Intercellular Connectivity and Multicellular Bioelectric Oscillations in Nonexcitable Cells: A Biophysical Model

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ABSTRACT: Bioelectricity is emerging as a crucial mechanism for signal transmission and processing from the single-cell level to multicellular domains. We explore theoretically the oscillatory dynamics that result from the coupling between the genetic and bioelectric descriptions of nonexcitable cells in multicellular ensembles, connecting the genetic prepatterns defined over the ensemble with the resulting spatio-temporal map of cell potentials. These prepatterns assume the existence of a small patch in the ensemble with locally low values of the genetic rate constants that produce a specific ion channel protein whose conductance promotes the cell-polarized state (inward-rectifying channel). In this way, the short-range interactions of the cells within the patch favor the depolarized membrane potential state, whereas the long-range interaction of the patch with the rest of the ensemble promotes the polarized state. The coupling between the local and long-range bioelectric signals allows a binary control of the patch membrane potentials, and alternating cell polarization and depolarization states can be maintained for optimal windows of the number of cells and the intercellular connectivity in the patch. The oscillatory phenomena emerge when the feedback between the single-cell bioelectric and genetic dynamics is coupled at the multicellular level. In this way, the intercellular connectivity acts as a regulatory mechanism for the bioelectrical oscillations. The simulation results are qualitatively discussed in the context of recent experimental studies.

INTRODUCTION

The spatio-temporal coordination of biological processes requires signal transmission and processing across a wide range of scales from the single-cell to the multicellular level. In the case of ensembles of non-neural cells, simple diffusion alone does not allow a rapid and efficient propagation of signals without significant distortion, and bioelectricity is emerging as a complementary mechanism because of some essential characteristics: (i) bioelectrical signals can act in concert with biochemical and biomechanical signals to orchestrate large-scale outcomes; (ii) electrical potential and current are especially suited for information processing because they can modulate the single-cell state via the membrane ion channels and the multicellular connectivity via the intercellular gap junctions; and (iii) using modern experimental techniques, it is currently possible to associate bioelectrical magnitudes such as cell membrane potentials $V$ with molecular biology components such as the specific ion channel proteins that regulate $V$. Remarkably, the above experimental facts are common not only to networks of excitable cells in the brain but also to nonexcitable cellular ensembles in tissues.

Signaling ions and charged molecules such as calcium and serotonin can influence transcriptional, translational, and post-translational processes. Therefore, genetic and bioelectric networks should be interrelated because the local concentrations of these signaling ions and molecules over multicellular ensembles depend on the spatio-temporal map of cell electric potentials. Increasing experimental evidence shows a complex feedback between multicellular bioelectrical states and gene expression patterns in embryogenesis, regeneration, and tumorogenesis. Hence, a better understanding of bioelectrical magnitudes should allow complementing the currently dominant bottom-up molecular approaches with top-down descriptions based on macroscopic concepts that may be useful for tissue engineering and regenerative medicine.

Experimentally, the dynamic monitoring and spatio-temporal control of bioelectrical states described by cell potentials could be based on electrical double-layer-gated field-effect transistor biosensors, the binding of nanoparticles to the cell membrane, the external application of electric fields and voltage pulses, and the induction of polarized/depolarized cell states by means of pharmacological, optogenetic, and molecular genetic techniques including the local injection of mRNAs that encode specific ion channels. Theoretically, the biophysical description of the above processes requires new conceptual schemes that incorporate not only the single-cell characteristics but also the intercellular connectivity because the control...
of large-scale multicellular ensembles can constitute a convenient alternative to acting on individual cells.

The question of how biological systems process information constitutes one of the current problems in modern biophysical chemistry. It has been shown that multicellular aggregates of nonexcitable cells can store bioelectrical memories in the form of spatio-temporal patterns that encode information for specific biological outcomes.\textsuperscript{1−4,19} In particular, Levin and co-workers have emphasized that these patterns act as a software that allows the communication among cells using both biochemical and bioelectrical signals.\textsuperscript{5−7} In a similar way, synaptic transmission in excitable cells also involves chemical and electrical signals, and these two forms of neuronal communication are crucial for brain development and function.\textsuperscript{2,4,19}

Following an admittedly simplistic but vivid analogy,\textsuperscript{2,4,19} the genome would encode the hardware—for example, the ion channel proteins that regulate cell membrane potentials and intercellular connectivity—but a better knowledge of the software—for example, the spatio-temporal maps of cell electric potentials and signaling molecules—might allow the control of biological outcomes at a different level than that of single-cell molecular genetics. Experimentally, this bioelectrical control has been studied in the regulation of cell proliferation and differentiation,\textsuperscript{6} the plasticity of predifferentiated mesenchymal stem cells,\textsuperscript{5} and the differentiation of the mammalian lens.\textsuperscript{2} Interestingly, it has also been shown that the same body with the same genome can store different bioelectrical patterns acting as distinct memories for regeneration in flatworms,\textsuperscript{3} which suggests the different roles of biological hardware and software in this model system.

Oscillatory phenomena are central to biology, and it has been demonstrated that information processing in non-neuronal cells and bacterial communities makes use of oscillatory biochemical and bioelectrical patterns. For instance, low-frequency current noise and membrane potential oscillations have been detected in glioma cells where specific K\textsuperscript{+} and Na\textsuperscript{+} ion channels coordinate electric responses throughout large cell populations.\textsuperscript{21} Cell electric potentials and metabolic oscillations are closely connected in bacterial communities where the intercellular bioelectrical communication at the long-range level is also based on K\textsuperscript{+} ion channels and extracellular concentrations.\textsuperscript{22} In particular, two biofilm communities undergoing metabolic oscillations can be coupled through electrical signaling in order to synchronize their growth dynamics.\textsuperscript{23} Other experimental examples concern the gap junction-mediated electrical coupling characteristic of the cardiac oscillations in the heart\textsuperscript{24} and the metabolic oscillations in pancreatic islets.\textsuperscript{25} Remarkably, oscillations between polarized and depolarized cell potentials can also be coupled with genetic pathways, as observed in the development of the two sides of an embryo.\textsuperscript{26} In single-neuron models, bistability and oscillatory phenomena have been shown to arise from the coupling between voltage pulses and gene expression.\textsuperscript{27}

It is important to note the central role played by the ion channel proteins in the above experimental systems, although the specific function of a particular channel is often difficult to ascertain because of the complex nonlinear interactions between the different channels involved in each particular case. In the case of neurons, for instance, it has been experimentally demonstrated that a balance between outward and inward-rectifying channels is required for generating slow oscillatory activity.\textsuperscript{28} Recently, a synthetic excitable tissue composed of a small number of functional ion channels and pumps has been described.\textsuperscript{29} The system of optically reconfigurable bioelectric oscillators can perform information processing tasks via propagation of electrical waves based on cell potentials.\textsuperscript{30} Could bioelectrical oscillations allow the coordination of spatially separated cells across long distances?

We attempt to model the oscillations arising from the feedback between the genetic and bioelectric descriptions in a multicellular ensemble of coupled nonexcitable cells where the individual cell properties are regulated by ensemble-averaged magnitudes such as electric potentials. Because ion channels and membrane potentials are significant to organism morphology and tumor initiation,\textsuperscript{4,6−8,15,30,31} we focus on the transcription and translation rates of an ion channel protein that regulates the single-cell membrane potential.\textsuperscript{32} We assume that these rates can be spatially heterogeneous and study the bioelectrical consequences of this assumption. Our previous work concerns the theoretical description of the coupling between the bioelectrical and genetic descriptions\textsuperscript{10} and the simulation of abnormal depolarization processes in model multicellular ensembles.\textsuperscript{33} In the latter case, a first attempt to establish the conditions needed for oscillations was made. However, we did not carry out a complete study of the optimal windows allowing oscillatory signals nor systematically simulated the effects of intercellular connectivity and protein transcription constants on bioelectrical patterning. We connect here the genetic spatial prepatterns with the resulting map of cell potentials, describe the long-range effect caused by the polarized membrane potentials of distant cells on locally depolarized cell potentials, show that oscillatory signals alternating polarized and depolarized cell potentials can be maintained for optimal windows of the intercellular connectivity, and suggest that remodeling this connectivity can act as a regulatory mechanism for the multicellular ensemble.

## Model Genetic and Bioelectric Networks

Biological systems show often a multilayer network structure; in the brain, individual neurons are coupled through gap junctions both via chemical synapses and via electrical synapses.\textsuperscript{34} The collective patterns emerging from the dynamical processes that occur in multilayer networks are much richer than those corresponding to single-layer networks.\textsuperscript{34} In our case, the intercellular coupling is regulated by the feedback between the genetic and the bioelectric layers.\textsuperscript{17,35} On the basis of previous and emerging experimental data (see, e.g., refs 2−4,7 and references therein), we assume that the central biological parameter connecting these layers is the transmembrane potential.\textsuperscript{19} This bioelectrical magnitude is defined as the electrical potential difference \( V < 0 \) between the cell cytoplasm and the extracellular environment. Under conditions of zero total current, \( V \) is usually termed the resting membrane potential.\textsuperscript{35} The potential difference \( V \) is regulated by the extracellular and intracellular ion concentrations together with the conductances of specific ion channels inserted in the cell membrane.\textsuperscript{6,7,30,36−39} This potential constitutes a significant readout of the cell bioelectrical state.\textsuperscript{38,39} For instance, proliferating cancerous cells show that abnormally low values of \( V \) and high values of \( V \) are characteristic of differentiated cells,\textsuperscript{6,7,38} though the particular role of \( V \) in tumorigenesis is still under discussion.\textsuperscript{39} It has been shown experimentally and theoretically that a simplistic but qualitatively useful description of \( V \) can be obtained using a minimal model with two voltage-gated channels \( \text{pol} \) and \( \text{dep} \), one regulating the current around
Figure 1. Genetic and bioelectrical feedback at the single-cell level is shown for the case of a channel protein (pol) whose transcription and translation kinetic equations are based on the central dogma (left, bottom) for the information flow from DNA to mRNA (transcription) to protein (translation). A specific mRNA of concentration \(m\) regulates the channel protein concentration \(p\), where \(m\) and \(p\) are relative values that depend on the biochemical system considered. (Adapted from Figures 1 and 2 of ref 33, published by the PCCP Owner Societies Royal Society of Chemistry.)

The kinetic equations that describe the intracellular mRNA \((m)\) and protein \((p)\) concentrations of one of the ion channels are coupled with the cell potential because the spatial distribution of the signaling ions and molecules that regulate the genetic transcriptional and translational networks depends on the local values of \(V\). In this way, the role of a particular ion channel is sensitive to the other functional channels that may upregulate or downregulate its expression via genetic processes.\(^{43}\) The rate constants for mRNA transcription \((r_m)\) and protein translation \((r_p)\) and the respective degradation rate constants \((d_m\) and \(d_p)\) may depend on multiple kinetic steps.\(^{17,27,44,45}\) The cell potential-dependent concentration \(S\) of a specific signaling ion or molecule is assumed to influence the effective potential-dependent protein transcription rate \(r_m(V)\) (Figure 1, left, bottom). For the dependence of the channel conductance on the protein intracellular concentration \(p\), we consider the Hill kinetics \(G_{pol} = G_{pol}^m[p/(p_0 + p)]\), where \(p_0 = 60\) corresponds to \(G_{pol}^m/2\) and \(G_{pol}^m\) is the maximum conductance (Figure 1, left, top). The cell potential \(V\) is regulated by the conductances \(G_{pol}\) and \(G_{dep}\) with \(G_{pol}/G_{dep} = 1.5\), together with the equilibrium potentials \(E_{pol}\) and \(E_{dep}\) (Figure 1, left, top). The values \(E_{pol} = -60\ mV\) and \(E_{dep} = 0\ mV\) assumed here do not change with time provided that the intracellular and extracellular ion concentrations are approximately constant.\(^{36,47}\)

The interplay between the bioelectric and genetic descriptions is extended from the single-cell to the multicellular ensemble by the intercellular gap junctions of effective conductance \(G_j\) coupling the cells \(i\) and \(j\) (Figure 1, right, bottom). Remarkably, the electrical synapses and the channels that join plant cells have some qualitative similarities to gap junctions concerning the transfer of intercellular information. In nonexcitable cells, which is the case studied here, these intercellular connections are found in a multitude of animal cells. Experimentally, \(G_j\) is a bell-shaped function of the potential difference \(V_j - V_i\) (Figure 1, right, top), where \(G^0\) is the maximum conductance of the junction and the potential \(V_j = 18\ mV\) gives the width of the experimental distribution of conductances.\(^{17,46,48}\) In this way, the cell potential \(V_i\) evolves with time \(t\) because of the single-cell currents \(I_{pol}\) and \(I_{dep}\), and the intercellular current regulated by \(G_j\) and \(V_i - V_j\). The sum over \(j\) in \(nn\) considers only the nearest-neighbor (nn) cells around the central cell \(i\).

As mentioned previously, the experimental basis of the model of Figure 1 is that the electric potential influences the local concentration \(S\) of a signaling ion (e.g., calcium) or molecule (e.g., serotonin) that regulates in turn the transcription rate constant \(r(V)\) of the channel (Figure 1, left, bottom). Certainly, the model is an oversimplification of real biological problems, but it provides a simple description of the bioelectrical and genetic feedback in terms of a reduced number of concepts that...
can be extended to more complex cases. The input parameters for the model of Figure 1 are chosen within a range of biologically relevant values that were previously justified. Note that for the particular case of zero current $I_{pol} + I_{dep} = 0$ between the external microenvironment and the cell cytoplasm of an isolated cell, the transmembrane potential $V$ of Figure 1 (left) reduces to the single-cell resting potential. The equations for the currents $I_{pol}$ and $I_{dep}$ qualitatively describe the observed experimental trends in terms of a small number of phenomenological parameters: the effective charge $z = 3$ for channel gating and the threshold potentials $V_{th,pol} = V_{th,dep} = -V_T$, with $V_T = RT/F = 27$ mV for the thermal potential, where $R$ is the gas constant, $T$ is the temperature, and $F$ is the Faraday constant. Note that the conductances $G_{pol}$ and $G_{dep}$ contribute differently to the total membrane conductance. In particular, when the conductance ratio $G_{pol}/G_{dep}$ takes low values, $V_{mem}$ is decoupled from the normal normalized value $E_{pol}$ and $V_{mem}$ assumes depolarized potentials close to $E_{dep}$.

Experimentally, the concentration $S$ of a signaling ion regulating the genetic rates can change with the cell potential $V_{mem}$. The model of Figure 1 (left, bottom) considers the Hill kinetics $r_m = r_{m0}/[1 + (S/S_0)^n] = r_{m0}/(1 + e^{(V/V_H)}$ for the protein transcription rate, where $r_{m0}$ is the maximum transcription rate that is attained in the absence of a specific signaling ion, $S = 0$, and $S_0$ is a reference concentration. This equation shows a potential-dependent negative regulation of the protein because an increase in $IV$ decreases the production rates of mRNA.

Note that the bioelectric and genetic descriptions of Figure 1 are strongly coupled: the potential $IV$ characteristic of the cell regulates the concentration $p$ of the channel protein that gives the conductance $G_{pol}$ (Figure 1, left, bottom); in turn, $G_{pol}$ modulates the total membrane conductance that gives the potential $V$ (Figure 1, left, top). Recent experimental and theoretical results strongly suggest that there is a significant feedback between bioelectric and biochemical networks (see, e.g., ref 19 and references therein).

Interestingly, the single-cell state described by Figure 1 can be modulated at the ensemble level because of the coupling of the central cell with the neighboring cells. This coupling is allowed by the intercellular gap junctions that permit the transference of electric currents and signaling molecules between two adjacent cells (Figure 1, right, bottom). In particular, every central cell experiences the average electric potential because of its nearest-neighbor cells, as shown explicitly by the sum $\sum_{i \in \text{NN}} G_i (V_i - V_j)$ (Figure 1, right, bottom), where the contribution of the neighbor cell potential $j$ is weighted by its junction conductance $G_i$. The intercellular coupling is described by the conductance ratios $G_{pol}/G_{dep}$ and $G_{pol}/G_{dep}$ for the relative contributions of the intercellular ($G'$) and single-cell ($G_{pol}$) effective conductances with respect to the common reference value $G_{dep}$. In this way, $G'/G_{dep}$ constitutes a measure of the intercellular connectivity degree: large values of $G'/G_{dep}$ should give isopotential multicellular regions, whereas low values of $G'/G_{dep}$ should give isolated cells with no bioelectrical communication. Abnormal intercellular communication resulting in largely autonomous cells appears to be involved in the initial states of some cancerous processes.

The numerical algorithm used to solve the system of coupled equations of Figure 1 has been described in detail previously; see, in particular, the Methods section of ref 35 together with ref 33 for additional explanations. The cells in the multicellular ensemble are assumed to form an elliptic monolayer initially at the same potential $V_i(t = 0)$, with $i = 1, ..., N$, with $N = 304$ cells. The initial concentrations of mRNA and protein are obtained by solving the respective equations of Figure 1 under steady-state conditions. The evolution of the system for times...
t > 0 is given by the N equations for the cell potentials $V_i(t)$ of Figure 1 (right, bottom). The characteristic time $C_i/G_i^{pol}$ gives an electrical response lower than 1 s for capacitances and conductances within the ranges $C_i = 10–100$ pF and $G_i^{pol} = 0.1–1$ nS, respectively. On the contrary, the genetic processes in the cell are relatively slow because transcription and translation rate constants ($r_m^p$ and $r_p$) within the range $0.1–1$ min$^{-1}$ give times between 1 and 10 min, whereas degradation rate constants ($d_m^p$ and $d_p$) within the range $0.003–0.1$ min$^{-1}$ give times between 0.1 and 5 h.

### RESULTS AND DISCUSSION

The results shown in Figure 2a–d are obtained by assuming different values for the genetic rates $r_m^p$ and $r_p$ of Figure 1 in the small patch and the rest of the multicellular domain (Figure 2a). The cells outside the patch have $r_m^p = 1$ min$^{-1}$ and $r_p = 1$ min$^{-1}$, while these rates are decreased to $r_m^p = 0.25$ min$^{-1}$ and $r_p = 0.25$ min$^{-1}$ for those cells in the patch. According to the model of Figure 1, this decrease in the rates would give a reduced expression of the ion channel protein of conductance $G_{pol}$ at the patch and then a local depolarization$^{33}$ with respect to the rest of the domain.

The initial ($t = 0$) conditions used in the multicellular ensemble correspond to the steady-state solution for the dominant polarized state of the cells outside the patch. However, the spatial differences assumed for the genetic rates tend to produce a regionalization of the domain polarization in the long term: the decrease of the long-range coupling values.

At intermediate values of $N_p$ (Figure 2b) and $G^{pol}/G_{dep}$ (Figure 2d), oscillatory cell potentials are obtained. To better understand these bioelectrical oscillations, it should be noted that when the pol ion channel protein transcription and translation rate constants rates are sufficiently low there is only one stable (depolarized) potential, which is the case of the cells in the patch.$^{33}$ On the contrary, when these rate constants take high values, there is only one stable (polarized) potential, which is the case of the cells outside the patch.$^{33}$ Thus, the stable bioelectrical states for the cells in the patch and the rest of the domain are unique and opposite. Initially, all cells in the domain are assumed to have the same polarized potential that characterizes the normal cell state (Figure 2b,d). However, this polarized potential is not stable for the cells in the patch, which have relatively low rates $r_m^p$ and $r_p$ with respect to the rest of the domain. These low protein production rates eventually give low values of the channel conductance $G_{pol}$ for the cells in the patch. Therefore, these cells tend to reach the stable depolarized potential consistent with the locally low rates assumed. In the long range, however, the cells in the rest of the polarized domain act as a bioelectrical buffer forcing the repolarization of the patch for low $N_p$ (Figure 2a) and high $G^{pol}/G_{dep}$ (Figure 2c). For intermediate cases, bioelectrical oscillations in the patch can be sustained (Figure 2b,d) because of the interplay between the genetic (protein concentration in Figure 1, left, bottom) and electric (cell potential in Figure 1, left, top) mechanisms.

We establish now the experimental window where bioelectrical oscillations would be possible. Figure 3 shows the phase diagram obtained for the cell potentials in the patch as a function of number of cells, $N_p$, and the degree of intercellular connectivity, $G^{pol}/G_{dep}$. The insets schematically show the different time evolutions of the cell potentials for three bioelectrical states of the patch.

The simulations of Figures 2 and 3 suggest that modifying the coupling degree provided by the gap junctions of Figure 1 (right) allows the flexible topology, which is needed to establish or abolish single-cell bioelectrical oscillations between the depolarized and polarized single-cell states. In the limits of weak and strong coupling represented by low and high values of the intercellular connectivity, the cells in the patch exhibit only weak and strong coupling represented by low and high values of the intercellular connectivity. In this way, the long-range effects on the small patch to be expected in each experimental case can be predicted and optimal windows for the patch size and intercellular coupling can be established. In particular, the different regions of Figure 3 clearly suggest that (i) the patch size is critical for bioelectrical oscillations to occur and (ii) remodeling the intercellular connectivity constitutes a regulatory mechanism for establishing spatio-temporal bioelectrical patterns. Both theoretical predictions are in qualitative agreement with experiments conducted on model systems.$^{2,4,19,26,36,55}$

The simulation results of Figures 2 and 3 suggest that modifying the coupling degree provided by the gap junctions of Figure 1 (right) allows the flexible topology, which is needed to establish or abolish single-cell bioelectrical oscillations between the depolarized and polarized single-cell states. In the limits of weak and strong coupling represented by low and high values of the intercellular connectivity, the cells in the patch exhibit only one dynamical solution which corresponds to the depolarized or polarized state, respectively. However, other dynamical solutions including oscillatory states emerge at intermediate intercellular coupling values.

The extensions of the depolarized and polarized regions of Figure 3 also suggest that the regimes of weak and strong intercellular coupling where no oscillation is possible should be robust. In the first case, the cells in the patch are essentially isolated from the rest of the domain and remain thus in the depolarized state because of their low pol channel protein.
transcription rates. In the second case, these cells can shift to the opposite polarized state because of the coupling with the surrounding polarized cells in the rest of the domain in spite of the locally low rate constants in the patch favoring depolarization.

Experimentally, weakly connected multicellular ensembles show spatial heterogeneities of the cell polarization that are crucial in embryogenesis where the local expression of ion channels and pumps usually gives bioelectrical regionalization.2,14,41 On the contrary, strongly connected ensembles should give isopotential ensembles where no patterning information can be stored.17 In this case, the strong coupling of the patch with the majority of cells in the rest of the domain would provide bioelectrical stabilization against any spatial fluctuation of the genetic rates favoring the patch abnormal depolarization. Note that this long-range stabilization could be useful to avoid the autonomous behavior of the patch cells promoted by abnormally low local genetic rates.17,33

It has been reported that endogenous bioelectric gradients act as instructive factors for morphogenetic processes and that the intercellular connectivity experiences dynamic changes during embryonic development.2,3 Indeed, the electric potentials of distant cells can reverse local biological processes by long-distance signaling.26,55 Indeed, it has been experimentally observed that opposite local and distant bioelectric signals can counterbalance crucial cellular processes such as proliferation and apoptosis.55 In developing embryos, for example, the brain development that was disrupted by the local perturbation of cell potentials could be reversed by long-distance electrical signals.55 As it could be expected, the efficient transduction of these distant bioelectric signals requires the presence of active gap junctions:55 the disruption of the gap junction-based intercellular communication on one side of the embryo can affect electrically driven physiological changes on the other side56 and influence apoptosis in the developing brain.55 In the above processes, the dynamic nature of the intercellular gap junctions appears to be crucial.

Figure 4 provides some qualitative insights relevant to these experimental observations.26,55 In particular, we illustrate how dynamic, time-dependent gap junction conductances allow the transitions between the cell membrane potentials in the small patch. Initially (t = 0), the cells in the patch are assumed to be in the polarized (red) state. However, the low intercellular connectivity \( G_o/G_{dep} = 0.4 \) considered initially in Figure 4 cannot allow the distant normally polarized cells to enforce this state for a long time because the local genetic prepattern in the patch favors the opposite abnormally depolarized (blue) state. In this case and because of the patch isolation, the genetically favored depolarized state is eventually reached (Figure 4). At intermediate times, the increase assumed for the intercellular coupling (\( G_o/G_{dep} = 0.5 \)) allows the distant cells to start influencing the patch cells and thus oscillatory potentials are obtained. Finally, at a longer time, the increase of the intercellular connectivity to \( G_o/G_{dep} = 0.6 \) can allow the distant polarized cells to reverse the local electric potentials of the depolarized cells in the small patch (Figure 4).

Figure 5 is obtained at the intermediate intercellular conductance \( G_o/G_{dep} = 0.5 \), and the protein transcription rate \( r_p \) is changed. As in Figure 4, the cells in the patch that were polarized at \( t = 0 \) could depolarize with time because of the low values initially assumed for the \( pol \) channel protein transcription rate. However, these cells become polarized when this rate is increased, assisted also by the long-range action exerted by the polarized cells outside the patch. In between these cases, an oscillatory regime emerges, which is determined by the number of cells in the patch and the intercellular coupling (Figure 3).

Taking together, the simulation results of Figures 4 and 5 show the counterbalancing effect caused by the stable polarized membrane potentials of the distant cells on the stable depolarized potentials of those cells localized in the patch, suggesting a binary short-/long-range control of the patch membrane potentials that can be regulated by the intercellular connectivity. Therefore, the long-distance transduction of electrical signals through dynamic gap junctions shows that the bioelectrical state of individual cells within a small group can be influenced at the ensemble level by a majority of surrounding cells.55

It must be mentioned that in real cases, the long-distance regulatory mechanisms also typically involve the spatio-temporal distribution of signaling ions and molecules such as calcium, butyrate, and serotonin.24,19 This effect is not explicitly accounted for in the model, but the fact is that the local concentrations of these ions and molecules are influenced by time-dependent maps of electric potentials similar to those described here.59 For instance, the membrane potentials of Xenopus embryos hyperpolarized cells have been found to influence a distant tumorigenic site with oncogene-expressing cells.60 In this case, the butyrate influx into these cells and subsequent inhibition of histone deacetylation resulted in tumor cell proliferation arrest and the reduction of tumorigenic structures.60 Although these fluxes are not explicitly accounted for in the model, we believe that the results of Figures 2−5

Figure 4. Remodeling the intercellular connectivity emerges as a regulatory mechanism. The cells in the small patch have locally low values of the protein rate constants \( r_p \) and \( r_m \). The snapshots correspond to the different multicellular bioelectric states obtained when the dynamic intercellular conductance follows the time sequence \( G_o/G_{dep} = 0.4, G_o/G_{dep} = 0.5, \) and \( G_o/G_{dep} = 0.6 \) schematically shown by the abrupt conductance steps. The spatio-temporal maps of potentials are obtained for a fixed number of cells \( N_p = 55 \) in the patch and a total number of cells \( N = 304 \) in the multicellular domain. Other conditions and parameters are the same as that of Figure 2.
emerge as collective phenomena because of the coupling between the single-cell (Figure 1, left) bioelectric and genetic descriptions at the multicellular level (Figure 1, right). Note that we have not assumed here any periodic variation for the biological magnitudes characteristic of the single cell. It is the average electric potential resulting from the intercellular coupling that makes the cells to act as an oscillating multicellular patch in the model. This fact is evident from the significant role played by the number of interacting cells (Figure 2a) and the intercellular coupling degree (Figure 2c) in the patch.

(iv) The collective nature of our simulation results immediately suggests that the combination of local genetic prepatterns with dynamic gap junctions may allow implementing distributed biological memories via spatio-temporal maps of cell potentials, a question of experimental significance. Note in this context that the local position of the cells within the patch is not sufficient to establish their bioelectric state in Figures 4 and 5, rather it is the long-range communication with the remaining outer cells that determines the patch multicellular outcome (depolarized–oscillatory–polarized). In Figure 4, it is the dynamic evolution of the gap junctions that allows implementing different bioelectric patterns with the same genetic prepattern, which suggests that different spatio-temporal maps can be dynamically established and maintained by modulating the intercellular coupling.

## CONCLUSIONS

There are clear experimental motivations for the model simulations described here. For instance, bioelectrical oscillations modulated by different ion channels are observed in glioma cells, β-cells in pancreatic islets, bacterial communities, and multicellular domains in the heart. In the latter case, bioelectrical signals can influence cardiac function and modify the gene expression of extracellular matrix components. Experimentally, spatio-temporal bioelectric patterns can be monitored, for example, by a protein whose fluorescence intensity varies with the electric potential across the membrane. In particular, fluorescent membrane-potential indicators show the significance of bioelectric patterning during oogenesis in Drosophila ovarian follicles. Also, the central role played by particular channels can be demonstrated by effectively abolishing their electrical activity using specific pharmacological inhibitors. In general, the bioelectrical control of multicellular ensembles can be attempted by targeting specific ion channel and gap junction proteins at the transcriptional and post-translational levels. Although different drugs targeting ion channels have been approved for human use, a serious limitation is that externally modifying the membrane potential could have unexpected effects on the cellular microenvironment.

The simulations presented here show that a small number of cells within a multicellular domain can act as a single oscillatory population for optimal levels of intercellular coupling, suggesting collective regulatory mechanisms of single-cell polarization states. The physical basis for this regulation is the system-level bioelectrical response to local genetic changes: the oscillations in the patch arise because every single-cell state is determined not only by the individual potential $V_i$ but also by the potential difference $V_j - V_i$ relative to the neighboring cells $j$ (Figure 1, right). This theoretical prediction has an

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**Figure 5.** Dynamic changes in the single-cell balance between the dep and pol channels caused by an increase in the transcription rate constant $r_n$ of the pol channel protein at fixed intercellular conductance. Initially ($t = 0$), the cells in the small patch are assumed to be in a polarized state that is not stable because of the locally low values of the transcription rate $r_n$ corresponding to the pol channel protein. The subsequent snapshots correspond to the different multicellular bioelectrical states obtained when the patch rate $r_n$ is increased from 0.1 to 0.5 min$^{-1}$, with $r_p = 0.2$ min$^{-1}$ and a fixed intercellular conductance $G/G_{dep} = 0.5$ in the whole ensemble. The spatio-temporal maps of potentials are obtained for a fixed number of cells $N_p = 55$ in the patch and a total number of cells $N = 304$ in the multicellular domain. Other conditions and parameters are the same as those of Figure 4.
experimental basis\textsuperscript{3,20,26} and suggests that some bioelectrical control of multicellular patches should be possible, as opposed to the need of acting on each cell individually. In this context, we must note that biomechanical oscillations in cell monolayers can also show a collective dynamics regulation.\textsuperscript{32} In our case, the external regulation and collective control of depolarized/polarized cell states should be of biological significance because of the instructive role of membrane potentials in cell proliferation and differentiation,\textsuperscript{6–8} the plasticity of predifferentiated mesenchymal stem cells,\textsuperscript{7} and regeneration processes.\textsuperscript{4}

Recent experiments on model animals have also related bioelectrical oscillations with long-range information processing in nonexcitable cells,\textsuperscript{26} showing that the electric potentials of distant cells can reverse local processes by long-distance signaling.\textsuperscript{7} Unfortunately, the oversimplified model used here does not allow a direct comparison with real biological systems. However, the simulations suggest that genetic prepatterns, established, for example, by the distribution of signaling molecules over the ensemble, acting in concert with intercellular connectivity can produce maps of electric potentials that can be useful as information processing mechanisms for multicellular outcomes.

Note finally that the model implicitly assumes that the electrical potential maps may influence the single-cell transcription processes because of the feedback between the genetic and the bioelectrical levels of description (Figure 1). In this way, individual cells could display different memories stored in the form of distinct bioelectrical states (Figure 3) that could be retrieved by appropriate changes in the spatial distribution of the signaling molecules that regulate specific rate constants (Figure 5) and the intercellular communication (Figure 4). Future work currently in progress will explore the interplay between genetic prepatterns of ion channel expression and the remodeling of the intercellular connectivity as possible mechanisms for establishing distributed memories in ensembles of non-neural cells.

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Notes
The authors declare no competing financial interest.

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