Metabolic basis of brain-like electrical signalling in bacterial communities

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Information processing in the mammalian brain relies on a careful regulation of the membrane potential dynamics of its constituent neurons, which propagates across the neuronal tissue via electrical signalling. We recently reported the existence of electrical signalling in a much simpler organism, the bacterium Bacillus subtilis. In dense bacterial communities known as biofilms, nutrient-deprived B. subtilis cells in the interior of the colony use electrical communication to transmit stress signals to the periphery, which interfere with the growth of peripheral cells and reduce nutrient consumption, thereby relieving stress from the interior. Here, we explicitly address the interplay between metabolism and electrophysiology in bacterial biofilms, by introducing a spatially extended mathematical model that combines the metabolic and electrical components of the phenomenon in a discretized reaction–diffusion scheme. The model is experimentally validated by environmental and genetic perturbations, and confirms that metabolic stress is transmitted through the bacterial population via a potassium wave. Interestingly, this behaviour is reminiscent of cortical spreading depression in the brain, characterized by a wave of electrical activity mediated by potassium diffusion that has been linked to various neurological disorders, calling for future studies on the evolutionary link between the two phenomena.

This article is part of the theme issue ‘Liquid brains, solid brains: How distributed cognitive architectures process information’.

1. Introduction

Local interactions between individual cells are known to generate complex emergent behaviour in multicellular organisms, which underlie the functionalities of tissues, organs and physiological systems [1]. The human brain provides one of the finest examples of such phenomena, with its complexity ultimately emerging from the interactions between neuronal cells [2]. Similarly, unicellular organisms can also self-organize into communities with complex community-level phenotypes [3]. Bacterial biofilms, in particular, provide a good model system for the study of collective behaviour in biological systems [4].

We have previously shown that biofilms of the bacterium Bacillus subtilis can display community-level oscillatory dynamics [5]. These oscillations ensure the viability, under low-nitrogen (low-glutamate) conditions, of the cells in the centre of the community, which are crucial for community regrowth upon external sources of stress like antibiotics. In our experimental set-up (figure 1a), growing two-dimensional biofilms that reach a certain size develop oscillations [6], such that peripheral cells periodically stop their growth (see black dotted line in figure 1b,c). This behaviour was attributed to a metabolic codependence...
between central and peripheral cells mediated by ammonium [5] (see introduction to §2 below).

Besides exhibiting metabolic oscillations in growth rate, our biofilms display periodic changes in extracellular potassium (figure 1b) and cellular membrane potential (figure 1c), as reported by the fluorescent dyes APG-4 and Thioflavin-T (ThT), respectively. The membrane potential changes reported by ThT are caused by the periodic release of intracellular potassium ions [7] and figure 1d). Potassium propagates from the centre of the biofilm to its periphery according to the following scenario: potassium released by glutamate-deprived (and thus metabolically stressed) cells in the centre diffuses towards neighbouring cells, causing depolarization, stress and subsequent potassium release (figure 2, right), which thereby keeps propagating along the biofilm in a bucket-brigade manner [7].

Notably, potassium and glutamate are the two most abundant ions in living cells [8]. In addition to their roles in osmoregulation, pH maintenance and nitrogen metabolism, they are essential for information propagation in animal nervous systems. In particular, potassium is one of the key ions involved in the modulation of the membrane potential in neurons, and glutamate is a central excitatory neurotransmitter. Besides the presence of the same ions in the two scenarios, there are also phenomenological links. In a normally functioning brain, electrical signalling typically propagates along neurons through action potentials mediated by sodium and potassium that can travel at speeds up to metres per second [9]. In addition, under pathological conditions, electrical activity can also propagate over the brain on a much slower timescale, on the order of millimetres per minutes (reviewed in [10]), similar to the biofilm potassium waves. This occurs during the phenomenon of cortical spreading depression, a wave of intense depolarization followed by inhibition of electrical activity that entails a strong metabolic disturbance of neuronal function. Cortical spreading depression has been shown to occur in the brains of multiple species, from insects [11,12] to mammals [13], where it has been regarded as an evolutionary conserved process [10] that has been linked to human pathologies like migraine, and to the propagation of brain damage during ischemic stroke. The phenomenon has been modelled as a reaction–diffusion process [14,15] according to the following mechanism: an initial stimulus leads to an increase in extracellular potassium, causing the membrane potential to rise beyond a critical threshold that triggers a self-amplifying release of potassium, a major ionic redistribution of sodium, calcium and chloride, and release of neurotransmitters like glutamate. Diffusion of potassium and glutamate to neighbouring cells leads to subsequent depolarization beyond a threshold, thus causing ionic release and self-propagation of the wave [10,16,17].

The widespread importance of potassium and glutamate, and the links between electrical signalling in bacteria and neuronal dynamics led us to investigate in more detail the roles of those two ions in the oscillations exhibited by B. subtilis. In this work, we unify in a discretized reaction–diffusion scheme the metabolic and electrical components of the system. Assuming a homogeneous population of cells, the model reveals the spontaneous emergence of two phenotypically distinct populations of cells as a consequence of the spatio-temporal

**Figure 1.** Biofilms of *B. subtilis* display oscillations in growth rate and electrical signalling activity. (a) Scheme of the microfluidics device used to grow biofilms. Biofilms grow around the central pillar or attached to the wall, as in the cartoon. Biofilm dynamics can be represented as a kymograph, where each row represents a one-dimensional cross-section of the biofilm area at a given movie frame. (b–d) Experimental kymographs of a cross-section of a biofilm, as depicted in (a), for different channels. Each pixel is the average of a region of 5.2 × 5.2 μm. The dotted black line denotes the limit of the biofilm as established from the phase-contrast images. (b) Oscillations in extracellular potassium, reported by APG-4. (c) Oscillations in membrane potential, reported by ThT. (d) Extracellular potassium and ThT time traces corresponding to the position denoted by the white line in (b,c). Data have been smoothed with an overlapping sliding window of size 3 frames. (Online version in colour.)
dynamics of the system, which causes oscillations to start beyond a critical size. We begin by developing a model that accommodates the interactions considered in previous works both for the metabolic and electrical components of the phenomenon. Then we show that the details of the ammonium metabolism are not required to explain the key aspects of the dynamics. The behaviour can be explained by a model with minimal interactions between glutamate metabolism and potassium signalling, thus clarifying the process in bacteria and providing further insight into the roles of these ubiquitous biological ions.

2. A unified spatially extended model of biofilm oscillations

Conceptually, biofilm oscillations can be understood to arise as result of a delayed, spatially extended negative feedback of metabolic stress [6]. A first molecular model was proposed in [5] (left half of figure 2), based on the fact that cells need to create glutamine from glutamate and ammonium for biomass production. Since ammonium is not present in the MSgg medium used in the experiments, it must be synthesized by the cells from glutamate. The model assumed that only cells in the interior of the biofilm produce ammonium (through the glutamate dehydrogenase enzyme GDH, $H$ in figure 2), part of which diffuses away to the periphery of the biofilm, where it is used by the peripheral cells to grow. In turn, peripheral growth reduces glutamate availability in the centre, and subsequently ammonium availability in the periphery. As a result, peripheral cells stop growing, thus allowing the centre to recover glutamate and produce ammonium again, leading to a new oscillation cycle. The model considered two different cell populations—interior and peripheral—and was subsequently implemented on a continuous spatial domain in [18], with still pre-defined interior and peripheral cell types.

This model can readily generate oscillations, which crucially rely on the assumption of a strongly nonlinear activation of GDH by glutamate in the interior population of cells (modelled with a Hill function with large Hill coefficient). However, it does not account for the electrical signalling component of the phenomenon, which we incorporate next.

(a) Full model description

We consider a horizontal cross-section through the middle of a biofilm (as depicted in figure 1a) which allows us to simplify the system into a one-dimensional lattice, as shown in figure 3. The left-most lattice sites are ‘biofilm’ (shaded squares, ‘b’), followed by ‘non-biofilm’ sites (white squares, ‘n’) on the right. We begin by a more detailed model (figure 3) that includes all the aforementioned metabolic interactions [5] (left half of figure 2) in addition to the electrical signalling propagation [7] (right half of figure 2). The details of the equations can be found in the electronic supplementary material, and we next describe the main points of the model (see also table 1).

‘Non-biofilm’ sites have extracellular variables only: extracellular glutamate ($G_e$), ammonium ($A_e$, assumed to have the same concentration inside and outside cells, following [5]) and extracellular potassium $K_e$. These variables diffuse between

Figure 2. Metabolic (left) and electric (right) processes involved in the response of the biofilm to a gradient of nutrient-limitation-drive stress from the interior to the periphery. The left-hand side schematizes the interactions considered in the metabolic model of [5], whereas the right-hand side schematizes the electrical signalling propagation model proposed in [7]. In the diagram on the left, $H$ represents the enzyme GDH. In the diagram on the right, $E_k$ stands for excess extracellular potassium, $V$ for the membrane potential and $n_k$ for the gating variable of the potassium channel. (Online version in colour.)
neighbouring lattice sites. In addition, we also account for the fact that media is flowing constantly through the microfluidics chamber such that their concentration tends to become close to that in the media (electronic supplementary material, equations (S1), (S2) and (S17)).

‘Biofilm’ sites correspond to small biofilm regions, such that variable values would correspond to averages across multiple cells. As schematized in figure 3, in these sites, there are also the extracellular variables (also subject to diffusion and media flow) in addition to the intracellular ones that take part in the various biochemical reactions. We model explicitly the dynamics of extracellular ($G_e$) and intracellular ($G_i$) glutamate in the biofilm (electronic supplementary material, equations (S5) and (S6)), where we consider glutamate uptake into the cells and the conversion of glutamate into ammonium by the action of the enzyme GDH. In turn, we model GDH synthesis in its inactive form ($h$) and subsequent activation ($H$) (electronic supplementary material, equations (S8) and (S9)). We assume that high concentrations of glutamate inhibit GDH synthesis. This follows from the original

Table 1. Main assumptions and comparison between the full and simplified models.

<table>
<thead>
<tr>
<th>model assumption</th>
<th>full model (§2)</th>
<th>simplified model (§3)</th>
</tr>
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<tbody>
<tr>
<td>extracellular glutamate flow and diffusion</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>ammonium flow and diffusion</td>
<td>yes</td>
<td>no (no ammonium present)</td>
</tr>
<tr>
<td>extracellular potassium flow and diffusion</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>decay of flow and diffusion rates towards the biofilm interior as a consequence of cellular density</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>glutamate uptake favoured by hyperpolarization and disfavoured by depolarization</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>glutamate uptake enhanced in metabolically active cells</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>saturable glutamate uptake</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>GDH dynamics regulated by glutamate</td>
<td>yes</td>
<td>no (no GDH present)</td>
</tr>
<tr>
<td>ammonium produced from intracellular glutamate by GDH</td>
<td>yes</td>
<td>no (no ammonium present)</td>
</tr>
<tr>
<td>growth favoured by high levels of intracellular glutamate</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>through the effect of biomass-producing biomolecules</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>through intermediate biomass-producing biomolecules</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>metabolic stress inhibited by intracellular glutamate</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>potassium channel opening probability ($n_k$) increased by stress</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>potassium released as a result of the opening probability ($n_k$) and membrane potential</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>potassium uptake governed by homeostatic processes and the metabolic state of the cells (intracellular glutamate)</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>membrane potential determined by intracellular and extracellular potassium concentrations</td>
<td>yes</td>
<td>yes</td>
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<tr>
<td>ThT as a reporter of membrane potential</td>
<td>yes</td>
<td>yes</td>
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observation that GDH overexpression in the periphery kills biofilm oscillations [5], suggesting that GDH expression is restricted to the centre, where glutamate levels are low. Moreover, we assume that there is a threshold for GDH activation by glutamate, as in the original metabolic model. Glutamate and ammonium are assumed to be used for the production of biomass-producing biomolecules such as ribosomal proteins, denoted by $r$ (electronic supplementary material, equation (S11)). We also account explicitly for the glutamate transporter concentration ($R_g$), which we assume to saturate for large enough $C_0$. In order to accommodate the assumption that cells with a higher metabolic activity consume more glutamate, we consider that $R_g$ is subject to an inducible activation that depends on the presence of biomass-producing biomolecules (electronic supplementary material, equation (S7)).

In [7], the YuoO potassium channel, which has a TrkA gating domain, was determined to be responsible for potassium release in this phenomenon. TrkA proteins are potassium channel regulatory proteins with nucleotide-binding domains that bind NAD/NADH [19,20] and ADP/ATP [21], thus coupling potassium translocation to the metabolic state of the cell. Glutamate is a central amino acid at the crossroad between metabolism and electrical signalling. Our assumption is that low glutamate levels lead to imbalances in the aforementioned nucleotides that are sensed by TrkA and trigger potassium release [7]. Therefore, we assume that high levels of intracellular glutamate inhibit the production of stress-related biomolecules, resulting in the following equation for stress dynamics:

$$\frac{dS}{dt} = \frac{S_0}{1 + (G_i/C_0)} - \gamma_i S. \quad (2.1)$$

In turn, $S$ enhances the opening probability of the potassium channel, given by $n_0$. This results in a link from the metabolic component of the phenomenon to the electrical counterpart.

As the potassium channel opens, potassium is released to the extracellular environment. Conversely, potassium uptake is assumed to be governed by homeostatic processes that tend to keep its intracellular concentration at a fixed value. In addition, it is assumed to depend on the cellular metabolic state (glutamate level), to account for the energy demand of the process (electronic supplementary material, equations (S13) and (S14)). Extracellular and intracellular potassium concentrations determine the membrane potential ($V$), whose dynamics is described by a Hodgkin–Huxley-like conductance-based model (electronic supplementary material, equation (S18)). Finally, we include the ThT reporter $T$ downstream of the membrane potential, increasing when the cells become hyperpolarized owing to potassium release (electronic supplementary material, equation (S20)).

In B. subtilis, glutamate is imported by pumps such as the symporter GltP [22], which uses the proton motive force as a source of energy, and this is in turn influenced by the membrane potential [23]. We therefore consider that glutamate transport into the cell is modulated by the membrane potential $V$, such that depolarization reduces entry, and hyperpolarization enhances it. This provides the link from the electrical to the metabolic component of the phenomenon, and is represented with the following switch-like import-modulation term:

$$F(V) = \frac{1}{1 + \exp(g_s(V - V_0))}. \quad (2.2)$$

The biofilm is assumed to expand proportionally to the biomass-producing biomolecules. In order to simulate growth, we consider that ‘non-biofilm’ lattice sites neighbouring biofilm sites become occupied with cells (and thus become a lattice site of type ‘biofilm’) with probability $P_{\text{grow}} dt r$, with $dt$ being the simulation time step. Importantly, each new biofilm lattice site inherits the intracellular variables of the ‘mother’ site. Owing to this particularity, the continuum approximation is unsuitable, and we simulate the system as a coupled map lattice.

(b) Simulations of the model

The diffusion coefficient in water for ions and small molecules such as potassium and glutamate is of the order of approximately $10^6 \mu m^2 h^{-1}$. We thus fixed the diffusion coefficient of potassium and ammonium in the media to this value, and assumed the diffusion coefficient of glutamate to be half that value, owing to its larger molecular weight. Regular glutamate concentration in the media is 30 mM and potassium concentration is 8 mM. Because there is no ammonium in the medium, $A_m = 0$. We fixed the resting membrane potential to $-150$ mV. Furthermore, oscillations experimentally start at a biofilm size of around 600 $\mu m$ with a period of around 2 h [6], and potassium and ThT signals should be correlated [7]. With these constraints, we manually adjusted the rest of the parameters (electronic supplementary material, table S1) in order to reproduce the experimentally observed oscillatory dynamics.

Figure 4 shows kymographs for extracellular potassium and ThT (variables that can be experimentally monitored as explained above, see figure 1), as well as for extracellular glutamate, stress, active GDH and ammonium, which have not been experimentally quantified. Oscillations with a period of about 2 h emerge in all variables once the biofilm becomes large enough. Moreover, in agreement with the experimental data, the top left plot shows that potassium peaks are likely to coincide with periods of no growth.

In contrast to the original model of [5] (see also [18]), where two populations of cells (central and peripheral) were pre-defined regarding GDH production, in the current model an a priori separation of cell types is no longer required, but emerges spontaneously. This can be seen in the bottom left kymograph of figure 4, which shows that a central population with higher levels of GDH activity and stress emerges over time. Notably, this model does not require strong cooperativity in GDH activation for the oscillations to emerge ($n_{H} = 2$, in contrast to the value of 7 in [5] and 12 in [18]). This is likely to be the result of the electrical component of the model. In order to test the relevance of this component of the oscillations, we next simplify the metabolic details and consider only the interplay between glutamate metabolism and electrical signalling.

3. Oscillations emerge from the interplay between glutamate and electrical signalling

We now simplify the metabolic aspects of the full model introduced above, eliminating GDH, ammonium and biomass-producing biomolecules (electronic supplementary material, equations (S8)–(S11)), and supposing instead that intracellular glutamate directly increases the glutamate
transporter and directs growth (figure 5a). With this, the equations governing the metabolic part of the phenomenon become:

$$\frac{dG_e}{dt} = -\alpha_5 \xi(V) R_g \frac{G_e}{K_g + G_e} + A_\delta \phi(G_m - G_e)$$

$$+ A_3 K_g \nabla^2 G_e,$$  

(3.1)

$$\frac{dG_i}{dt} = \alpha_3 \xi(V) R_g \frac{G_e}{K_g + G_e} - \delta_3 G_i,$$  

(3.2)

$$\frac{dR_g}{dt} = \alpha_g - \delta_g R_g + \alpha_7 \frac{G_m^u}{K_m^u + G_m^u},$$  

(3.3)

in addition to considering glutamate diffusion in the ‘non-biofilm’ sites (electronic supplementary material, equation (S1)).

In this case, the probability with which the biofilm grows depends directly on the concentration of intracellular glutamate at the biofilm boundary according to $P_{\text{grow}} dt G_i$. The dynamics of the electrical part of the model remain unchanged (electronic supplementary material, equations (S12)–(S20)).

The kymographs in figure 5b show that oscillations beyond a critical size also emerge in this case. The results suggest the following mechanism for the oscillations: once the biofilm reaches a critical size, glutamate in the centre of the biofilm is too low and leads to metabolic stress. As a result, cells release

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**Figure 4.** Oscillations in the full metabolic-electrophysiological model. Kymographs of the model simulation results, showing oscillations in various model variables. (Online version in colour.)

**Figure 5.** Oscillations in a simplified model, with glutamate and the electrical signalling components. (a) Scheme of the model. (b) Kymographs of various variables of the system. (c) Electrical signalling wave (reported by ThT) in an experimental biofilm triggered by an increase of potassium in the media from 8 mM to 300 mM during 3 min (denoted by the orange solid bar at $t = 0$ in the non-biofilm area). The wave propagates inwards, from the periphery (right) to the centre (left). (Online version in colour.)
potassium, that actively propagates towards the edge owing to depolarization and subsequent metabolic stress. Depolarization leads to a drop in glutamate consumption levels in the peripheral region, owing to the immediate effect on the transport efficiency, and a slightly delayed effect through the reduction in the glutamate transporter. As a result, glutamate can diffuse inwards to allow stress relief in the centre, and a new oscillation cycle can start.

According to this, oscillation onset requires stress in the centre to surpass a threshold that leads to potassium release. As we have recently described [6], oscillations can be triggered in experimental biofilms by transiently stopping the media flow of the microfluidics chamber. This is also the case in this model, where oscillations can be triggered by transiently setting the flow-rate parameter (d) to zero. If the biofilm is sufficiently large, such a perturbation leads to a sudden reduction in the glutamate availability and stress increase, with the subsequent potassium release and oscillatory onset (electronic supplementary material, figure S1).

Moreover, the model predicts that depolarizing any region of the biofilm should be sufficient to trigger a self-propagating wave of stress and potassium release, with the associated changes in membrane potential. This is reproduced experimentally as shown in figure 5c: a short increase in the concentration of potassium in the media triggers a wave in experimental biofilms, which propagates inwards from the original (peripheral) depolarization site.

4. Glutamate metabolism and stress release determine oscillation onset size and period

According to the aforementioned mechanism for the oscillations, if a biofilm increases its glutamate consumption, or if the glutamate concentration in the media is reduced, oscillations should start earlier because the centre becomes stressed at a smaller biofilm size. This expectation is fulfilled when simulations are performed with halved glutamate concentration in the media (G_m), in good agreement with the experimental data (compare the first two conditions in figure 6a, see also [5,6]). Similarly, bacteria that cannot synthesize glutamate owing to a deletion in the gltA gene are expected to consume more glutamate from the media. We model this condition as an increase in both glutamate uptake rate (α_g) and degradation rate (δ_g), as a result of higher glutamate demand. Also in this case oscillations start earlier both in the model and the experiments (third condition in figure 6a).

In these two cases of reduced onset size, the onset period is also smaller than in the reference situation (first three conditions in figure 6b). As we have described in previous work [6], larger biofilms tend to have higher periods, the explanation being that the larger a biofilm is, the more time it takes for the stress signal to propagate from centre to periphery, and thus the longer the oscillation period.

However, according to the oscillation mechanism that this model suggests, the period of the oscillations must also depend on the metabolism dynamics and the stress relieving capabilities of the cells owing to the potassium effects. The ΔtrkA mutant strain was characterized in [7] to be deficient in electrochemical signalling, owing to the deletion of the gating domain of the YugO channel. The mutation can be interpreted to render a channel with reduced conductance and increased basal leak [7]. This can be modelled with increased a_0 (to simulate a leaky channel), and reduced gK (to simulate reduced conductance once the channel is open). The model simulations predict that oscillations start at the same size as with the basal parameters, but with a reduced period, in good agreement with the experimental data (last condition in figure 6). The unaffected onset size is consistent with the fact that glutamate metabolism is largely unperturbed in this mutant, whereas the altered potassium channel leads to less effective stress relief during wave propagation (electronic supplementary material, figure S2), and thus facilitates a subsequent cycle. Therefore, the relationship between biofilm size and oscillation period is not universal, but depends upon the metabolic and electrical signalling capabilities of the cells and thus on the genetic background of the strain.

5. Discussion

The oscillations in growth rate and membrane potential exhibited by B. subtilis biofilms is an instance of self-organizing, emergent behaviour similar to the complex collective dynamics that characterizes neuronal tissue. Here, we have proposed a unified conceptual framework for these bacterial oscillations that combines glutamate metabolism with potassium wave propagation. Our discrete reaction—diffusion scheme assumes an initially homogeneous population of cells, and exhibits a spontaneous emergence of oscillations beyond a critical biofilm size. Our work shows that oscillations are triggered by a decrease in glutamate levels in the biofilm centre, that lead to metabolic stress and potassium release. Potassium diffusion to the neighbours interferes with glutamate metabolism, thus...
causing a self-propagating wave of membrane potential changes that allows glutamate recovery in the centre and thus explains the oscillation cycle.

With some notable recent exceptions in multicellular eukaryotes [24], electrophysiology has only been considered relevant so far for excitatory cells such as those in cardiac and neural tissue. The resemblance, both at the molecular and functional level, between excitatory wave propagation in these tissues and the communication of metabolic stress among bacterial cells calls for an evolutionary perspective on the phenomenon of electrical signalling. Animal nervous systems have evolved intricate electrochemical circuits that allow sensing and responding to both internal and external stimuli, with high integrative and computational capabilities clearly evidenced by the human brain. How this complexity arose is still a matter of active investigation [25–28] but it is tempting to speculate that the bacterial oscillations reported here may represent an ancient instance of electrically mediated information transmission.

Primitive prokaryotic potassium channels are regarded as the ancestors of the animal cation channels whose diversification has been linked to the evolution of nervous systems [29,30]. Given that high intracellular concentrations of potassium are essential for bacterial pH maintenance [8,31], it is natural to expect that potassium channels were used in early bacteria for osmoregulation [27]. The biofilm oscillations studied here suggest that potassium also acts as a signalling intermediate in modern bacteria, communicating information among cells of both the same and different bacterial species [32].

The link between metabolism and electrical signalling described here is also reminiscent of the relationship between cellular energy and neuronal function [33]. Neuronal dynamics is highly dependent upon cellular metabolism and energy state: the high energy requirements of the ion pumps that maintain proper electrochemical gradients are well known [34]. In addition, some animal voltage-gated ion channels directly respond to energy-related metabolites like NAD(H) and ADP/ATP through direct ligand binding [35], as in the bacterial YugO channel.

While the mechanisms that allow information processing in bacterial biofilms share similar chemical and electrical processes with animal brains, the latter compute at much faster speeds and thus in much more complex ways. This can be partly ascribed to the reliance of animal brains on faster voltage-gated ion channels, such as those dependent on sodium, which evolved only when rapid responses were increasingly beneficial (due for instance to the appearance of predators) [27]. Nevertheless, slow propagation of electrical activity is still present in modern animal brains. During the phenomenon of cortical spreading depression, for instance, a slow wave of electrical activity propagates over brain tissue, with massive ionic redistribution involving, critically, potassium and glutamate accompanied by a strong metabolic disturbance. This phenomenon has been regarded as intrinsic to neurons and is evolutionarily conserved across animal species [10]. In the light of these facts, it is tempting to see the potassium-mediated transmission of stress among bacterial cells as a precursor of more recent electrically-mediated information propagation tasks in animal brains. The study of membrane potential dynamics and electrical signalling processes in other bacterial species will be important to further illuminate this issue.

6. Methods

(a) Experimental data

(i) Strains and plasmids

All experiments were performed using *B. subtilis* strain NCIB 3610. For the ΔtrkA mutant, we deleted the C-terminal portion of *yugO* (amino acids 117328), leaving only the N-terminal ion channel portion of YugO (amino acids 1116). For the ΔgltA mutant, the *gltA* gene was replaced by kanamycin resistant gene.

(ii) Growth conditions

Biofilms were grown using the standard MSgg biofilm-forming medium [36]. In this media, glutamate is the only nitrogen source for the bacteria. We explored biofilm dynamics at glutamate concentrations of 1 × 30 (mM) and 0.5 × 15 (mM). For microfluidics, we used the CellASIC ONIX Microfluidic Platform and the Y04D microfluidic plate (EMD Millipore). Details can be found in our previous work [5,37].

(iii) Time-lapse microscopy

Biofilms were monitored using time-lapse microscopy, using Olympus IX81 and IX83 inverted microscopes. To image entire biofilms, 10× lens objectives were used. Images were taken every 10 min. We tracked membrane potential dynamics using the fluorescent dyes Thioflavin T (10 μm) and APC-4 (2 μm), from TEF Labs [7].

(b) Modelling

(i) Simulation methods

Simulations were performed using custom-code in C, and analysis and plotting was done in Python. We used a first-order approximation of the discrete Laplacian operator [38] and the time evolution of the variables was obtained using a fourth-order Runge–Kutta method. Boundary conditions were reflective: the outside neighbours of the lattice sites at the boundary of the system are the boundary sites themselves (such that the discrete normal derivatives of the variables at the boundary are zero). The simulation time step was set to 5 × 10^−6 h, and each lattice site corresponds to 10 μm. The system was allowed to relax towards the steady state at the beginning of the simulation by preventing expansion during 1 h of simulated time. The initial conditions for the intracellular variables are *S* = 0 μm, *n* = 0, *V* = −156 mM, *K* = 300 mM, ThT = 0 μm, *R* = 1 μm, *H* = 0.5, *H* = 0.25, *R* = 1 μm, and *G* = log-normally distributed around 3 mM. In the biofilm cells, *K* = log-normally distributed around 8 mM, and *A* = 1 mM. In the non-biofilm cells, *A* = 0. Initial *G* = 30 mM (or 15 mM in reduced glutamate concentrations).

(ii) Numerical perturbations

In order to study the effect of halved glutamate in the media, and the *trkA* and *gltA* deletions, we assumed the following parameter values with respect to the wild-type: ΔgltA mutant: \( \frac{a_0}{a_0} = 1.5 \times \), \( a_0 = 1.3 \times \); ΔtrkA mutant: \( \frac{a_0}{a_0} = 2 \times \), \( g_x = 0.5 \times \). We performed 30 simulations per condition starting from a random initial radius between 150 and 250 μm. Potassium peaks were defined based on a threshold of 9.5 mM (pulse begins when crossing the threshold). Onset period is defined to be the time between the beginnings of the first two peaks, and onset size that of the first beginning.

Data accessibility. This article has no additional data.

Competing interests. We declare we have no competing interests.

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