	43	SAP
8	0	0	0	12	83	99	50	0	0	0	0
8.5	0	0	0	10	79	97	27	0	0	0	0
115	0	0	0	7	59	53	8	0	0	0	0

3.4. Mechanisms behind the increased proportions of CB.

As demonstrated in Sec. 3.3, a smaller d_{cleft} can either prevent or promote CB depending on parameter range. In wider lattices, CB is more frequent in the

402 presence of ephaptic coupling when $0.4 \leq p \leq 0.6$.

403 We now explore the mechanism behind this observation by using a repre-
 404 sentative example of 6×75 lattice at $p = 0.6$. A similar analysis can be per-
 405 formed in other cases as well. In Figs. 10 - 13, EAT is plotted at different it-
 406 erations ($i = 9, 20, 35, 50$, respectively). In Figs. 10A, 11A, 12A, and 13A,
 407 $d_{\text{cleft}} = 2 \text{ nm}, 3.5 \text{ nm}, 8 \text{ nm}$ and 8.5 nm , respectively. In Figs. 10B, 11B, 12B and
 408 13B, $d_{\text{cleft}} = 115 \text{ nm}$. In each figure, panels A and B have the same distribution
 409 of Cx43 expression. We plotted EAT for instances in which CB is seen in the
 410 presence of ephaptic coupling ($d_{\text{cleft}} = 2 \text{ nm}, 3.5 \text{ nm}, 8 \text{ nm}, 8.5 \text{ nm}$) but not in
 411 its absence ($d_{\text{cleft}} = 115 \text{ nm}$). At the location where CB is observed (denoted by
 412 $\text{EAT} = 0$) when ephaptic coupling is present (solid box in panel A), there is al-
 413 ways a plateau phase of EAT when ephaptic coupling is absent (solid box in panel
 414 B). Similarly, when there is a gap in EAT in the presence of ephaptic coupling
 415 (dashed box in panel A), a plateau phase in the EAT is seen in its absence (dashed
 416 box in panel B). This implies the presence of relatively large cell clusters that are
 417 excited almost simultaneously when ephaptic coupling is absent, but are difficult
 418 or impossible to excite when ephaptic coupling is present.

419 In light of the foregoing, we may describe the mechanism as follows. For
 420 $0.4 \leq p \leq 0.6$, Cx43KO and WT cells are mixed in roughly equal proportion.
 421 The random distribution of cell genotypes results in local clusters of WT cells
 422 coupled through normal levels of gap junctions, which form electric loads that
 423 may substantially impair the conduction. When an action potential propagates
 424 into such cell clusters, the electric loads can lead to delayed activation or even CB
 425 if the activating current is not sufficiently strong.

426 In the presence of ephaptic coupling (at smaller d_{cleft}), R_c is high, and the cleft

potential may drop low enough to excite the end membrane of cell downstream, but the resulting inward current may not be sufficient to excite the entire cell. The high resistance creates a bottleneck that limits the amount of current into the cleft space and across the end membrane of neighboring cell. Ephaptic coupling may thus lead to a decrease in activating current for cells downstream, which together with the increased load discussed above (when $0.4 \leq p \leq 0.6$), may result in a prominent current-to-load mismatch.

4. Conclusions and Discussions

In this manuscript, we explored the interplay between reduced heterogeneous Cx43 expression and ephaptic coupling on cardiac action potential propagation. Under severely reduced Cx43 expression, we identified three new phenomena in the presence of ephaptic coupling: alternating conduction, in which ephaptic and gap junction-mediated mechanisms alternate; instability of planar fronts; and SAP which has a smaller potential amplitude than the normal action potential. We then examined the effects of heterogeneous Cx43 expression and ephaptic coupling on CB using a 2D cell lattice as our model system. We found that ephaptic coupling can either prevent or promote CB depending on the Cx43KO content. When Cx43KO content is relatively high ($0.7 \leq p \leq 0.9$), ephaptic coupling reduces the possibilities of CB, an effect that is greater in narrower lattices. In wider lattices, ephaptic coupling promotes CB when the Cx43KO content $0.4 \leq p \leq 0.6$. This increase in the probabilities of CB can be attributed to an increase in current-to-load mismatch.

We demonstrated the biphasic behavior of CV_L as a function of d_{cleft} when Cx43KO content is 100% (Sec. 3.1). This nonmonotonicity of CV_L qualitatively

451 agrees with previously published results shown in Kucera et al. (2002); Hand and
 452 Peskin (2010). In Kucera et al. (2002), the maximal CV_L (15 cm/s) under 1% of
 453 normal gap junction is achieved at $d_{\text{cleft}} = 40$ nm. In Hand and Peskin (2010), the
 454 maximal CV_L (20 cm/s) is obtained at $d_{\text{cleft}} = 11$ nm under 1% of normal gap
 455 junction expression. Although the optimal d_{cleft} values are quantitatively different
 456 from ours, the qualitative behavior of CV_L is similar.

457 This is the first study to examine the interaction between heterogeneous Cx43
 458 expression and ephaptic coupling on the occurrence of CB (Sec. 3.3). CB, espe-
 459 cially unidirectional CB (UCB) has been considered a substrate for fatal reentrant
 460 arrhythmias and sudden cardiac death Waldo and Wit (1993). Therefore, over the
 461 last decades, CB or UCB has become the major focus of research. We reported
 462 that ephaptic mechanism can lower the possibilities of CB when Cx43KO content
 463 $p \geq 0.7$, which implies an anti-arrhythmic role of ephaptic coupling when gap
 464 junctions are reduced. This finding is consistent with the result shown in Fig. 2A
 465 of Sec. 3.1 and earlier numerical (Mori et al. (2008); Hand and Peskin (2010);
 466 Lin and Keener (2014)) and experimental results (Yao et al. (2003); Veeraragha-
 467 van et al. (2012)), which suggested that ephaptic coupling can increase conduc-
 468 tion speed when gap junction expression is low. On the other hand, we showed
 469 that ephaptic coupling can increase the proportion of CB when Cx43KO content
 470 $0.4 \leq p \leq 0.6$, which points to a disadvantageous role of ephaptic coupling in this
 471 context. This finding is also consistent with the result shown in Fig. 2B of Sec.
 472 3.1, which suggests that ephaptic coupling can reduce conduction velocity when
 473 gap junctional coupling is sufficiently high. It is notable that similar results have
 474 been reported elsewhere in Kucera et al. (2002); Mori et al. (2008); Hand and Pe-
 475 skin (2010); Lin and Keener (2014). It is important to note, however, that previous

papers Yao et al. (2003); Mori et al. (2008); Hand and Peskin (2010); Veeraraghavan et al. (2012); Lin and Keener (2014) studied ephaptic coupling by examining its effect on conduction speed with a homogeneous change in gap junctional conductance. Our study focused on the reliability of cardiac signal propagation by studying CB in the presence of heterogeneous Cx43 expression.

Finally, we mention that all the effects of ephaptic coupling mentioned in our findings (results in Sec. 3) are shown to be highly dependent on the localized Na^+ channels, which are consistent with the experimental and numerical results in Cohen (1996); Maier et al. (2002); Kucera et al. (2002); Sperelakis and McConnell (2002); Hand and Peskin (2010); Veeraraghavan et al. (2014a); Lin and Keener (2014).

In this paper, we adopted a simplified geometry, cell discretization (each cell is treated to be isopotential) and arrangement of cells. We believe that using realistic cell shape or delicate cell arrangement (Prudat and Kucera (2014); Lin and Keener (2014)) will not qualitatively change our conclusions, but may lead to new phenomena. Although we identified some important features of conduction, alternating conduction, instability of planar fronts and SAP, the detailed mechanism behind these phenomena are still unclear. A future study with simplified models may allow us to obtain better understanding. Finally, we mention that the role of Cx43 may not be restricted to electrical conduction, as suggested in Lübke et al. (2013). This is beyond the scope of our current paper, and is a subject for future study.

498 **Author Contributions**

499 N.W., Y.M. and E.G.T. designed research; N.W. performed the research; N.W.,
500 Y.M. and E.G.T. analyzed the data; and N.W., Y.M. and E.G.T. wrote the manuscript.
501 All authors approved the final version of the paper.

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613 6. Figure captions

614 Fig. 1: A lattice view of a 2D bidomain model in the presence of ephaptic cou-
 615 pling (A) and circuit diagram for two adjacent cells coupled through a shared cleft
 616 in the presence of end-to-end gap junctions (GJ_{end}) (B), side-to-side gap junctions
 617 (GJ_{side}) and resistive connections between extracellular spaces (R_{ee}) are not shown
 618 here. This diagram is not to scale, as the cleft width is three orders of magnitude
 619 smaller than the length of cell. $\phi_i^{(i,j)}$ and $\phi_i^{(i,j+1)}$ represent intracellular potentials
 620 of cells at location (i, j) and $(i, j + 1)$, respectively. $\phi_e^{(i,j)}$ and $\phi_e^{(i,j+1)}$ represent
 621 extracellular potentials of cells (i, j) and $(i, j + 1)$, respectively. Cleft space was
 622 modelled as a narrow compartment between cells (i, j) and $(i, j + 1)$, which is
 623 resistively connected (R_c) to the extracellular space. Cleft potential $\phi_c^{(i,j+\frac{1}{2})}$ is de-
 624 fined at location $(i, j + \frac{1}{2})$. The space, which lies in the extracellular space and
 625 stays adjacent to the aforementioned cleft is called the extracellular-cleft space.
 626 The potential in the extracellular-cleft space is denoted by $\phi_{ec}^{(i,j+\frac{1}{2})}$. Resistive con-
 627 nections between extracellular space and the extracellular-cleft space are denoted
 628 by R_{ec} .

629 Fig. 2: CV_L as a function of d_{cleft} for $p = 1$ (A, Cx43KO) and $p = 0$ (B, WT).

630 Fig. 3: EAT as a function of N at $d_{\text{cleft}} = 8.5$ nm (A), 8.6 nm (B), 8.7 nm (C),
 631 8.9 nm (D).

632 Fig. 4: End transmembrane potential ($\phi_{ic} = \phi_i - \phi_c$) traces of the left (ϕ_{icL})
 633 and right (ϕ_{icR}) sides of cells at location $(3, 24)$, $(3, 25)$, $(3, 26)$ and $(3, 27)$, where
 634 $d_{\text{cleft}} = 5$ nm (A), 8.9 nm (B) and 10 nm (C).

635 Fig. 5: Instability of planar fronts. A sequence of snapshots of propagation, il-
 636 lustrating ϕ at $t = 89$ ms (A), 112 ms (B), 139 ms (C) and 173 ms (D), respectively
 637 at $d_{\text{cleft}} = 9.8$ nm.

Fig. 6: SAP. A sequence of snapshots illustrate ϕ at $t = 13$ ms (A), 51 ms (B), 80 ms (C) and 126 ms (D), respectively for $d_{\text{cleft}} = 3.5$ nm.

Fig. 7: Intermediate wave. A sequence of snapshots illustrate ϕ at $t = 13$ ms (A), 54 ms (B), 77 ms (C) and 107 ms (D), respectively for $d_{\text{cleft}} = 3.8$ nm.

Fig. 8: Activation gating variable (m) profile of Na^+ channels residing on the end membrane (A) and ϕ profile (B) of cell (3, 25) at $d_{\text{cleft}} = 3.5$ nm.

Fig. 9: Activation gating variable (d) profile of L-type Ca^{2+} channels residing on the end (A), side (B) membranes and ϕ profile (C) of cell (3, 25), where $d_{\text{cleft}} = 3.5$ nm (blue), 3.8 nm (red), and 115 nm (green).

Fig. 10: Representative plots of EAT along a 6×75 lattice at the 9th iteration, where $p = 0.6$, $d_{\text{cleft}} = 2$ nm (A), 115 nm (B). Conduction delays and CB (A) are denoted by dashed and solid boxes, respectively. Plateau phase of EAT (B) corresponding to conduction delays and CB are denoted by dash and solid boxes, respectively.

Fig. 11: Representative plots of EAT along a 6×75 lattice at the 20th iteration, where $p = 0.6$, $d_{\text{cleft}} = 3.5$ nm (A), 115 nm (B). Conduction delays and CB (A) are denoted by dashed and solid boxes, respectively. Plateau phase of EAT (B) corresponding to conduction delays and CB are denoted by dash and solid boxes, respectively.

Fig. 12: Representative plots of EAT along a 6×75 lattice at the 35th iteration, where $p = 0.6$, $d_{\text{cleft}} = 8$ nm (A), 115 nm (B). Conduction delays and CB (A) are denoted by dashed and solid boxes, respectively. Plateau phase of EAT (B) corresponding to conduction delays and CB are denoted by dash and solid boxes, respectively.

Fig. 13: Representative plots of EAT along a 6×75 lattice at the 50th iteration,

663 where $p = 0.6$, $d_{\text{cleft}} = 8.5$ nm (A), 115 nm (B). Conduction delays and CB (A)
664 are denoted by dashed and solid boxes, respectively. Plateau phase of EAT (B)
665 corresponding to conduction delays and CB are denoted by dash and solid boxes,
666 respectively.

667 7. Figures

Figure 2

Figure 3

Figure 4

Figure 5

A **B** **C** **D****Figure 6**

A **B** **C** **D****Figure 7**

A

B

Figure 8

A

B

C

Figure 9

Figure 10

Figure 11

Figure 12

Figure 13