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Biofilms as Bioelectric Networks: Understanding Their Response to Neural Signals

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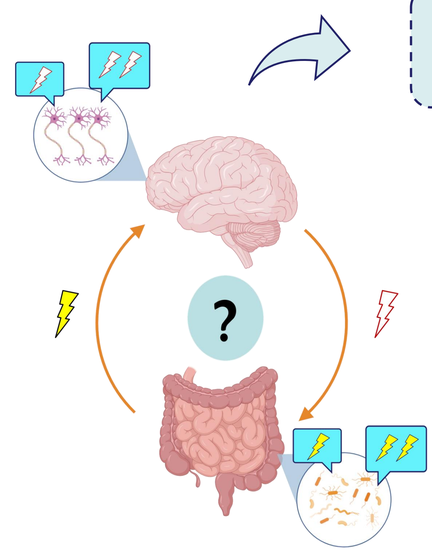
Introduction

The gut microbiota plays a pivotal role in the pathophysiology of metabolic and neuropsychiatric disorders through the microbiota–gut–brain (MGB) axis. Within this ecosystem, bacterial biofilms represent intelligent multicellular communities capable of coordinating collective behaviors, resisting stress, and processing information. Recent studies suggest that biofilms use bioelectrical signals—analogous to neuronal activity—to synchronize their functions (1). *Escherichia coli*, a common member of the gut microbiota, is emerging as a potential modulator of host physiology through such bioelectric mechanisms (2).

Could neurons and bacteria communicate using bioelectrical signals?

In this work, we characterized the bioelectrical profile of *E. coli* biofilms under controlled conditions and evaluated their response to the neurotransmitter GABA. Our findings reveal a dynamic bioelectric landscape within biofilms and show that neuroactive molecules can reprogram their membrane potential. Together with our integrated biophysical and computational approach, these observations provide a framework to investigate bacterial bioelectricity as a communication mechanism and support future studies on neuron–bacteria interactions within the MGB axis.

Hypothesis & Objectives

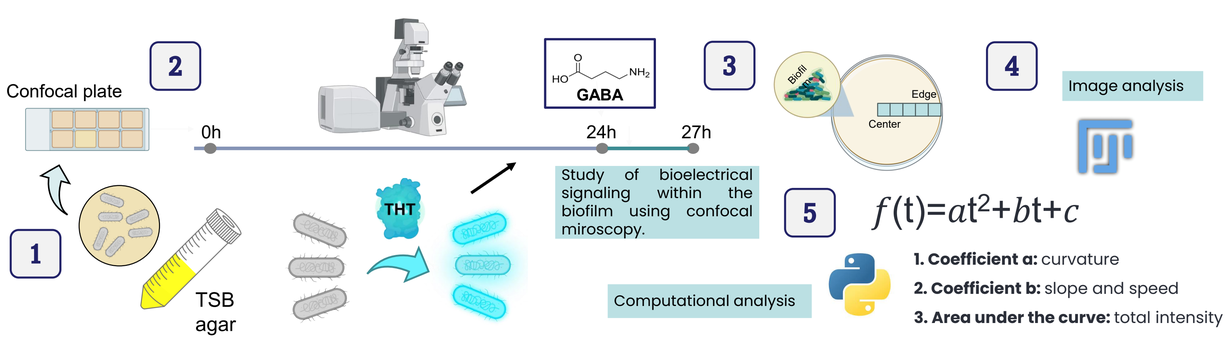


Is bioelectricity a shared communication language between bacteria and neurons in the MGB axis?

Objectives

1. **Assess the ability of E. coli to form viable biofilms.**
2. **Validate Thioflavin-T (THT) as a reliable probe for monitoring membrane potential.**
3. **Characterize spatio-temporal bioelectrical dynamics during biofilm maturation.**
4. **Determine the effect of GABA on the bioelectrical profile of E. coli biofilms.**

Material & Methods

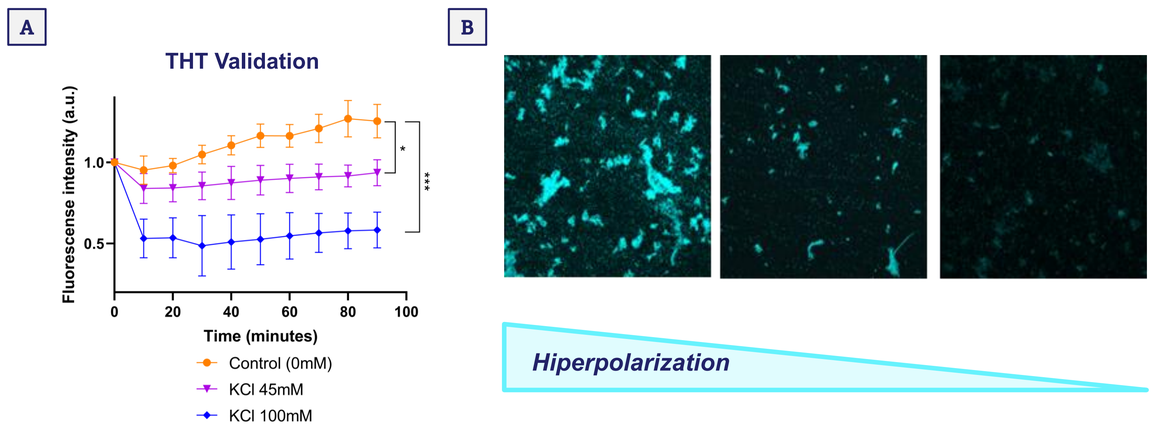


Experimental design to study bioelectrical patterns in *E. coli* biofilm in basal conditions and after adding the neurotransmitter GABA

1. ***E. coli* was cultured in a minimal medium (nutrient broth) for subsequent biofilm formation on a confocal plate for 24h.**
2. **After 24 h, the voltage-sensitive fluorescent dye (validated THT) and the neurotransmitter (GABA) were added.**
3. **Mosaic images were acquired from the center of the biofilm to the periphery in each well (under both control and neurotransmitter conditions).**
4. **Image analysis was performed using ImageJ software.**
5. **We conducted the statistical analysis of the data in Python using a quadratic function and generated a 3D visualization of the results to compare bioelectrical patterns.**

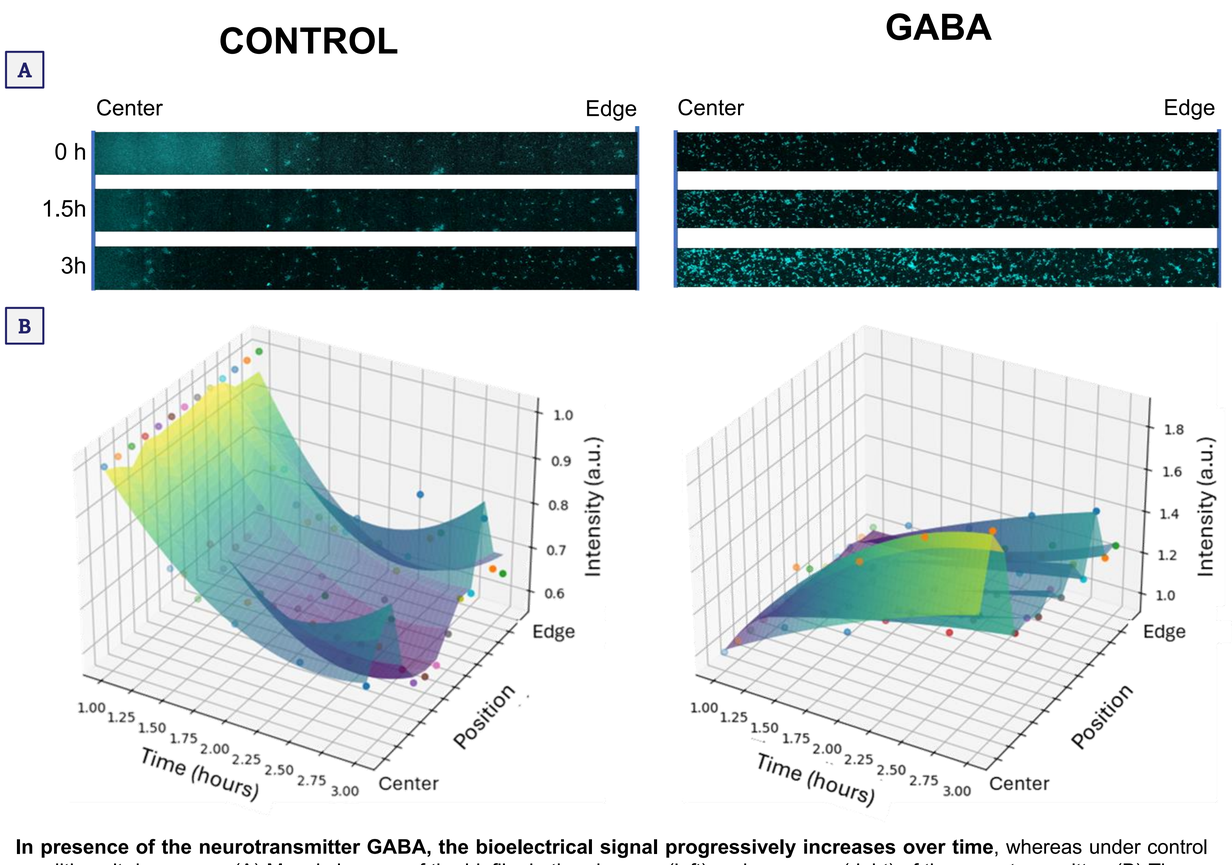
Results

1. Thioflavin T (THT) is a reliable dye for monitoring membrane potential in *E. coli* biofilms over extended periods without compromising viability, enabling long-term tracking of bioelectrical changes.



THT as a reliable Vmem reporter. (A) Graphical representation of fluorescence intensity in response to different KCL concentrations in *E. coli* biofilms. Results were analyzed by Kruskal-Wallis test in Graphpad PRISM9 software. (B) Images of hyperpolarization response detected by THT as Vmem sensible dye.

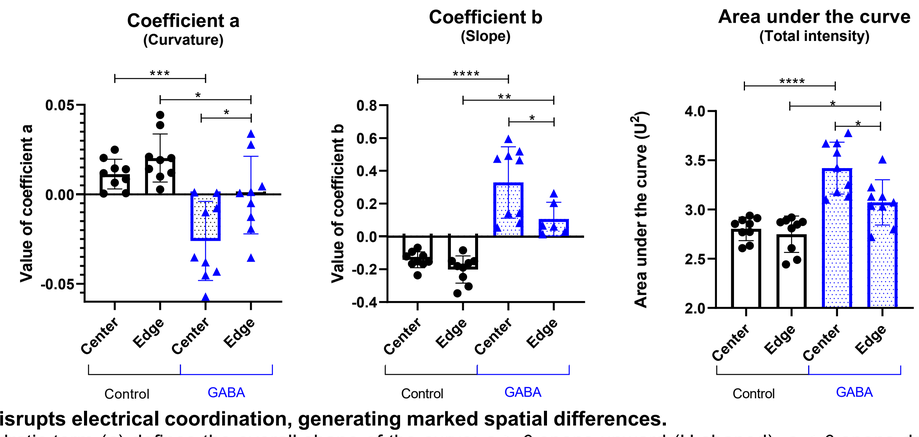
2. In basal conditions the *E. coli* biofilm display a coordinated bioelectricity, which is fully disrupted by the presence of neurotransmitter GABA.



In presence of the neurotransmitter GABA, the bioelectrical signal progressively increases over time, whereas under control conditions it decreases. (A) Mosaic images of the biofilm in the absence (left) and presence (right) of the neurotransmitter. (B) Three-dimensional plot of fluorescence intensity (arbitrary units, Y-axis) as a function of time (hours, X-axis) and mosaic position (from center to periphery, Z-axis).

3. GABA induces a hyperpolarization state and generates two bioelectrical patterns center vs periphery

$$f(t)=at^2+bt+c$$



GABA disrupts electrical coordination, generating marked spatial differences.
The quadratic term (*a*) defines the overall shape of the curve: $a > 0$ opens upward (U-shaped), $a < 0$ opens downward (∩-shaped), and larger $|a|$ values result in steeper curvature. The linear term (*b*) controls the mean slope, indicating rate and direction: $b > 0$ reflects an ascending trend, whereas $b < 0$ reflects a descending trend. The constant (*c*) represents the estimated initial value, which in this case is consistently close to 1.

The results of this study reinforce the role of bacterial bioelectricity as a dynamic and measurable feature of microbial communities (3).

Our findings suggest that bacterial biofilms may actively respond to neurochemical cues, according with their planktonic counterparts (4), supporting the hypothesis of a bioelectric dialogue within the microbiota–gut–brain axis. Further research may help to elucidate the underlying mechanisms and their implications in host–microbiota communication.

Conclusions

1. ***E. coli* biofilms show a coordinated decrease in hyperpolarization during maturation, suggesting synchronized bioelectrical dynamics. Under basal conditions, the biofilm gradually depolarizes over time.**
2. **GABA reverses this trend, inducing sustained hyperpolarization and spatial heterogeneity.**
3. **Bacterial bioelectricity emerges as a dynamic, plastic process responsive to neuroactive molecules.**
4. **Our integrated platform supports future studies exploring bioelectricity as a candidate mechanism for neuron–bacteria communication in the MGB axis.**

Acknowledgments

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