E. coli and the Microbiota-Gut-Brain Axis: Bioelectrical Connection

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the microbiota-gut-brain (MGB) axis is involved in both physical (Duvallet C, 2017) and mental pathologies (Cheung SG 2019).

Escherichia coli is a bacteria of the gut microbiota and a promising probiotic with the potential to regulate various metabolic and multifactorial diseases.

Exploring the communication pathways between *E. coli* populations and neurons could unveil novel nutritional approaches to target the MGB axis. We analyze the bioelectrical profile of *E. coli* throughout its growth. Furthermore, we evaluated bioelectrical changes induced by nervous signals and their impact on bacterial growth, viability and culturability.

Material and Methods

Bacteria: *Escherichia coli* NCTC9001

Nervous signals: γ-aminobutyric acid, GABA (inhibitory neurotransmitter) and Glutamate. Glu (excitatory neurotransmitter)

Bacteria bioelectricity: Bis-1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC4 (3) was used as membran potential (Vmem) reporter. *E. coli* cultures were treated with EDTA to permeabilize the bacterial membrane. After incubation for 15 min with Valinomycin (5 µM) and DiBac (50 µM), they were observed under an inverted epifluorescence microscope (LEica DMi8). The images obtained were treated with Image J

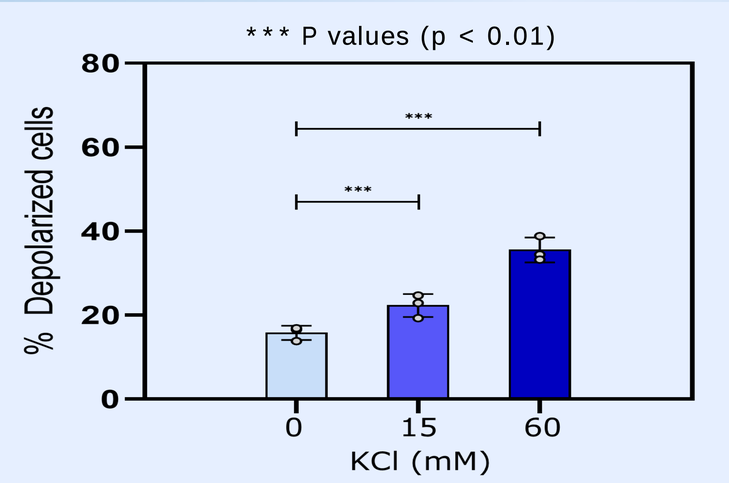
Bacteria growth: *E. coli* growth in TSB (Tryptic Soy Broth) was analyzed by meassuring absorbance at 600 nm

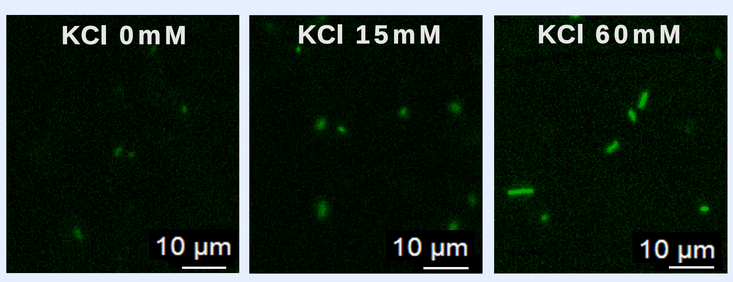
Bacteria cultivability: It was determined by viable counting in TSA (Tryptic Soy Agar)

Bacteria viability: LIVE/DEAD™ BacLight™ Bacterial Viability Kit, Invitrogen) was used as fluorescent viability probe

Statistical study: The statistical treatment was done with Generalized Estimating Equations (GEE).

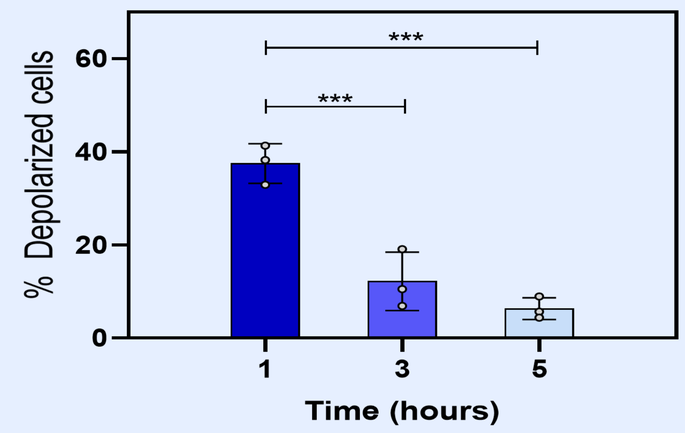
DiBAC as a reliable Vmem reporter





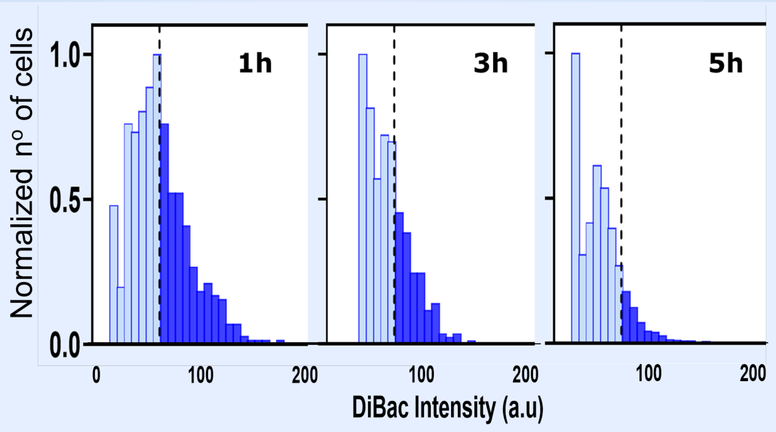
A significant increase in the percentage of depolarized cells was observed as the KCl increases in the extracellular medium

Bioelectrical profile changes along *E. coli* growth



*** P values (p < 0.001)

% of depolarized cells decreased significantly with growth time

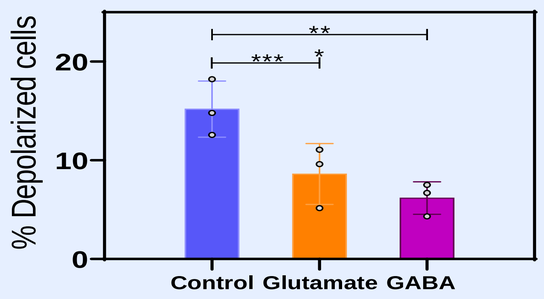


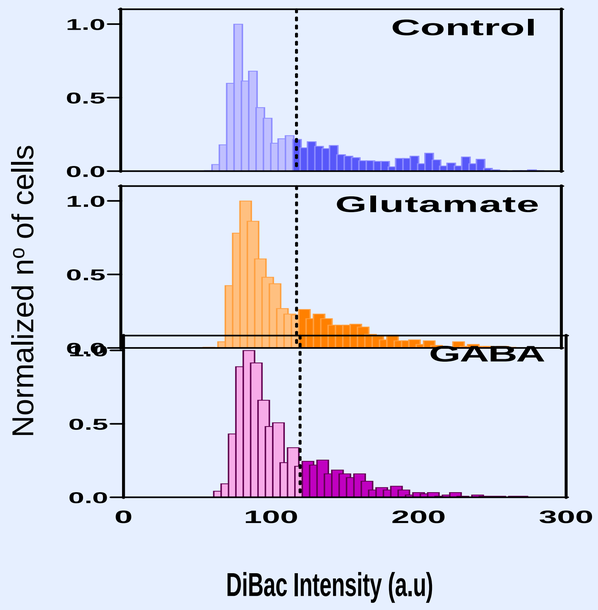
Nº of cells normalized to the nº of those exhibiting the most frequent intensity value

Depolarization threshold (average DiBAC fluorescence intensity at 1 h) was 65,32 a.u

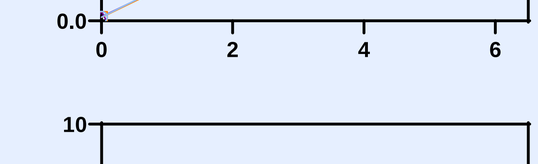
Neurotransmitters alter Vmem but not its growth, culturability or viability

After 4,5 hours, both neurotransmitters decrease % of depolarizated *E. coli*

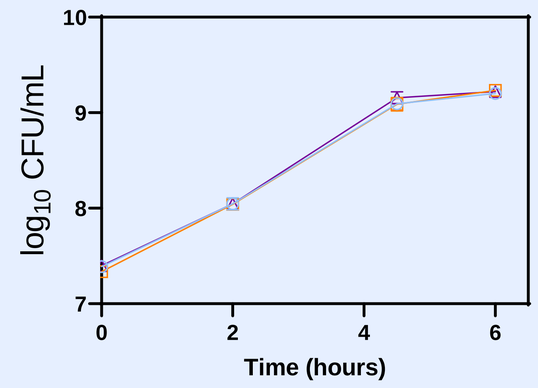




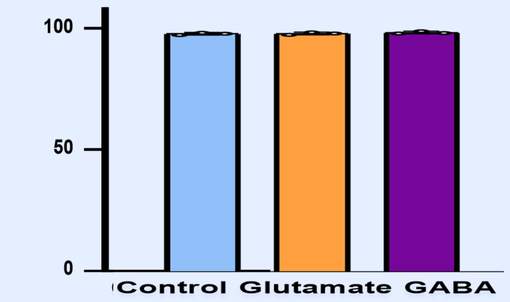
Depolarization threshold: 119.75 a.u



no effect on *E.coli* growth along 6h of exposition



No effect on *E. coli* culture viable count along 6h of exposition



No effect on % of alive *E. coli* along 6h exposition

Conclusions

There are significant alterations in the bioelectrical profile of E. coli (decrease in depolarization) depending on the growth phase and neurotransmitter exposure

Neurotransmitters do not affect bacterial viability or cultivability, so changes in Vmem are attributed to neural stimuli but not to fundamental shifts in bacterial physiology.

References

Cheung SG y col. 2019. *Front Psychiatry*, 10: 34

Duvallet C y col. 2017. *Nature Communications*, 8(1): 1784