Synchronization of bioelectric oscillations in networks of non-excitable cells: from single-cell to multicellular states

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ABSTRACT

Biological networks use collective oscillations for information processing tasks. In particular, oscillatory membrane potentials have been observed in non-excitable cells and bacterial communities where specific ion channel proteins contribute to the bioelectric coordination of large populations. We aim at describing theoretically the oscillatory spatio-temporal patterns that emerge at the multicellular level from the single-cell bioelectric dynamics. To this end, we focus on two key questions: (*i*) *what single-cell properties are relevant to multicellular behavior* and (*ii*) *what properties defined at the multicellular level can allow an external control of the bioelectric dynamics*. In particular, we explore the interplay between biochemical pre-patterns and oscillatory cell potentials in a model multicellular ensemble, describe the spatio-temporal patterns that arise when the average electric potential allows groups of cells act as a coordinated multicellular patch, and characterize the resulting synchronization phenomena. The simulations concern bioelectric networks and collective communication across different scales based on oscillatory and synchronization phenomena, thus shedding light on the physiological dynamics of a wide range of endogenous contexts across embryogenesis and regeneration.

INTRODUCTION

Pattern regulation, in embryogenesis and regeneration, requires complex spatio-temporal coordination of cell behavior to achieve long-range anatomical order.[1,2] Robustness of patterning and physiology is the result of complex signalling pathways mediated by not only biochemical and transcriptional, but also by bioelectrical signalling: note the use of ion channel- and pump-mediated ion flows and voltage gradients by all cells (not only neurons and muscle) to communicate on the tissue and organ levels.[3,4] Mutation of electrogenic proteins often leads to characteristic birth defects,[5] and targeted modulation of the endogenous bioelectric patterns result in downstream changes of gene expression, cell differentiation, and morphogenesis. Thus, recent progress in this field has highlighted the importance of this control system for regenerative medicine,[6] cancer,[7] immunology,[8] and synthetic biology.[9,10] Despite this progress in the molecular details of how voltage patterns control downstream events,[3,4] crucial knowledge gaps remain about the bioelectric code itself: the global spatio-temporal dynamics of bioelectrical signalling within tissues. This is a pre-requisite for understanding the evolutionary implications of developmental bioelectrics and for the design of rational strategies for biomedical intervention.[11,12]

There exists a significant interplay between the bioelectrical and biochemical signals of non-excitable cells both at the intra and intercellular levels, though the mechanisms involved are challenging to study because of the complex feedback between these different signals. While emphasis is usually made on positional information and spatial bioelectric pre-patterns,[12] oscillatory phenomena and synchronization are basic components in computational procedures[13] and can also be exploited in collective information processing. Oscillations are characteristic of cell populations and developmental patterning,[14,15] as well as in primitive cognition in slime

molds.[16,17] In particular, oscillatory cell membrane potentials have been observed in glioma cells,[18] bacterial communities,[19] and pancreatic islets[20] where specific ion channel proteins contribute to the coordination of large cell populations. Recently, bioelectric oscillations and dynamic gap junction networks have been experimentally characterized in the development of chicken embryos.[21] Describing collective oscillations is difficult, however, because of the complex spatio-temporal patterns that emerge at the multicellular level from single-cell dynamics.[22] At these different levels of description, two closely related questions immediately arise: (*i*) *what single-cell properties are relevant to multicellular behavior* and (*ii*) *what biophysical properties defined at the multicellular level can allow an external control of the system dynamics*.[3,23–25]

Conceptual approaches for describing bioelectric oscillatory phenomena in networks of non-excitable (non-neural) cells can be based on the ion channel proteins inserted in the cell membrane.[24] These approaches have a solid experimental basis because ion channels form the aqueous pores at the cell membrane that are responsible for the exchange of biological information between the cell inside and the outer environment, including the neighboring cells.[26,27] In particular, these channels regulate the transmembrane potential V_{mem} defined as the electrical potential difference through the cell membrane, which plays a crucial role in proliferation and differentiation.[5,7,23] While persistent deviations of V_{mem} from the steady physiological values are not likely to occur because of the feedback mechanisms that regulate V_{mem},[7,27] transcriptional and translational changes affecting key ion channel proteins could compromise this control, though cells can also control these channels at the post-translational level. Also, since bioelectrical signals are shared between neighboring cells, a key question is to find out if *individual transcriptional and translational processes could be influenced by changes*

of ensemble-averaged cell membrane potentials. Multicellular electric potentials can exert an endogenous control on the molecular random kinetics of cell biochemistry because they constitute ensemble-averaged macroscopic magnitudes defined at a higher level than single-cell characteristics.[24,25,28,29] The causal structure of physical systems can be studied at different spatial and temporal scales and it is an open question as to whether this structure could be fully captured from the most detailed microscale descriptions.[30] This is an important issue, because efforts to control bioelectric dynamics in contexts such as tumor normalization[31] or repair of birth defects[32] need to be informed by models that facilitate the design of interventions at the most effective and causal level.

Although it has long being known that bioelectric events can determine cell behavior and gene expression, the large-scale code mapping between spatio-temporal bioelectric patterns and specific anatomical outcomes is still unknown.[3,23] It is clear however that transduction mechanisms enable *bioelectrical* signals to be integrated with other, often downstream, means of intercellular communication such as *biomechanical* and *biochemical* signals.[7,21,23,33] For instance, a perturbation of V_{mem} can be transduced in modifications of the distribution of negatively charged phospholipids over the membrane.[33] In turn, these conformational changes may influence the clustering of signaling proteins with positive residues around the negative lipids. Finally, these intracellular rearrangements can indirectly impact on downstream transcriptional pathways regulating cell proliferation.[33] Other end-effects on transcription can also result from the spatio-temporal distribution of signaling ions and molecules such as calcium and serotonin, which depend markedly on V_{mem}.[3,23,29,34] Moreover, changes in V_{mem} have often a post-translational effect because of the opening/closing of specific voltage-gated channels.[26] These experimental facts suggest that bioelectrical signals and cell states are not a mere consequence of

biochemical processes but may have also an *instructive role* in the observed feedback between biological mechanisms occurring at different levels.[3,35,36] In this way, while V_{mem} may not be a transcription factor itself, it can indirectly modulate transcription via other biochemical and biomechanical effects.[5,7,23,33,34]

In a different context, local potentials and currents allow information processing in electronic devices composed of many interconnected individual components, which suggests that bioelectrical signals should also be especially suited to provide information exchange in multicellular ensembles,[3,24] as is the case of neural networks. Following this analogy, it is conceivable that the spatio-temporal map of local V_{mem} values may allow some control of multicellular ensembles.[3,24,28,29] In particular, the intercellular gap junctions can provide a short-range connectivity for neighboring cells complementary to the spatio-temporal maps of signaling molecules involved in positional information and multicellular patterning processes. Note that molecular diffusion-reaction processes alone are relatively slow and messy for a precise regulation of these maps over long distances.[37,38] Moreover, signaling ions and molecules such as calcium and serotonin carry electrical charges and thus their distribution is markedly influenced by the map of V_{mem} values. It is then of interest to explore theoretically other long-range biomechanical[39] and bioelectrical[24] mechanisms that may coordinate spatially-separated cells.

We have studied recently the collective properties of gap junction-coupled multicellular ensembles. When a high enough number of cells in the ensemble share a common bioelectric state, they could impose this particular state to the rest of cells following a threshold mechanism[28,40,41] due to *system-level bioelectric responses to local transcriptional changes*. The multicellular oscillations are possible because the single-cell state is modulated not only by the individual membrane potential but also by the potential difference relative to the neighboring

cells. These theoretical predictions have an experimental basis,[3,24,36,42] suggesting that collective regulatory mechanisms of single-cell bioelectric states could be possible,[20,40] as in the cases of biomechanical oscillations in monolayers of coupled cells[39] and biochemical networks relevant to cell differentiation and tumorigenesis.[22] We aim here at exploring the coupling mechanisms that may allow and sustain bioelectric oscillations in multicellular networks based on the interplay between biochemical pre-patterns and oscillatory cell potentials. In particular, we consider the spatio-temporal oscillatory patterns and synchronization phenomena that arise when the average electric potential makes cell groups to act as a coordinated multicellular patch.[24,28,29] It is tempting to speculate that these modes of bioelectric communication across different scales may act as *distributed biological memories* in non-excitable cells.[42] Instead of focusing on a particular biological problem, we describe general results relevant to intercellular bioelectric communication that can be applicable to different contexts, emphasizing functional rather than structural aspects.

New biophysical approaches are timely in view of the recently available experimental techniques. For instance, spatially distributed gene expression can be monitored using microfluidic devices where confined compartments allow establishing the gene expression spatio-temporal patterns.[43] Also, patterns of cell potentials can be dynamically studied using electrically-gated FET biosensors,[44] nanoparticle binding to cell membranes,[45,46] and protein fluorescence based on electrically-dependent optical activity.[9] Note in addition that the cell membrane potential can currently be associated with the molecular characteristics of key ion channel proteins.[3,32] In this way, optogenetic and pharmacological techniques such as local injection of particular mRNAs that encode ion channel and gap junction proteins can modulate the cell polarization states.[8,23,24,31] Also, the electrical activity of specific channels can be

suppressed by specific pharmacological inhibitors.[18,47]

MODEL BIOCHEMICAL AND BIOELECTRIC NETWORKS

Biochemical and bioelectric networks are interrelated in an *instructive* way, as shown in a multitude of experimental contexts. For instance, the local concentrations of signaling ions and charged molecules such as calcium and serotonin that influence transcriptional, translational, and post-translational processes depend on the spatio-temporal map of cell electric potentials.[3,25,26,29,36] Also, the relationship between the membrane potential and cell proliferation and differentiation processes suggests that multicellular bioelectric states are significant to gene expression patterns in embryogenesis, regeneration, and tumorigenesis.[3,48–51] In these experimental contexts, a phenomenological description of the feedback between biochemical and bioelectric networks can complement *bottom-up* molecular approaches with alternative *top-down* mechanisms.[3,24,48,50,51]

There exists an electrical potential difference between the cell cytoplasm and the extracellular medium (Figure 1) that is regulated by the electrical conductance of specific ion channels in the membrane together with the intracellular and extracellular ionic concentrations.[7,52–55] For the particular case of zero current $I_{\text{pol}} + I_{\text{dep}} = 0$ between the external microenvironment and the cell cytoplasm, the transmembrane potential of Figure 1 would reduce to the steady-state resting potential,[26,28] which constitutes a bioelectric read-out of the cell state. For instance, differentiated cells tend to show relatively high values of $|V_{\text{mem}}|$ while proliferating cells are characterized by abnormally low values of $|V_{\text{mem}}|$,[7,49,56] although the role of V_{mem} in tumorigenesis is still a matter of controversy. Interestingly, a minimal model based on two

effective channels promoting low (depolarized, *dep*) and high (polarized, *pol*) values of $|V_{\mathrm{mem}}|$ allows a useful qualitative description of $|V_{\mathrm{mem}}|$.[24,28,53-55]

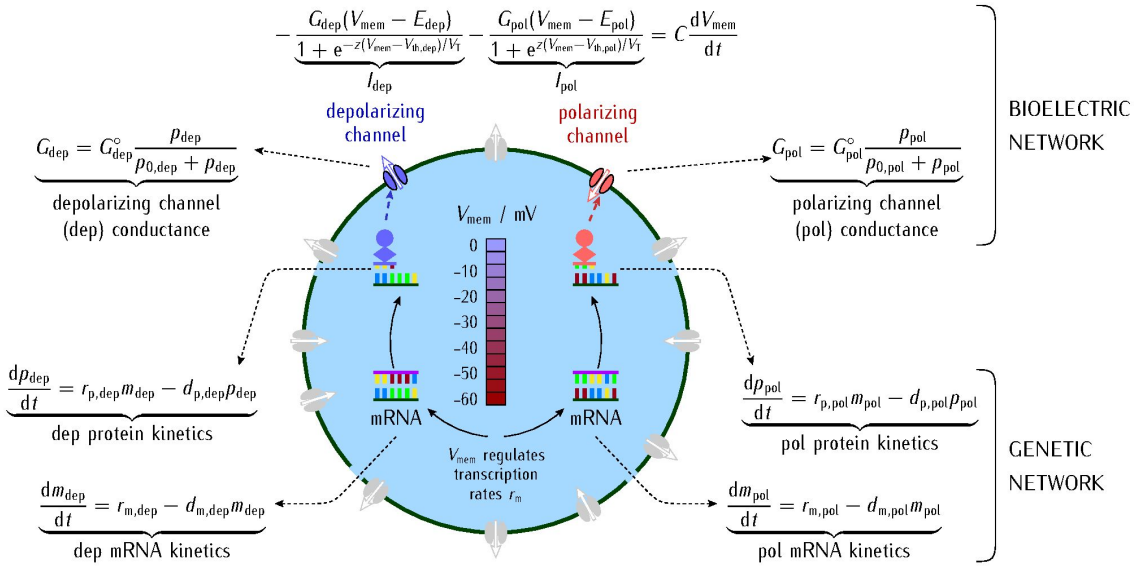


Figure 1. The single-cell description of the biochemical and bioelectrical feedback is shown for two channel proteins that regulate opposite cell polarizations, an extension of the model previously described in ref 24. The kinetic equations describe the protein transcription and translation. Two specific mRNAs of concentrations m_{dep} and m_{pol} regulate the respective protein concentrations p_{dep} and p_{pol}; these magnitudes are relative values that depend on the biochemical problem considered. As a first approximation, molecular diffusion within the cell is ignored because it is fast compared with genetic processes. The rate constants for mRNA transcription ($r_{\mathrm{m,k}}$), protein translation ($r_{\mathrm{p,k}}$), and degradations ($d_{\mathrm{m,k}}$ and $d_{\mathrm{p,k}}$) are effective values that may correspond to multiple kinetic steps. In the text, the protein transcription rates are written as $r_{\mathrm{m,k}}(V_{\mathrm{mem}})$ (k = pol, dep) to emphasize that they depend on V_{mem} because this cell potential can influence the concentrations S_{pol} and S_{dep} of the respective signaling ions or molecules. Note that this dependence provides the feedback between the biochemical and the bioelectric layers of description. In the figure, C is the cell capacitance and we consider the Hill kinetics $G_{\mathrm{k}}=G^{\mathrm{o}}_{\mathrm{k}}p_{\mathrm{k}}/(p_{0,\mathrm{k}}+p_{\mathrm{k}})$ for the dependence of the k channel conductance on the protein concentration p_{k}, where $G^{\mathrm{o}}_{\mathrm{k}}$ is the maximum

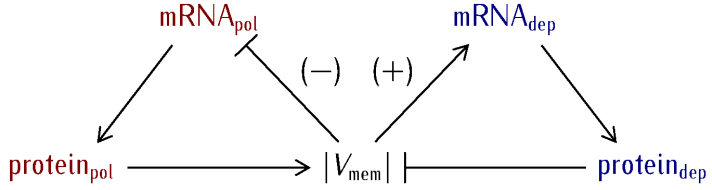
conductance and $p_0 = p_{0,k} = 60$ corresponds to $G_k^o / 2$. Note that the cell transmembrane potential V_{mem} is modulated by the *dep* and *pol* conductances G_{dep} and G_{pol} that act to establish the respective depolarized and polarized potentials potentials E_{dep} = 0 mV and E_{pol} = –60 mV. Note that the cell is assumed to be in contact with an external microenvironment that acts as a bioelectric buffer in the sense that the extracellular ionic concentrations are approximately constant and thus these potentials do not change with time.

Because of the feedback between the biochemical and bioelectric descriptions,[3,24,25,29,36,57,58] the kinetic equations for the concentrations of mRNAs (m_{dep} and m_{pol}) and ion channel proteins (p_{dep} and p_{pol}) are coupled with the cell potential V_{mem} (Figure 1). Therefore, the upregulation/downregulation of a specific ion channel depends on the other functional channels via the cell bioelectric state. Experimentally, this feedback between channels can lead to different compensatory and control mechanisms.[34,53,59] In addition to acting on the ion channel expression, V_{mem} can also manifest post-translationally by closing voltage-gated channels or by driving the channel blocking with specific molecules.[26,28,47,60]

Figure 1 is an extension of a biophysical model described in detail previously.[24] In this case however, both the *dep* and *pol* channel protein concentrations are influenced by V_{mem} because of the potential-dependent local concentrations S_{dep} and S_{pol} of the respective signaling ions and molecules that regulate the transcription rate constants $r_{m,dep}(V_{mem})$ and $r_{m,pol}(V_{mem})$ of the respective ion channel proteins, as shown in Figure 2. The typical input values assumed for the model parameters have already been justified.[25,40] Figures 1 and 2 constitute a minimal model for the complex feedback between the biochemical and bioelectric layers of description. This feedback scheme involves only a small number of basic concepts and can be extended further to

more realistic cases.[24,29,61] Interestingly, the biophysical description of Figure 1 can provide a *bistable memory* based on depolarized and polarized single-cell states.[25,54,55]

The phenomenological equations for the currents I_{dep} and I_{pol} of Figure 1 allow a qualitative description of typical current-voltage curves in terms of a reduced number of experimental parameters: the effective charge $z = 2$ for channel gating and the channel threshold potentials $V_{th,pol} = V_{th,dep} = -V_T$, where $V_T = RT/F = 27$ mV is the thermal voltage, with T the temperature, R the gas constant, and F the Faraday constant.[26,28] The different contributions of the *dep* and *pol* channels to the membrane conductance regulate the cell potential V_{mem}. For instance, when the conductance ratio G_{dep}/G_{pol} is high, V_{mem} decouples from the normal polarized potential E_{pol} and takes abnormally low potentials close to the depolarized potential E_{dep}.[28,54]



$$(-): \frac{r_{m,pol}}{r^{\circ}_{m,pol}} = \frac{1}{1+(S_{pol}/S_{0,pol})} = \frac{1}{1+e^{|V_{mem}|/V_T}} \qquad (+): \frac{r_{m,dep}}{r^{\circ}_{m,dep}} = \frac{(S_{dep}/S_{0,dep})}{1+(S_{dep}/S_{0,dep})} = \frac{e^{|V_{mem}|/V_T}}{1+e^{|V_{mem}|/V_T}}$$

Figure 2. The feedback between the mRNA transcription rate and the absolute value of the transmembrane potential regulates the protein concentrations, with $r_{m,k}(V_{mem} = 0) = r^{\circ}_{m,k}/2$ (k = pol, dep). The potential regulation is *negative* (–) for the *pol* channel protein and *positive* (+) for the *dep* channel protein. These bioelectric regulations thus allow the spontaneous emergence of the single-cell oscillations without the need of an outside pacemaker.

The concentration S_k of a signaling ion influencing the protein transcription rates can be regulated by V_{mem}.[29,34,62,63] The model of Figure 2 assumes that the protein transcription rates can follow *positive* or *negative* Hill kinetics. For the *positive* regulation (*dep* channel) the potential-dependent transcription rate is $r_{m,dep}(V_{mem}) = r^o_{m,dep} \cdot (S_{dep} / S_{0,dep}) / \left[1 + (S_{dep} / S_{0,dep})\right]$ $= r^o_{m,dep} e^{|V_{mem}|/V_T} / (1 + e^{|V_{mem}|/V_T})$ and for the *negative* regulation (*pol* channel) $r_{m,pol}(V_{mem})$ $= r^o_{m,pol} / \left[1 + (S_{pol} / S_{0,pol})\right] = r^o_m / (1 + e^{|V_{mem}|/V_T})$ where $S_{0,k}$ (k = pol, dep) is a reference concentration.[25] In this way, the biochemical and bioelectric processes become coupled: the cell potential V_{mem} regulates the channel protein concentrations p_k of conductances G_k and, in turn, the conductances G_k regulate the potential V_{mem}. Figure 2 shows that the potential V_{mem} has a central role in the interplay between the mRNAs, proteins, and cell potential because it regulates the feedback between the biochemical and bioelectric layers of description.

Figure 3 shows that the single-cell states of Figure 1 can be extended to the multicellular case by means of voltage-gated gap junctions[25,64,65] of conductance G_{ij} that couple two neighboring cells i and j through intercellular currents $I_{ij} = G_{ij} \cdot (V_i - V_j)$, where we omit the subscript (mem) in V_i for clarity. Experimentally, these junction conductances depend on the potentials of the neighboring cells and are involved in development, regeneration, and cancer growth and progression.[65–67] The multicellular ensemble is a planar monolayer of $N = 304$ cells assumed to be initially at the same cell potential $V_i(t = 0)$, $i = 1,\ldots, N,$. The initial mRNA and protein concentrations correspond to the depolarized potential case of the respective equations in Figure 1.[25] At time $t > 0$, the multicellular state changes according to the N equations for the cell

potentials $V_i(t)$. Electrical responses are characterized by a time C_i / G_{ref} of the order of 1 s for a cell capacitance C_i = 100 pF and a reference channel conductance G_{ref} = 0.1 nS.[25] On the contrary, the genetic processes are much slower because transcription and translation rate constants in the range 0.1–1 min^{-1} give characteristic time responses between 0.02 and 0.2 h while degradation rate constants in the range 0.003–0.1 min^{-1} give times between 0.1 and 5 h.[25,58,61]

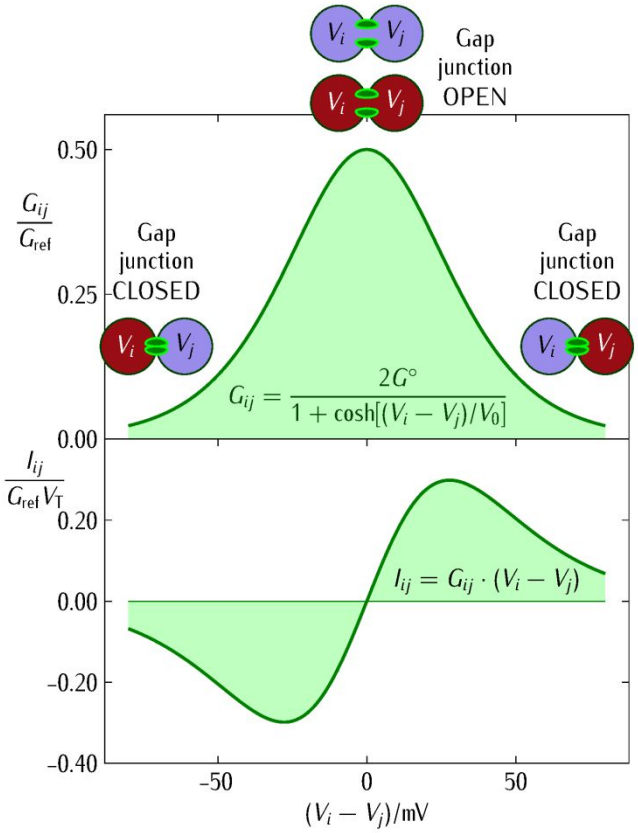


Figure 3. The feedback between the biochemical and bioelectric descriptions of the cell can be extended to the multicellular level by introducing intercellular gap junctions of conductance G_{ij} that couple two neighboring cells *i* and *j* (*up*). Experimentally, G_{ij} shows a bell-shaped dependence with the intercellular potential difference $V_i - V_j$, as in the figure. The maximum junction conductance $G^{\text{o}} = 0.5 G_{\text{ref}}$ and the reference potential $V_0 = 18$ mV characterize the experimental distribution of conductances. In this model,

the cell potential V_i changes with time t according to the intercellular current I_{ij} regulated by G_{ij} and $V_i - V_j$ (*bottom*) and the single-cell currents $I_{\mathrm{pol},i}$ and $I_{\mathrm{dep},i}$ of Figure 1. We incorporate only the nearest neighbors around the central cell i in the sum over the surrounding cells j.[24] In order to characterize the relative contributions of the intercellular G^{o} and single-cell $G^{\mathrm{o}}_{\mathrm{pol}} = G^{\mathrm{o}}_{\mathrm{dep}}$ conductances to the cell potential, all these conductances are scaled to the common reference value G_{ref}.

RESULTS AND DISCUSSION

Single cell

Figure 4 shows the single-cell oscillations of the transmembrane potential, the *pol* and *dep* channel protein concentrations, and the respective channel conductances. Note the close correspondence between protein concentrations and channel conductances as well as the effects of these conductances on the potential. The results show clearly the feedback between the biochemical and bioelectrical layers of description at the single-cell level (Figures 1 and 2).[25,57,58] Experimentally, the non-linear coupling of ion channel proteins with local cell potentials plays a role in morphogenetic phenomena.[3,23]

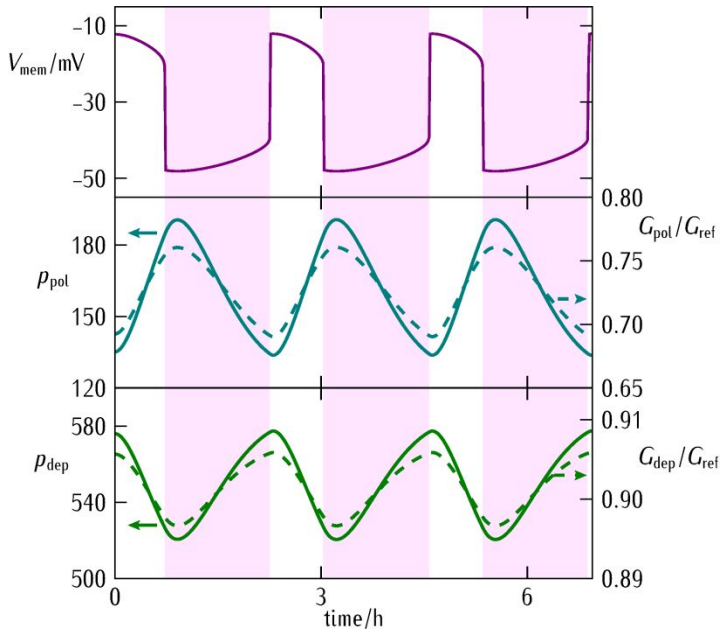


Figure 4. The single-cell bioelectric oscillations corresponding to the transmembrane potential (*up*) and the channel protein concentrations (*left* axis) and conductances (*right* axis) for the system of Figure 1. The *pol* and *dep* protein transcription and degradation rate constants are $r^{o}_{m,k} = r_{p,k} = 0.8$ min^{-1} and $d_{m,k} = d_{p,k} = 0.03$ min^{-1} (k = pol, dep). Note the time delay between the oscillations. Note that the electric potential regulations of Figure 2 explain the emergence of the single-cell oscillations directly from the physiological model circuit without any external pacemaker.

Figure 5 establishes the space phase region of genetic rate constants where the above oscillatory phenomena can be obtained for the particular biophysical model of Figures 1 and 2. The numbers in the curves correspond to the oscillation periods obtained. For a better understanding of the essential trends of the model, all transcription and translation rate constants take the same values, as is the case of the degradation constants. The ranges of rate constants where *coupled bioelectrical and biochemical oscillations* exist are given in the central region of Figure 5. Phase diagrams of increasing complexity can be constructed for the particular experimental parameters characteristic of each biological problem.[41]

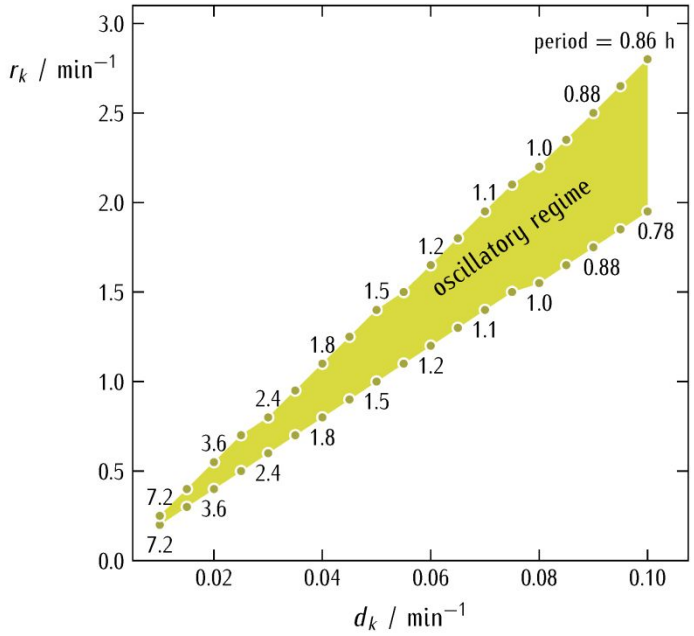


Figure 5. Phase diagram showing the *pol* and *dep* protein transcription and degradation rate constants where single-cell coupled bioelectrical and biochemical oscillations are obtained (central region). The numbers in the curves correspond to the oscillation periods (in hours, h). The system parameters are those given in the caption of Figure 1. Note that we assume $r^{o}_{m,k} = r_{p,k} = r_k$ and $d_{m,k} = d_{p,k} = d_k$ (k = pol, dep) with $r_{pol} = r_{dep}$ and $d_{pol} = d_{dep}$ for simplicity.

The phase diagram of Figure 5 establishes the region of *oscillatory* cell states as a function of genetic rate constants. In particular, Figure 5 associates individual oscillation frequencies with groups of rate constants. Because of cell heterogeneity, a wide range of individual time responses should then be expected for an uncoupled multicellular ensemble where no intercellular communication exists. It could be anticipated that intercellular connectivity will assure reliable average responses despite the individual cell variability. In other words, the intercellular coupling could act as an error-minimizing mechanism because the different frequency states resulting from the synchronization of the biochemical and bioelectric

layers at the single-cell level can be coupled together at the multicellular level. We consider this important question in the next sections.

Homogeneous multicellular ensemble

Figure 6 show the transmembrane potential oscillations in a multicellular ensemble characterized by zero (*up*) and non-zero (*bottom*) gap-junction conductances simulating weak and strong intercellular coupling, respectively. In both cases, the rate constants are in the oscillatory region of Figure 5. However, these rate constants take different values in the left and right regions of the multicellular ensemble to allow for different oscillatory periods. As a consequence, the oscillations in the left and right regions are independent in absence of intercellular coupling. On the contrary, the ensemble can oscillate as a whole for strong coupling despite the fact that the ion channel protein rate constants take different values in the left and right regions. These limiting cases suggest that a range of intermediate oscillatory patterns characterized by different spatio-temporal regionalizations of the rate constants should be possible; particular examples showing different *flexible bioelectric topologies* are given in ref 41.

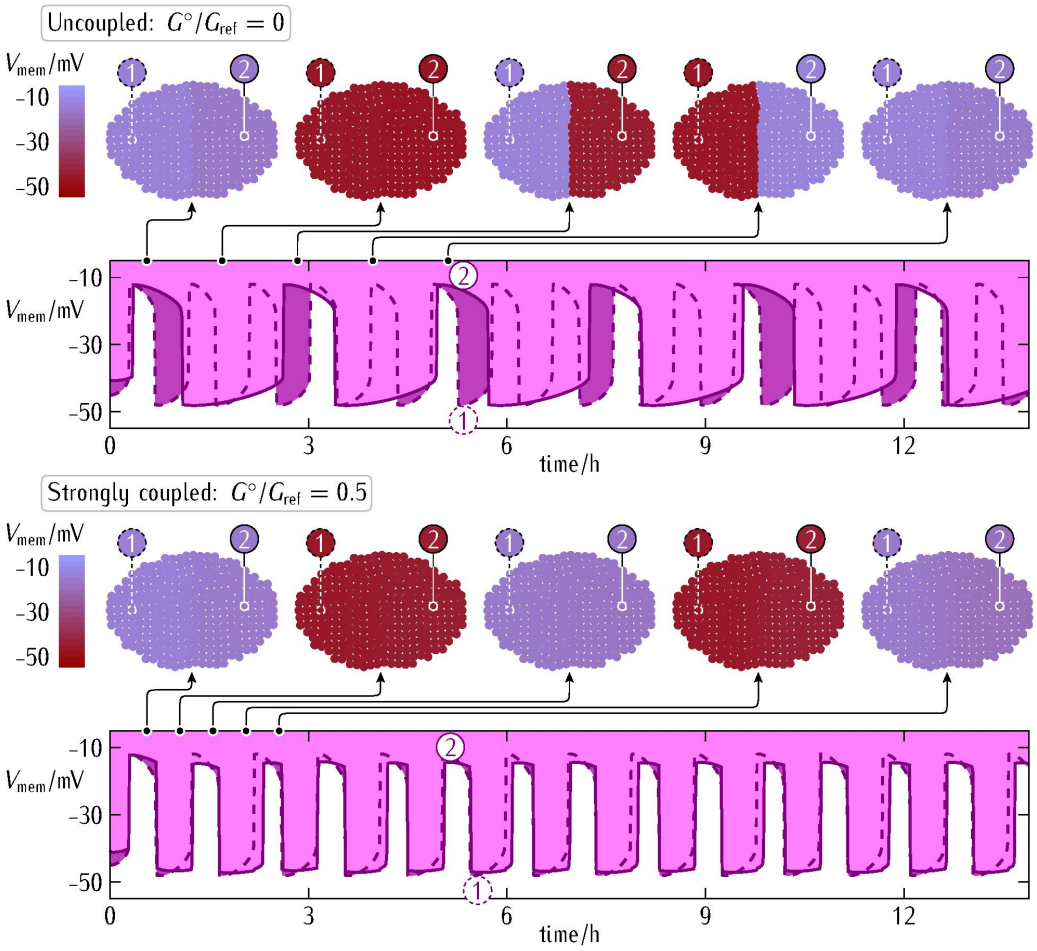


Figure 6. Snapshots showing the oscillations of the transmembrane potentials in the multicellular ensemble for the gap-junction conductance $G^{o}/G_{ref} = 0$ (zero intercellular coupling, *up* panel). Note that the periods of the left and right regions of the ensemble differ by a factor 2 approximately. The ensemble oscillates as a whole for the gap-junction conductance $G^{o}/G_{ref} = 0.5$ (strong intercellular coupling, *down* panel). As in Figure 5, the single-cell rate constants for the *pol* and *dep* proteins are equal, $r^{o}_{m,k} = r_{p,k} = 0.8\ \text{min}^{-1}$ and $d_{m,k} = d_{p,k} = 0.03\ \text{min}^{-1}$ (left region of the ensemble) and $r^{o}_{m,k} = r_{p,k} = 1.7\ \text{min}^{-1}$ and $d_{m,k} = d_{p,k} = 0.07\ \text{min}^{-1}$ (right region of the ensemble), k = pol, dep, with $G^{o}_{pol}/G_{ref} = G^{o}_{dep}/G_{ref} = 1$. Initially ($t$ = 0), the membrane potentials take the polarized value. Biochemical oscillations of the mRNA and protein concentrations are coupled to these

bioelectric oscillations (see Figure 4).

Experimentally, single-cell cycle regulation[49,62,68] and multicellular functions[20,39,42,52,69,70] may involve the coordination of oscillatory bioelectrical and biochemical signals. In this context, the above results provide clear physical insights at the single-cell (note the feedback between the biochemical and bioelectric layers of description in Figures 1, 2 and 4) and multicellular (note the intercellular coupling supporting the bioelectric oscillations of Figure 6) levels. For instance, Figure 6 suggests that the single-cell oscillation of Figure 4 consisting of two alternating polarized/depolarized cell states[40] can be extended at the multicellular level by: (*i*) changes in the spatial distribution of the signaling molecules that regulate the different protein rate constants assumed in the left and right regions of the ensemble and (*ii*) modifications of the intercellular coupling between cells.[41]

Endogenous bioelectric gradients are instructive factors in morphogenetic processes[3,36] that can be regulated by a dynamic intercellular connectivity during embryonic development.[42,71] For instance, the transduction of distant bioelectric signals through active gap junctions can influence the developing brain because local and distant bioelectric signals may have counter-acting actions.[32,71] Also, the transmembrane potential of somatic cells appears to influence oncogene-mediated tumorigenesis at long-range.[69] Figure 6 suggests that a distant oscillatory control should also be possible by showing two limiting cases for the bioelectric coupling of single-cell membrane potentials over a two-region ensemble. While long distance developmental mechanisms in real biological cases are regulated by the spatio-temporal distribution of signaling ions and molecules, the local concentrations of electrically charged messengers such as calcium, butyrate, and serotonin are influenced by the spatial maps of cell potentials.[24]

Heterogeneous multicellular ensemble

Self-organization of biochemical oscillators into spatio-temporal patterns have been previously observed during embryo development.[72,73] However, the possible role of biochemical and bioelectric coupling on the multicellular oscillations is not usually considered despite the fact that ensemble-averaged electric potentials may exert an endogenous control on the single-cell biochemical kinetics,[24,25,29] as shown in the case of pancreatic islets.[74] Individual variability and network stochasticity are inherent to multicellular aggregates. Therefore, it is important to show that synchronized bioelectric oscillations at the multicellular level are also possible in a heterogeneous ensemble.

We study the oscillation and synchronization of the cell potentials as a function of the intercellular coupling for a bimodal distribution of single-cell frequencies which are randomly distributed in the multicellular ensemble (Figure 7). For increasing values of the gap junction conductance G^{o}/G_{ref}, the initially assumed bimodal distribution for the individual cell frequencies eventually collapses into one multicellular effective frequency at long time. The ensemble shifts to lower periods (higher frequencies) because the average electric potential makes groups of cells to act as a coordinated multicellular patch. Note here that: *i*) single-cell electrical responses are much faster than transcriptional and translational processes[25,28] and *ii*) these biochemical processes are regulated by the cell transmembrane potentials in the model of Figure 1. Consequently, those cells with higher intrinsic frequencies eventually dominate the oscillatory behavior of the whole ensemble.

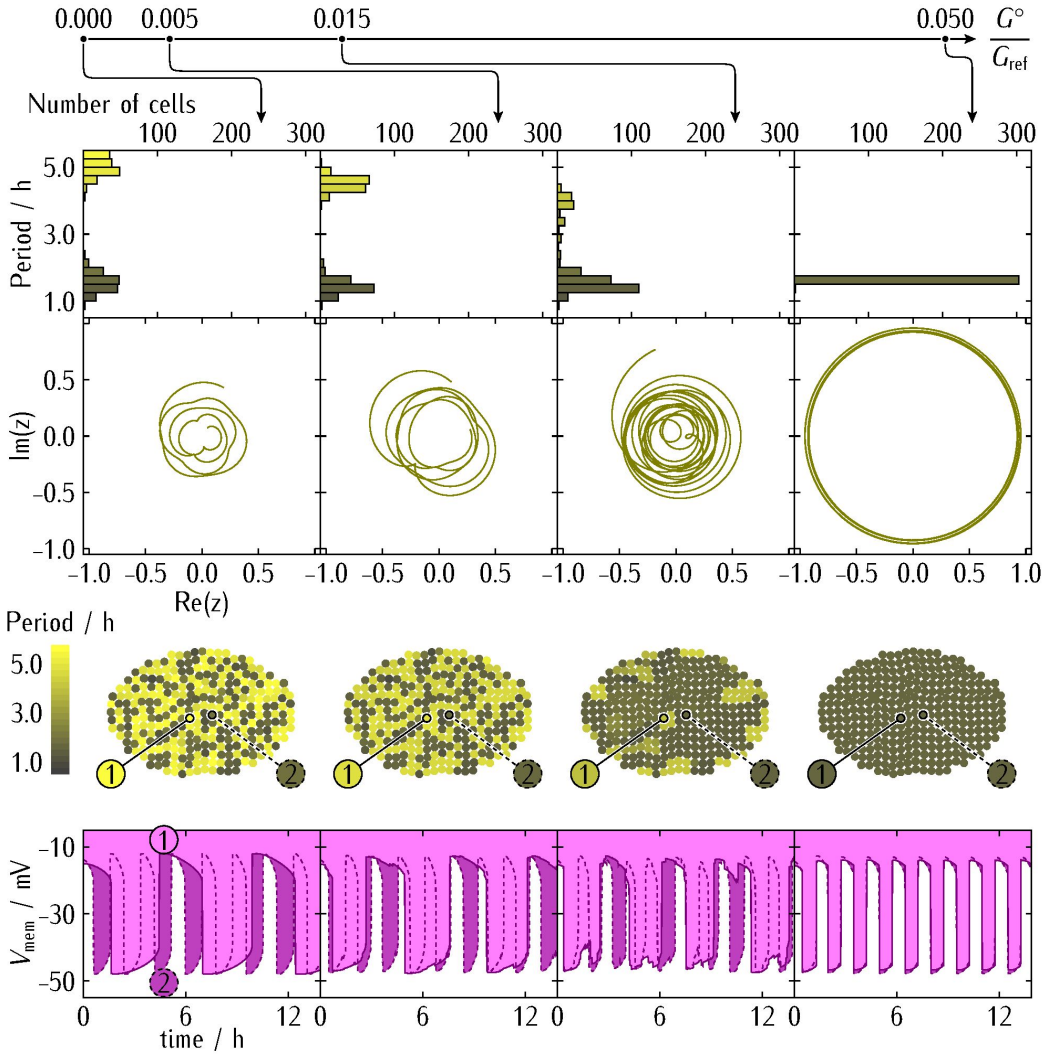


Figure 7. The synchronization of cell transmembrane potentials is shown as a function of the dimensionless gap-junction conductance G^{o}/G_{ref} (*up*) for the case of a spatially random bimodal distribution of single-cell frequencies at zero coupling (*left*) that collapses into one *effective* ensemble frequency for the case of high intercellular coupling (*right*). The trajectories of the multicellular ensemble order *z* parameter in the complex plane are shown together with the corresponding frequency maps. The maps show self-organized clusters of different frequencies that merge into a synchronized multicellular ensemble at high intercellular coupling. The transmembrane potential oscillations of two particular cells in the ensemble are also shown for different values of G^{o}/G_{ref} (*bottom*).

To better show this collective synchronization, Figure 7 shows also the trajectories of the

order parameter z in the complex plane Re(z)–Im(z) for different values of the intercellular coupling strength. This parameter is defined using an individual period obtained as the time between two consecutive protein concentration maxima (see Figure 4), $T_n = t_{n+1} - t_n$. The single-cell effective period is not in general constant and allows defining an individual phase $2\pi(t - t_n)/T_n$ at an intermediate time t. From the j single-cell phase θ_j, the complex order parameter of the ensemble can be written as $z(t) = \sum_{j=1}^{N} \exp(\mathrm{i}\theta_j)$. In the incoherent regime of zero coupling, the individual phases are uncorrelated. By increasing the intercellular conductance multicellular synchronization is gradually achieved, as shown by the limit circle[75] corresponding to the highest conductance. The frequency maps obtained at long times and the transmembrane potentials of two particular cells in the ensemble are also shown in Figure 7 for different values of $G^{\mathrm{o}} / G_{\mathrm{ref}}$.

Figure 8 considers the case of a spatially-structured *L/R* distribution of single-cell frequencies instead of the *spatially random* distribution of Figure 7. As in the case of Figure 7, the ensemble synchronization to a high effective frequency (low effective period) is achieved by increasing the gap-junction conductance. However, relatively high intercellular conductances are now needed for frequency locking: compare the $G^{\mathrm{o}} / G_{\mathrm{ref}}$ scale of Figure 8 with that of Figure 7. This result occurs because the high period *right* (*R*) ensemble of Figure 8 can resist better the driving effect of the low period *left* (*L*) ensemble than in the case of the high period small microdomains of Figure 7. This difference is also clearly shown in the two-circles (Figure 8) and one-circle (Figure 7) trajectories of the order parameter z. In both cases, however, desynchronization should be possible by changing the dynamic intercellular connectivity that modulates the spatial patterns; particular examples of this *reset* function are given in Figures 3

and 4 of ref 41.

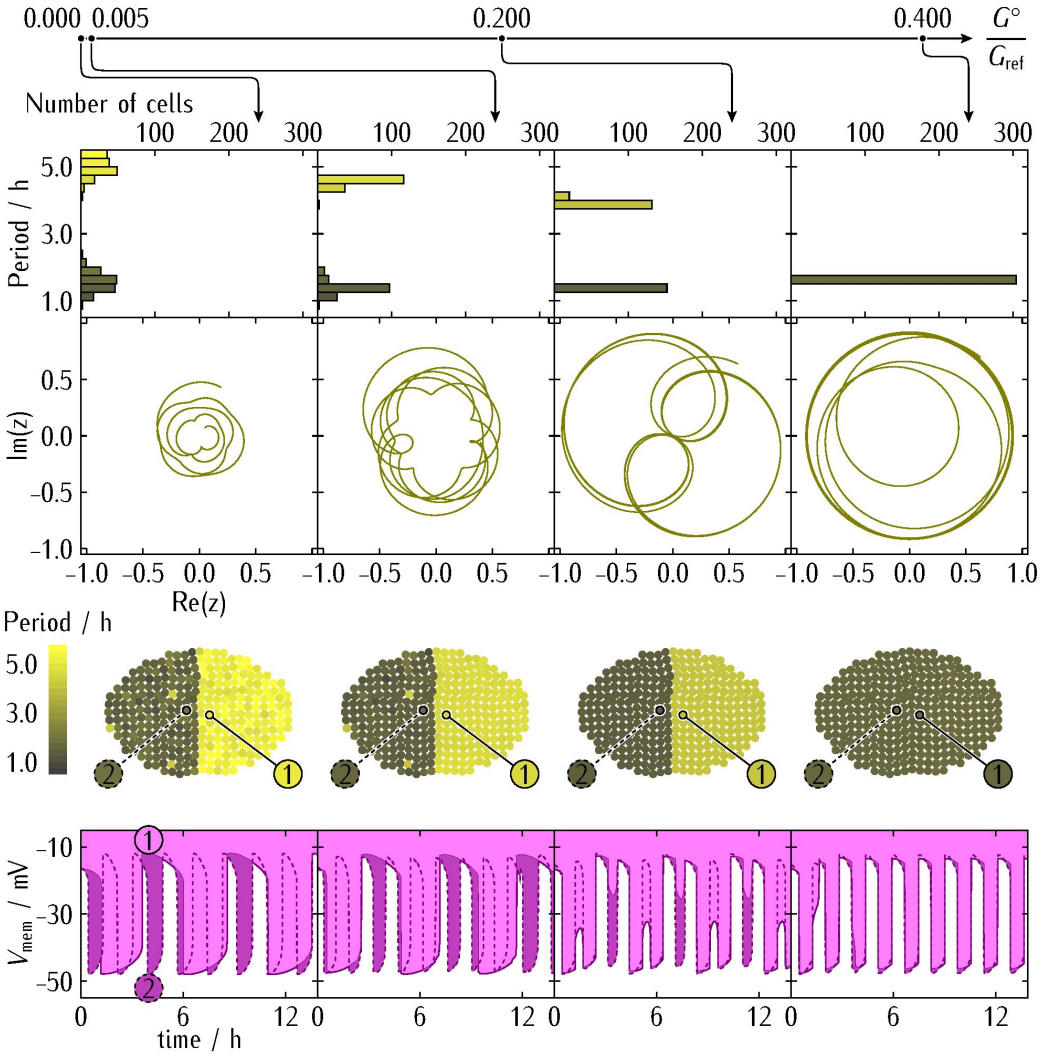


Figure 8. The synchronization of cell transmembrane potentials is shown as a function of the gap-junction conductance for the case of a spatially-structured *L/R* distribution of single-cell frequencies. The trajectories of the multicellular ensemble order *z* parameter in the complex plane are shown together with the corresponding frequency maps. The transmembrane potential oscillations of two particular cells in the ensemble are also shown for different values of G^{o} / G_{ref} .

From an experimental viewpoint, enhancing the intercellular coupling is analogous to increasing the density in quorum sensing bacterial ensembles[19,76] and cell populations,[15] which leads to collective behaviors at different levels of complexity. In this context, the results of

Figure 8 can also be extended to the case of spatially-structured oscillatory cell populations[15,72,73] by introducing pre-patterns of genetic rate constants and gap-junction conductances.[24,41] These patterns can define spatially-isolated domains in the multicellular ensemble where the cells coupled within a particular domain share the same oscillatory state which is different from those in the neighboring domains (see ref 41 for specific examples).

Note that the protein concentrations p_k (see Figures 1 and 2) should also synchronize with the cell potential oscillations of Figures 7 and 8, as shown in Figure 4. Increasing experimental data shows a significant interplay between the transmembrane potential and the progression through the cell cycle.[51,62,68,77] Therefore, the ensemble synchronization of electric potentials should have also feedback effects concerning the collective regulation of individual cell cycles. In a different context, the genes encoding the ion channels and synaptic proteins that regulate the local electric potentials in the brain are involved in the modulation of oscillatory patterns during memory encoding in particular areas, suggesting experimental correlations between gene expression and oscillating brain states.[78] Synchronization and oscillatory coherence can contribute to memory consolidation by acting on the expression of plasticity related proteins.[79,80]

In summary, the feedback between the bioelectric and biochemical descriptions (Figure 1) allows oscillatory and synchronization phenomena at the single-cell (Figure 4) and multicellular (Figures 6–8) levels. Remarkably, these collective modes of information processing emerge in a natural way without assuming any input periodic function for the biological magnitudes involved at the single-cell and multicellular levels. The voltage-dependent intercellular conductances of Figure 3 allow coherent multicellular states because the *average transmembrane potential* acts as an *ensemble controller* that couple together individually

different single-cell frequencies to produce a double biochemical/bioelectrical coherence (Figures 4 and 7) for high enough junction conductances.

The simulations are of qualitative relevance to oscillatory collective communication across different scales in distinct experimental contexts: aggregates of glioma cells,[18] bacterial communities,[19] pancreatic islets,[20] and the possible coupling between the expression of plasticity related proteins and oscillating brain states.[78,79] In all these cases, specific ion channel proteins contribute to the coordination of large cell populations by regulating endogenous electric pulses. It is conceivable that ensemble-averaged bioelectric magnitudes may assist in the control of single-cell characteristics by improving the system reliability at the multicellular level,[24,81] which suggests that external actions on cellular ensembles can be based on electric potentials and currents.[3,23,24,32,45,82]

It is tempting to speculate if the interplay between biochemical pre-patterns and cell potentials, regulated by the intercellular coupling, could provide a sort of *bioelectric software* implementing *distributed biological memories* via slow oscillatory patterns. In the synapses of neural networks, the functional feedbacks between biochemical and electrical signals allow for information processing and memory.[83] Following this analogy,[3,36,84] we note that the intercellular junctions that couple two neighboring cells in the model of Figure 3 are regulated by voltage-dependent conductances that change dynamically with the bioelectric states of the connected cells. From a *biological hardware* viewpoint, these conductances provide the junction plasticity[24] which is needed for a distributed memory where the network nodes are locally adapted to the dynamic state of the different neighboring regions.[41]

Most models of non-neural networks are based on stable attractors. However, the regulation of large-scale synchrony by a dynamic intercellular connectivity[41] may also allow

multiple attractors as transient spatial patterns reminiscent of brain oscillatory states.[79,80] In our case, the collective states are regulated by bioelectrical and biochemical layers of multicellular control showing both intra-layer and inter-layer synchronizations because of the controller role of ensemble-averaged transmembrane potentials.

CONCLUSIONS

Oscillations, synchronization, and pattern formation occur in biology across a range of spatial and temporal scales.[85,86] We have explored theoretically the bioelectric coupling between the *biochemical patterns* of ion channel protein expression and the *cell potential maps* as a plausible mechanism to control multicellular networks of non-neural cells. Because of its simplicity and independence from many structural details, the model suggests generic ion channel and gap junction dynamics that can be relevant to a range of functional interactions at the transcriptional and post-translational levels through the spatio-temporal maps of cell potentials.[23,31,32,36,48,51,60] Interestingly, the model predicts that coherent oscillations can emerge in a heterogeneous ensemble of non-neural cells without any centralized coordination.

The control of transmembrane potentials can be a powerful experimental strategy because patterns of electric potentials and currents are central to information processing not only in neural cells[79,80,87] but also in bacterial communities and non-neural cells.[18–20,33,42,52,82,88] In addition, the role of intercellular coupling on the regulation of multicellular dynamical states is crucial on cell differentiation and tumorigenesis.[22,42,89] The final goal here would be to devise new methods of acting on multicellular ensembles in terms of small number of average bioelectric magnitudes as a collective approach complementary to manipulating individual cell characteristics.[3,24,36,90]

We have suggested that a memory based on bioelectric patterns could permit a *top-down* control complementary to the dominant *bottom-up* approach based on biochemical pathways and individual cell genetics because multicellular ensemble-averaged properties such as the electric potential can be used to gain network control, as shown in experiments on model animals.[3,23,32,36,60] In our case, the simulations show that long lasted (several hours) electrical rythms can synchronize feedback processes between model biochemical and bioelectrical networks. Future work can extend this theoretical approach to include specific molecular mechanisms of feedback at both the single-cell and multicellular levels.[24,40]

The model results emphasize that experimentally testable actions concerning slow oscillatory phenomena in non-neural cells should consider the feedback between bioelectric and biochemical networks. It is well known that potassium channels can influence cell proliferation using different mechanisms.[62] In particular, slow electrical oscillations in the cell polarization are associated with phase-specific changes in the cell cycle (see Figure 2 of ref 62). Remarkably, a reduced number of key ion channels contribute to these transient hyperpolarization and depolarization processes characteristic of the cell cycle (see Figure 1 of ref 68). In a different context, neuron models of gene expression incorporate biochemical schemes with an activity-dependent ion channel expression.[63] The feedback between bioelectric and biochemical pathways is included here by channel mRNAs that are produced at a rate that depends on a calcium concentration-activated factor (see Figures 1 and 2 of ref 63 for the case of a Ca^{2+} integral controller). This approach shows that biophysical models incorporating a minimal number of biologically-consistent assumptions can be useful.[34]

For practical and testable actions concerning oscillatory phenomena, however, systems with a limited number of ion channels and gap junctions whose functional role is known[9,52,87]

should be used to avoid exceedingly complex nonlinear interactions. In real biological problems, a detailed identification of the particular channels entering the biochemical and bioelectric networks, together with a global understanding of the resulting feedback circuits, will be essential ingredients to developing control strategies for bioelectric signaling in applications.[21,32,48]

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Notes

The authors declare no competing financial interest.

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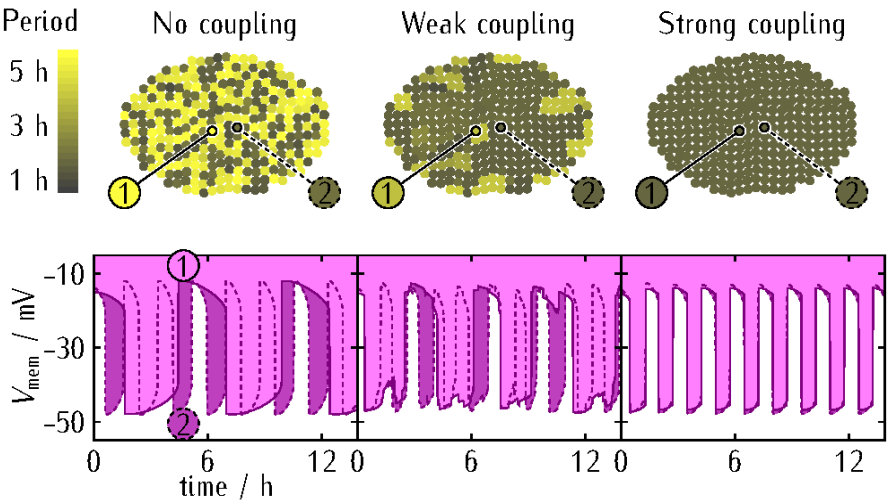
TOC


Period
5 h
3 h
1 h
No coupling
Weak coupling
Strong coupling
1
2
V_{mem} / mV
-10
-30
-50
0
6
12
time / h

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