SUPPLEMENTARY MATERIAL

Metabolic basis of brain-like electrical signalling in bacterial communities

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1 Model equations and description

As explained in the main text, we consider a one-dimensional array of simulation lattice sites where biochemical species react and diffuse. Here we describe in detail the model equations.

1.1 Metabolic component

In the 'non-biofilm' sites, we consider media flow and diffusion of glutamate (G) and ammonium (A) according to the following equations:

$$\frac{dG_e}{dt} = \phi\,(G_m - G_e) + D_g \nabla^2 G_e \tag{S1}$$

$$\frac{dA}{dt} = \phi\,(A_m - A) + D_a \nabla^2 A \tag{S2}$$

In the microfluidics chamber, media is flowing constantly. Thus, we simulate the effect of the media flow with the first term of each equation, such that with some rate ϕ, the concentration

of the chemical species tends to equate that in the medium (X_m). The second term models diffusion.

In the biofilm, we assume that the diffusion coefficient and flow rate of glutamate decay exponentially with the distance to the biofilm edge d_e, due to the extracellular matrix and high cell density, according to the following functions:

$$\Lambda_\phi = \frac{\exp(-\gamma_\phi\, d_e) + a_\phi}{1 + a_\phi} \tag{S3}$$

$$\Lambda_D = \frac{\exp(-\gamma_D\, d_e) + a_D}{1 + a_D} \tag{S4}$$

These functional forms are chosen such that at the edge $\Lambda_x = 1$, and flow and diffusion match those in the media. The coefficients decay towards the biofilm interior, tending asymptotically to $a/(1 + a)$ in the centre, which ensures some remaining flow and diffusion. We follow the original assumption [1] that ammonium diffusion over the biofilm is very fast, and do not apply these reduction terms to this variable.

The dynamics of extracellular (G_e) and intracellular (G_i) glutamate in the biofilm are modelled with the following dynamical equations:

$$\frac{dG_e}{dt} = -\alpha_g\, \mathcal{F}(V)\, R_g\, \frac{G_e}{k_g + G_e} + \Lambda_\phi\, \phi\, (G_m - G_e) + \Lambda_D\, D_g \nabla^2\, G_e \tag{S5}$$

$$\frac{dG_i}{dt} = \alpha_g\, \mathcal{F}(V)\, R_g\, \frac{G_e}{k_g + G_e} - \alpha_a\, H\, G_i - \delta_g\, G_i\, r\,, \tag{S6}$$

where, as mentioned, G_e is affected by media flow and diffusion. The first term in the right-hand side of the two equations represents glutamate transport into the cells. As explained in the main text, we consider that glutamate transport into the cell is modulated by the membrane potential V, such that depolarisation reduces entry, and hyperpolarisation enhances it, according to the functional form given in Eq. (2) of the main text.

In addition, we assume that glutamate is imported into the cells through the glutamate transporter R_g, which saturates for large enough G_e, with half-maximum concentration k_g. We describe explicitly the dynamics of R_g by:

$$\frac{dR_g}{dt} = \alpha_R - \delta_R\, R_g + \beta_R \frac{r^{n_R}}{k_R^{n_R} + r^{n_R}}\,. \tag{S7}$$

We thus assume that R_g is produced at a basal rate α_R and degraded at a rate δ_R. The last term accounts for the higher glutamate uptake by metabolically active cells, such that the presence of biomass-producing biomolecules, such as ribosomal proteins, denoted by r, enhances R_g synthesis via a Hill function with exponent n_R.

Equation (S6) also assumes that intracellular glutamate concentration decays due to ammonium production via the GDH enzyme (represented by H in the α_a-term at the right-hand side of

the equation) and through various metabolic tasks including in particular biomass production (δ_g-term).

The production of ammonium is regulated by the activity of the enzyme GDH. We describe the dynamics of the inactive and active forms of this enzyme, h and H respectively, by the equations:

$$\frac{dh}{dt} = \frac{\alpha_h}{1+(G_i/k_h)^{n_h}} - \alpha_H\, h\, \frac{G_i^{n_H}}{k_H^{n_H}+G_i^{n_H}} - \gamma_h\, h + \gamma_H\, H \tag{S8}$$

$$\frac{dH}{dt} = \alpha_H\, h\, \frac{G_i^{n_H}}{k_H^{n_H}+G_i^{n_H}} - \gamma_H\, H \tag{S9}$$

such that we account for synthesis and degradation of inactive GDH and its conversion into the active form (α_H-term in the two equations). As explained in the main text, we assume that high concentrations of glutamate inhibit GDH synthesis, whereas activation is positively regulated by glutamate via a Hill function with exponent n_H. We also consider deactivation at a constant rate γ_H.

Ammonium dynamics is affected by production from glutamate by active GDH, consumption for various metabolic processes such as biomass production, and diffusion:

$$\frac{dA}{dt} = \alpha_a\, H\, G_i - \delta_a\, A\, r + D_a \nabla^2 A \tag{S10}$$

Finally, biomass production is considered to increase with ammonium and intracellular glutamate, and to be subject to linear decay:

$$\frac{dr}{dt} = \beta_r\, A\, G_i - \gamma_r\, r \tag{S11}$$

1.2 Electrical signalling

Next we incorporate an adapted version of the electrical model introduced in [2]. As explained in the main text, we consider an inhibitory effect of intracellular glutamate on a stress variable S, whose production rate is modelled with an inhibitory Hill function:

$$\frac{dS}{dt} = \frac{S_0}{1+\left(\frac{G_i}{G_{s0}}\right)^{n_s}} - \gamma_s\, S \tag{S12}$$

We explicitly consider both extracellular (K_e) and intracellular (K_i) potassium, whose dynamics are given by:

$$\frac{dK_e}{dt} = F\, g_K\, n_k^4\, (V - V_K) - D_p\, G_i\, K_e(K_{i0} - K_i) + \Lambda_\phi\, \phi\, (K_m - K_e) + \Lambda_D D_k \nabla^2 K_e \tag{S13}$$

$$\frac{dK_i}{dt} = -F\, g_K\, n_k^4\, (V - V_K) + D_p\, G_i\, K_e(K_{i0} - K_i) \tag{S14}$$

Potassium uptake is assumed to be governed by homeostatic processes that tend to keep its intracellular concentration at a fixed value K_{i0}, described by the second term in the right-hand side of Eqs. (S13)-(S14). Uptake is also made to depend on the metabolic state (glutamate level), to account for the energy demand of the process. In addition, extracellular potassium diffuses and is subject to the media flow in the chamber.

Potassium flow through its ion channel (first term in the right-hand side of the K_e and K_i equations) is governed by the corresponding Nernst potential:

$$V_K = V_{K0}\,\ln\left(\frac{K_e}{K_i}\right), \tag{S15}$$

and depends on the opening probability of the potassium channel, n_k:

$$\frac{dn_k}{dt} = \frac{a_0\,S}{S_{th}+S}\,(1-n_k) - b\,n_k \tag{S16}$$

In the media lattice sites, as in the case of glutamate and ammonium [Eqs. (S1) and (S2)], the extracellular potassium dynamics is affected by diffusion and by the media flow:

$$\frac{dK_e}{dt} = \phi\,(K_m - K_e) + D_k\nabla^2\,K_e \tag{S17}$$

The membrane potential dynamics is described by a Hodgkin-Huxley-like conductance-based model containing potassium flux through the ion channel and a leak current [2]:

$$\frac{dV}{dt} = -g_K\,n_k^4\,(V-V_K) - g_L\,(V-V_L)\,, \tag{S18}$$

where the leak potential V_L is assumed to depend on the extracellular potassium [2] in a threshold-linear manner, such that when K_e is larger than its basal level in the medium, K_m, the leak potential V_L grows linearly (and the cell depolarizes), while when $K_e < K_m$ the leak potential stays at its basal level:

$$V_L = V_{L0} + d_L\frac{K_e - K_m}{1-e^{-(K_e-K_m)/\sigma}} \tag{S19}$$

Finally, we include the ThT reporter $\mathcal{T}$ downstream of the membrane potential, increasing when the cells become hyperpolarised due to potassium release:

$$\frac{d\mathcal{T}}{dt} = \frac{\alpha_{\mathcal{T}}}{1+\exp(g_{\mathcal{T}}(V-V_{0\mathcal{T}}))} - \gamma_{\mathcal{T}}\,\mathcal{T} \tag{S20}$$

1.3 Simplified model

In the simplified model, we keep the same dynamics for the electrical part (Eqs. (S12)-(S20)). The metabolic part is simplified as follows: Eqs. (S8)-(S11) are removed, the equations for extracellular glutamate dynamics, in both 'biofilm' and 'non-biofilm' sites (Eqs. S1, S5), are maintained, and Eqs. (S6) and (S7) become:

$$\frac{dG_i}{dt} = \alpha_g\,\mathcal{F}(V)\,R_g\,\frac{G_e}{k_g+G_e} - \delta_g\,G_i \tag{S21}$$

$$\frac{dR_g}{dt} = \alpha_R - \delta_R\,R_g + \alpha_r\frac{G_i^{n_r}}{k_r^{n_r}+G_i^{n_r}} \tag{S22}$$

2 Supplementary tables and figures

Parameter	Description	Full model	Simplified model	Units
α_g	glutamate uptake constant	36.0	24.0	mM /(μM h)
k_g	extracellular glutamate concentration at half-maximal uptake rate	0.75	0.75	mM
G_m	glutamate concentration in the media	30.0	30.0	mM
D_g	glutamate diffusion coefficient	4e+06	4e+06	μm^2/h
α_a	ammonium production constant	4.5	-	μM^{-1}h^{-1}
δ_g	glutamate degradation constant	0.525	4.8	mM^{-1}h^{-1}
α_R	glutamate receptor synthesis rate	6.75	4.5	μM/h
δ_R	glutamate receptor decay constant	36.0	24.0	h^{-1}
β_R	maximum rate of glutamate receptor induction	45.0	31.0	μM/h
k_R	threshold for R_g induction	5.0	2.25	mM
n_R	Hill coefficient for R_g induction	2.0	2.0	-
α_h	maximal GDH synthesis rate	0.075	-	μM/h
k_h	threshold for inhibition of GDH synthesis by glutamate	1.5	-	mM
γ_h	GDH decay rate	0.01	-	h^{-1}
n_h	Hill coefficient for GDH synthesis inhibition by glutamate	2.0	-	-
α_H	GDH activation constant	3.0	-	h^{-1}
k_H	intracellular glutamate concentration for half-maximal GDH activation	0.4	-	mM
n_H	Hill coefficient for GDH activation by glutamate	2.0	-	-
γ_H	GDH deactivation constant	5.0	-	h^{-1}
δ_a	ammonium consumption constant	0.135	-	mM^{-1}h^{-1}
A_m	ammonium concentration in the media	0.0	-	mM
D_a	ammonium diffusion coefficient	7e+06	-	μm^2/h
β_r	biomass-producing biomolecules synthesis rate	15.0	-	mM^{-1}h^{-1}
γ_r	biomass-producing biomolecules decay rate	6.0	-	h^{-1}
S_0	maximum stress production rate	1.12	1.12	μM/h
G_{S0}	threshold for stress inhibition by glutamate	0.2	0.2	mM
n_s	Hill coefficient for stress inhibition by glutamate	2.0	2.0	-
γ_s	stress decay constant	2.8	2.8	h^{-1}
a_0	maximum rate of increase of the potassium channel gating probability	91.0	91.0	h^{-1}
S_{th}	stress level for half-maximal gating activity	0.03	0.03	μM
b	opening probability decay constant	21.25	34.0	h^{-1}

Table S1 – *Continued on next page*

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Parameter	Description	Full model	Simplified model	Units
F	membrane capacitance	0.05	0.05	mM/mV
g_K	potassium channel conductance	70.0	70.0	h^{-1}
V_{K0}	Nernst potential prefactor	100.0	100.0	mV
D_p	potassium uptake constant	0.12	0.12	$mM^{-2}h^{-1}$
K_{i0}	intracellular potassium concentration	300.0	300.0	mM
D_k	potassium diffusion coefficient	7e+06	7e+06	$\mu m^2/h$
K_m	potassium concentration in the media	8.0	8.0	mM
g_L	leak conductance	18.0	18.0	h^{-1}
d_L	leak slope coefficient	4.0	4.0	mV/mM
V_{L0}	basal leak potential	-156.0	-156.0	mV
σ	leak threshold sharpness coefficient	0.1	0.1	mM
g_v	inverse sensitivity of glutamate uptake to membrane potential	1.0	1.0	mV^{-1}
V_0	resting membrane potential	-150.0	-150.0	mV
$\alpha_{\mathcal{T}}$	maximal rate of ThT uptake	20.0	20.0	μM/h
$\gamma_{\mathcal{T}}$	intracellular ThT decay constant	10.0	10.0	h^{-1}
$g_{\mathcal{T}}$	inverse sensitivity of ThT to membrane potential	0.3	0.3	mV^{-1}
P_{grow}	growth probability	0.3	0.5	h^{-1}
γ_ϕ	spatial decay rate of the flow rate within the biofilm	0.0085	0.0085	μm
a_ϕ	basal flow factor	0.012	0.012	-
γ_D	spatial decay rate of the diffusion coefficient within the biofilm	0.0085	0.0085	μm
a_D	basal diffusion factor	0.012	0.012	-
ϕ	flow rate	5.0	5.0	h^{-1}

Table S1: Parameter description and basal values for the two models.

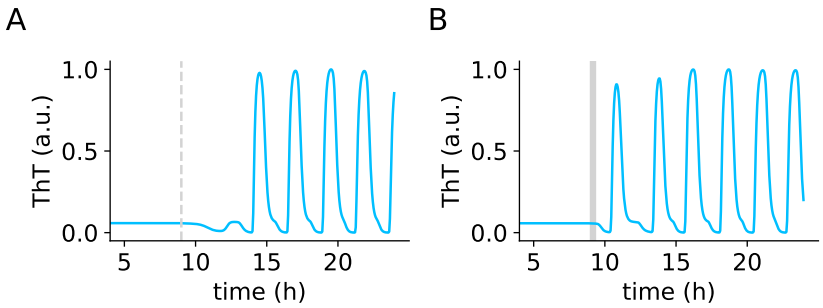

Figure S1: Stop-flow triggers oscillations in the simplified model. Normalised ThT time traces at the periphery (50 μm from the biofilm edge). A) Reference simulation. Vertical dashed line indicates the time of stop-flow in B. (This simulation is the same as in Fig. 5B). B) The biofilm was perturbed with a stop-flow-like perturbation ($\phi = 0$ during 20 min at $t = 9$ hours). The growth noise realisation is the same in both simulations, such that the only difference is the perturbation.

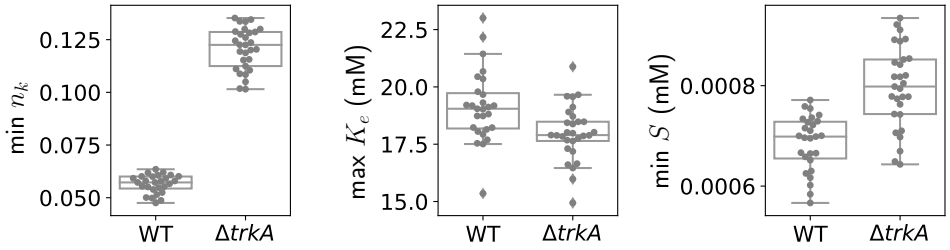

Figure S2: The $\Delta trkA$ mutation in the model leads to impaired stress relief. For each simulation, either the maximum or the minimum of the variable during the oscillations was computed for the peripheral region (outermost 100 μm). Each dot represents a simulation, from the same data as in Fig. 6 from the main text.

References

[1] J. Liu, A. Prindle, J. Humphries, M. Gabalda-Sagarra, M. Asally, D.-y. D. Lee, S. Ly, J. Garcia-Ojalvo, and G. M. Süel, "Metabolic co-dependence gives rise to collective oscillations within biofilms," *Nature*, vol. 523, no. 7562, pp. 550–554, 2015.

[2] A. Prindle, J. Liu, M. Asally, S. Ly, J. Garcia-Ojalvo, and G. M. Süel, "Ion channels enable electrical communication in bacterial communities," *Nature*, vol. 527, no. 7576, pp. 59–63, 2015.