



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



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Gut microbiota-brain axis seems to be involved in some physical and mental pathologies caused by microbial imbalance. Bioelectricity could be an important means of communication in the axis.

Enterococci, natural component of the human microbiota, have been used as probiotic food additives or supplements for the treatment of intestinal dysbiosis.

This work analyzes the relationship between nervous signals, microbiota and probiotics through bioelectrical profile of *E. faecalis*.

Material and Methods

Bacteria: *Enterococcus faecalis* ATCC 19433

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 (V_{mem}) reporter. After incubation for 15 min with Valinomycin (5 μ M) and DiBac (50 μ M), *E. faecalis* cultures were observed under an inverted epifluorescence microscope (LEica DMI8). The images obtained were treated with Image J

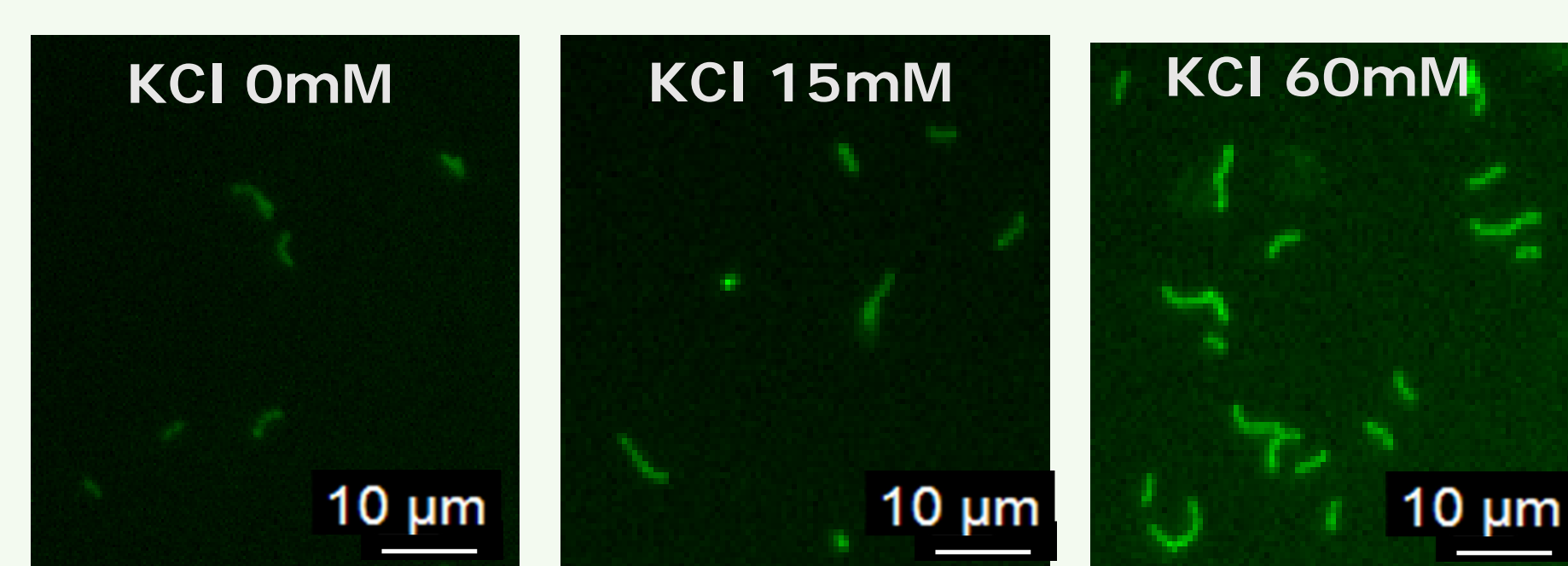
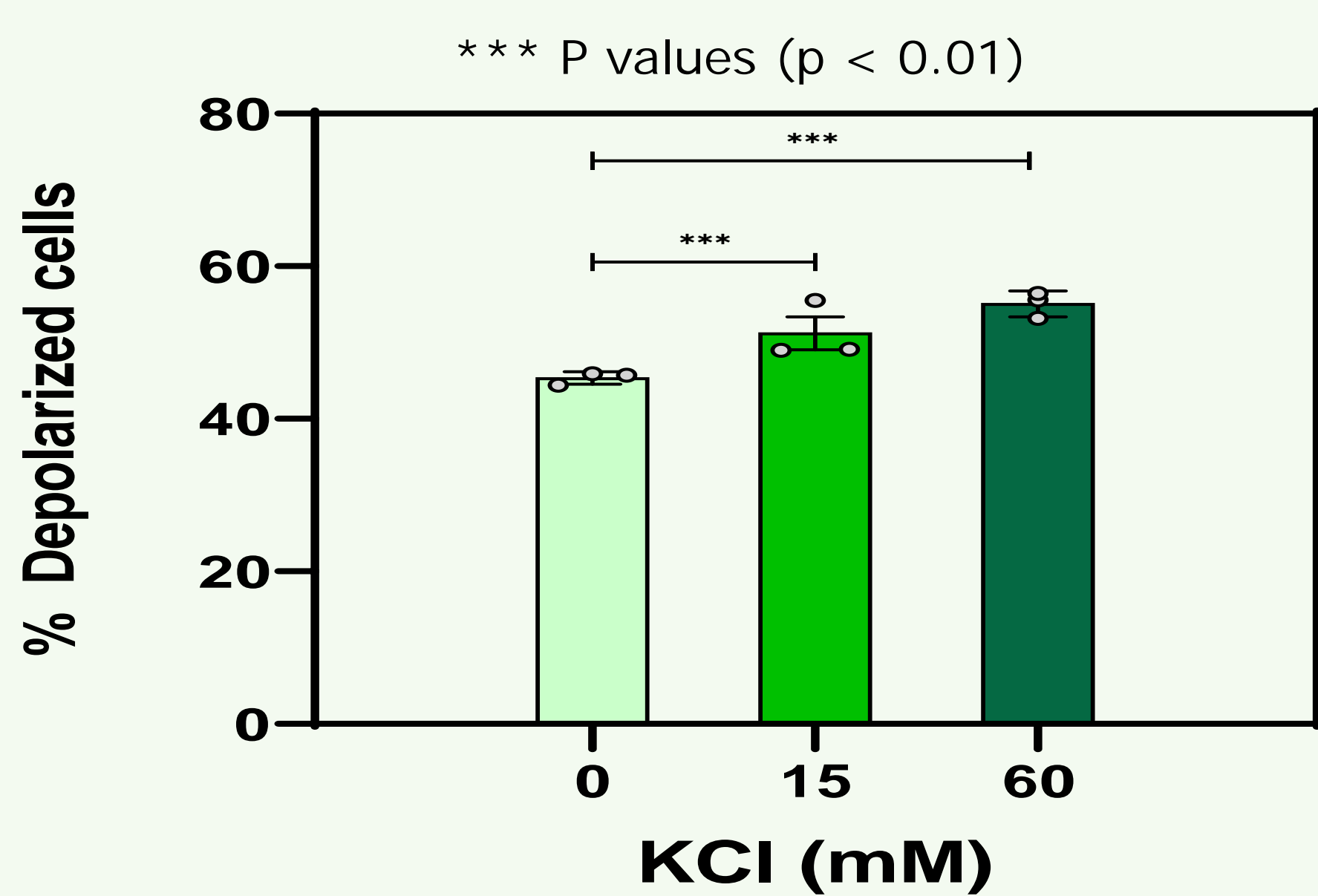
Bacteria growth: *E. faecalis* growth in TSB (Tryptic Soy Broth) was analyzed by measuring absorbance at 600 nm

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

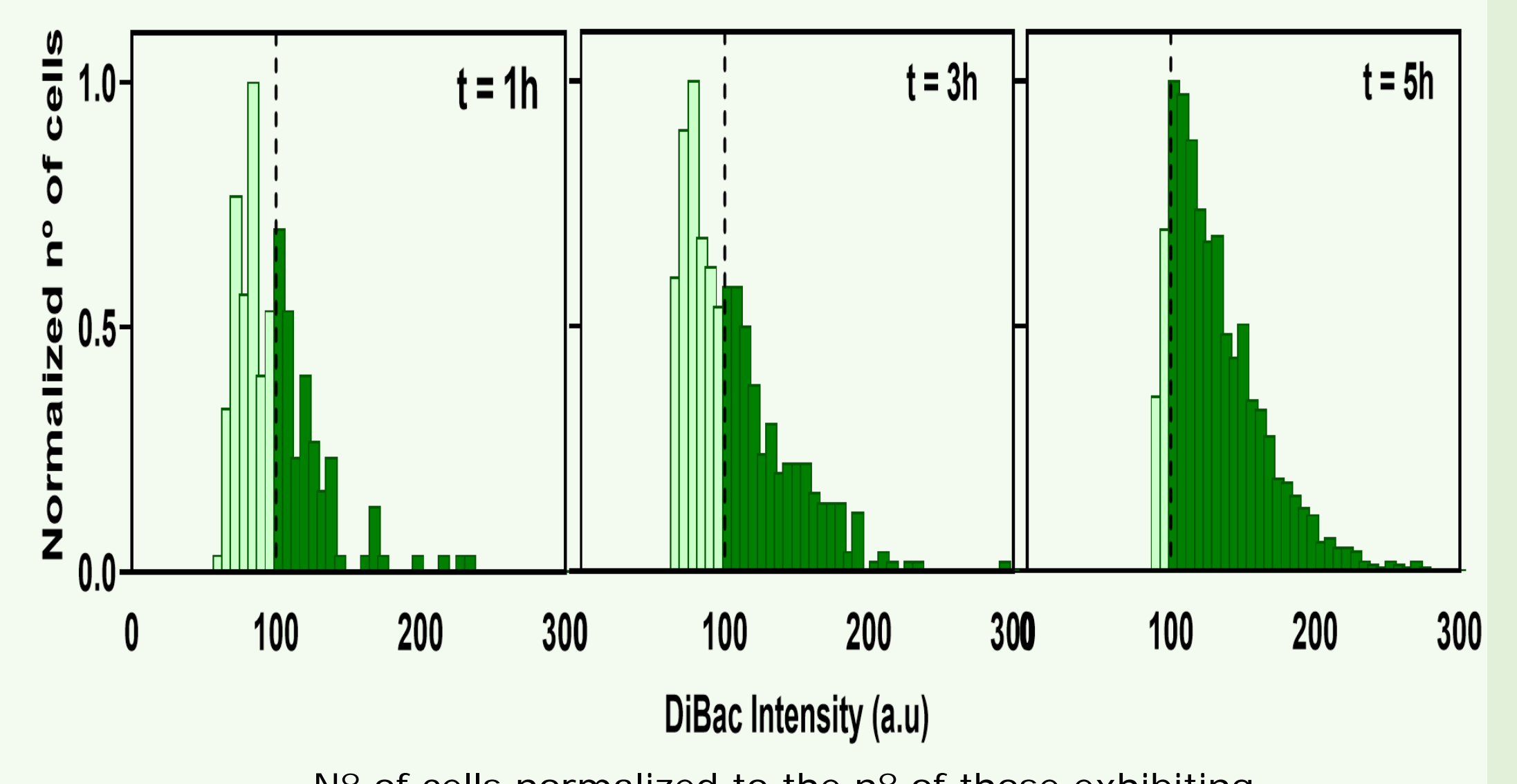
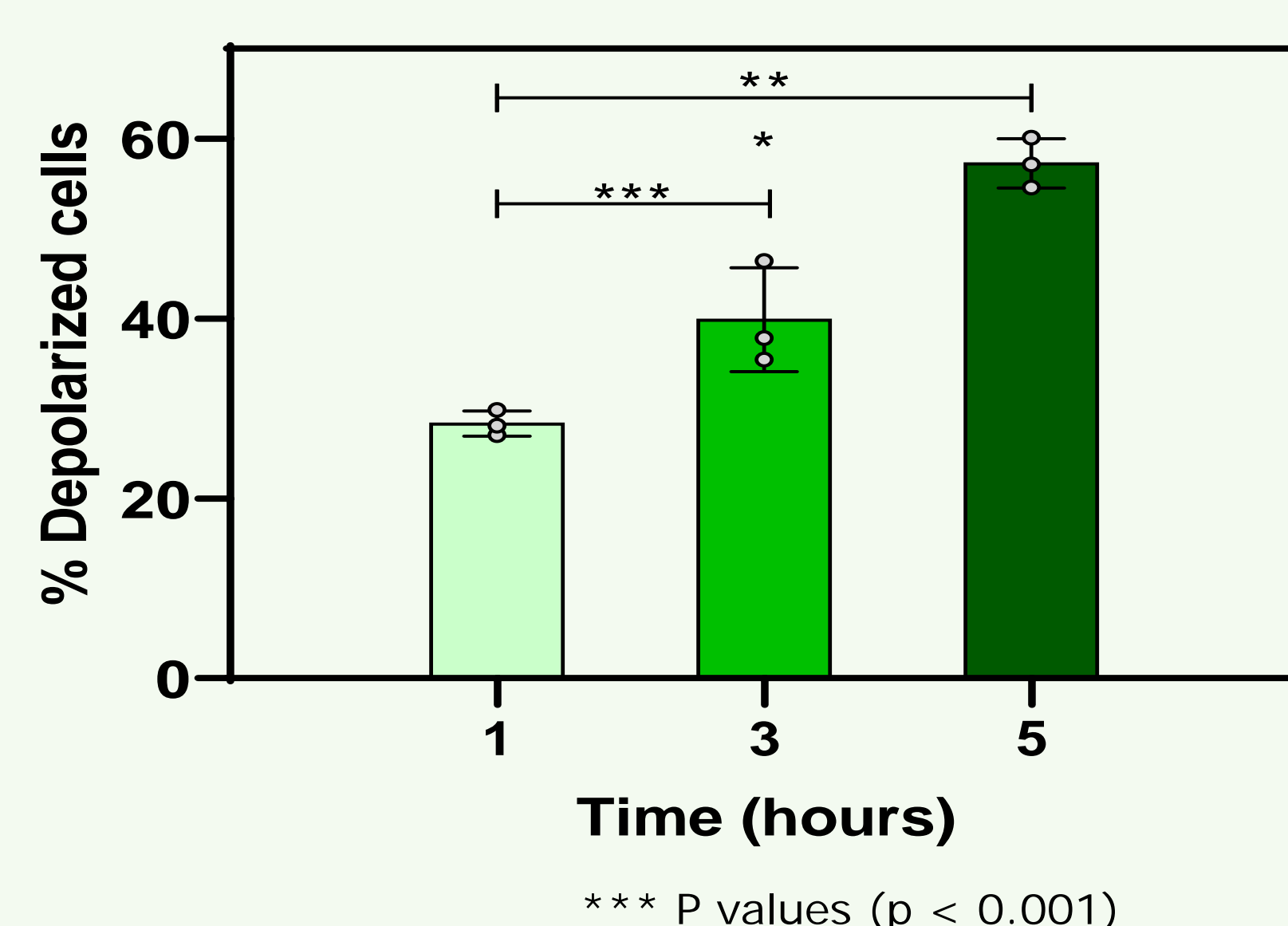
Statistical study: The statistical treatment was done with Generalized Estimating Equations (GEE).

DiBAC as a reliable V_{mem} 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. faecalis* growth

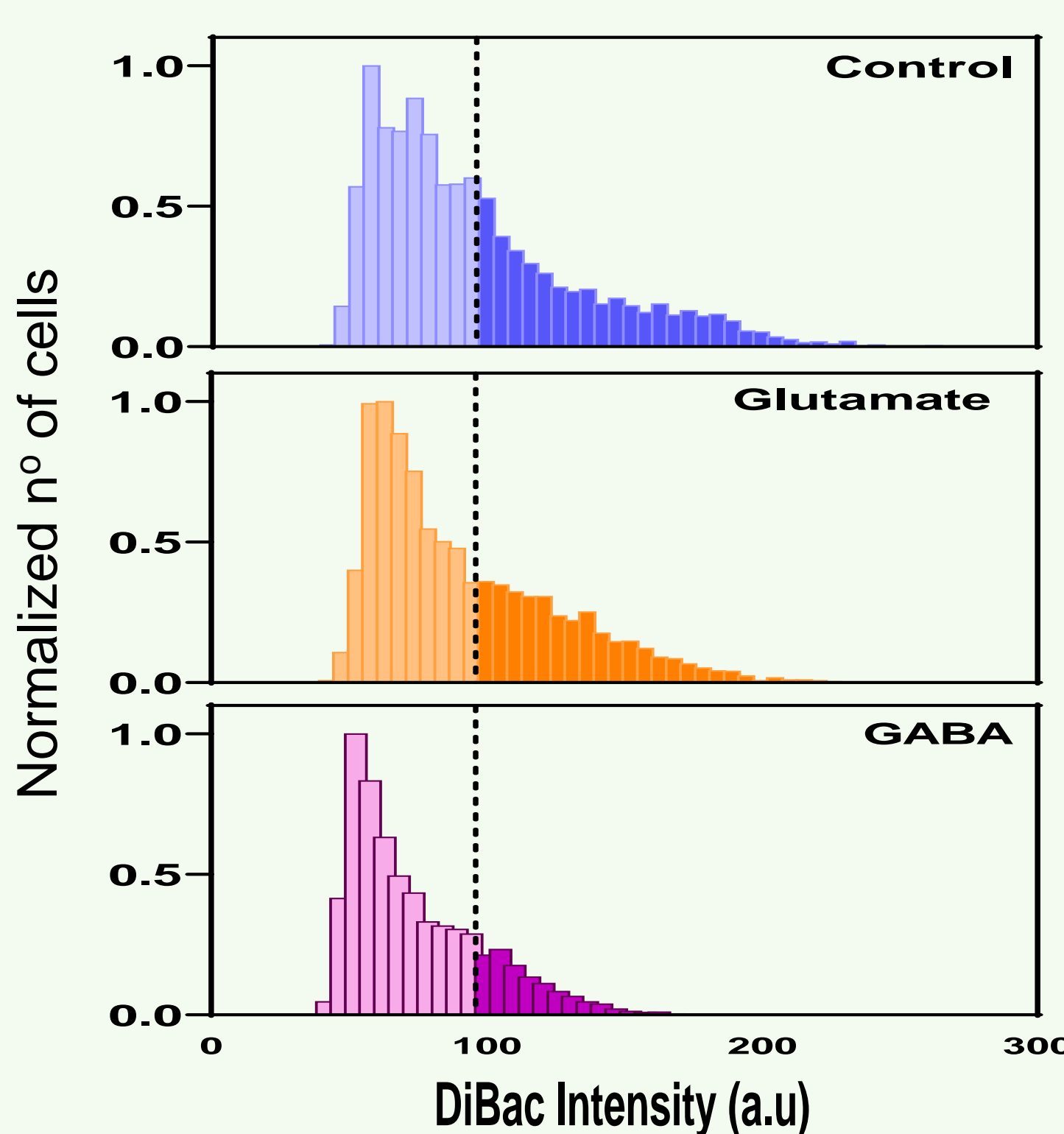
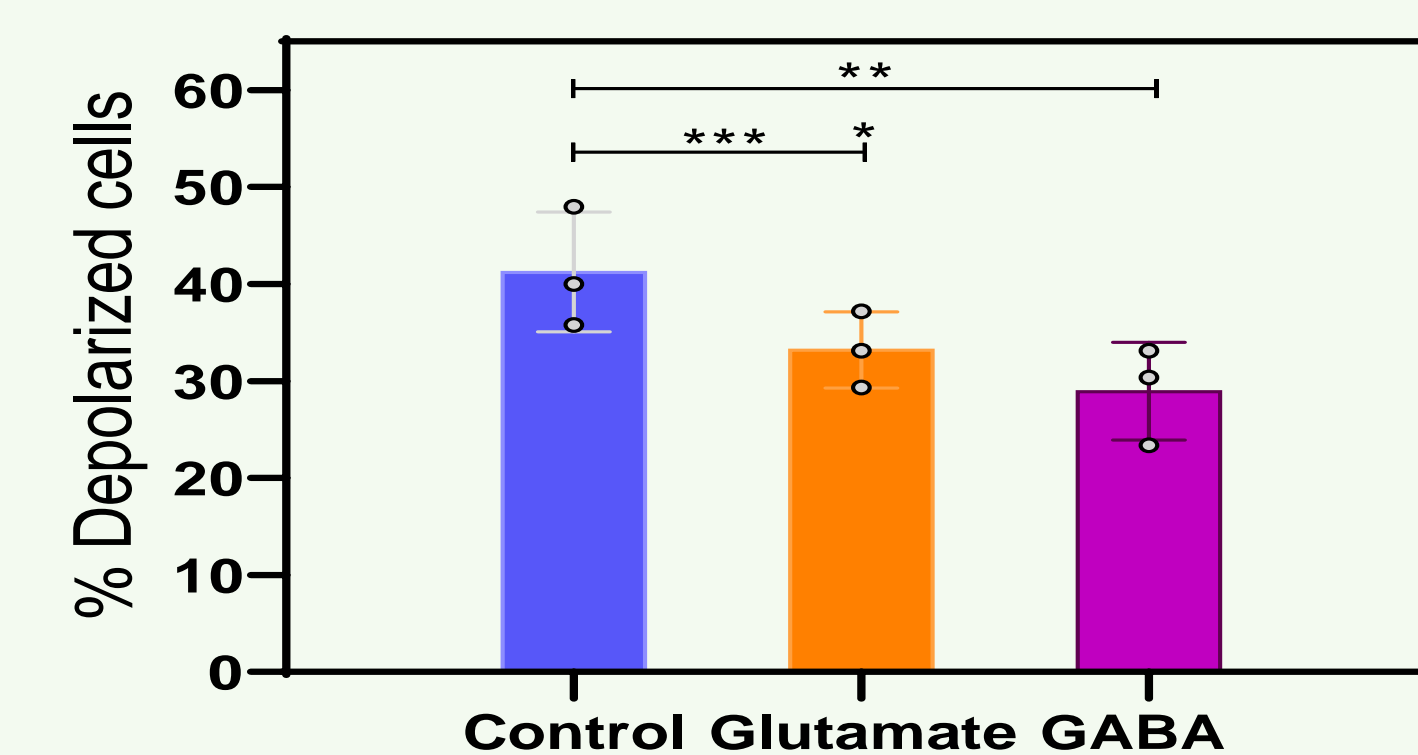


% of depolarized cells increased significantly with growth time

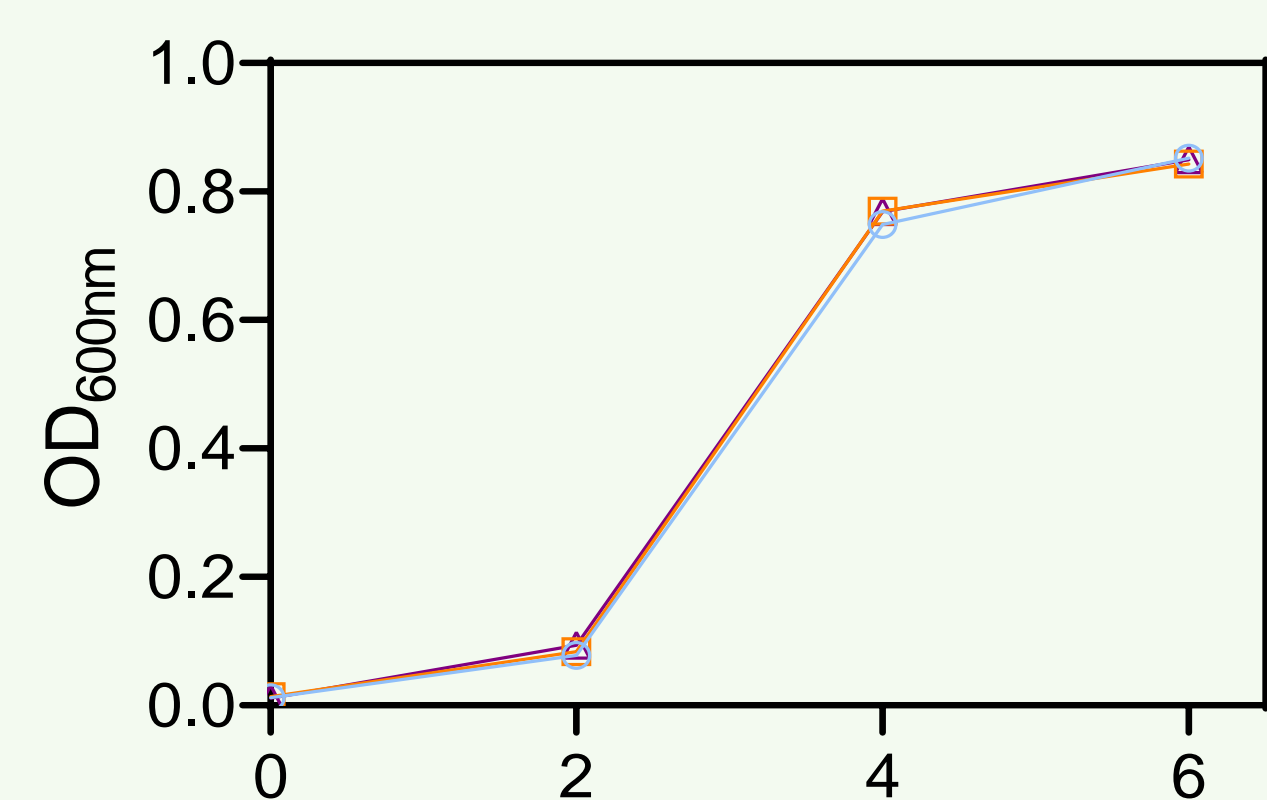
Nº of cells normalized to the nº of those exhibiting the most frequent intensity value
Depolarization threshold (average DiBac fluorescence intensity at 1 h) was 99,82 a.u

Neurotransmitters alter V_{mem} but not its growth, culturability or viability

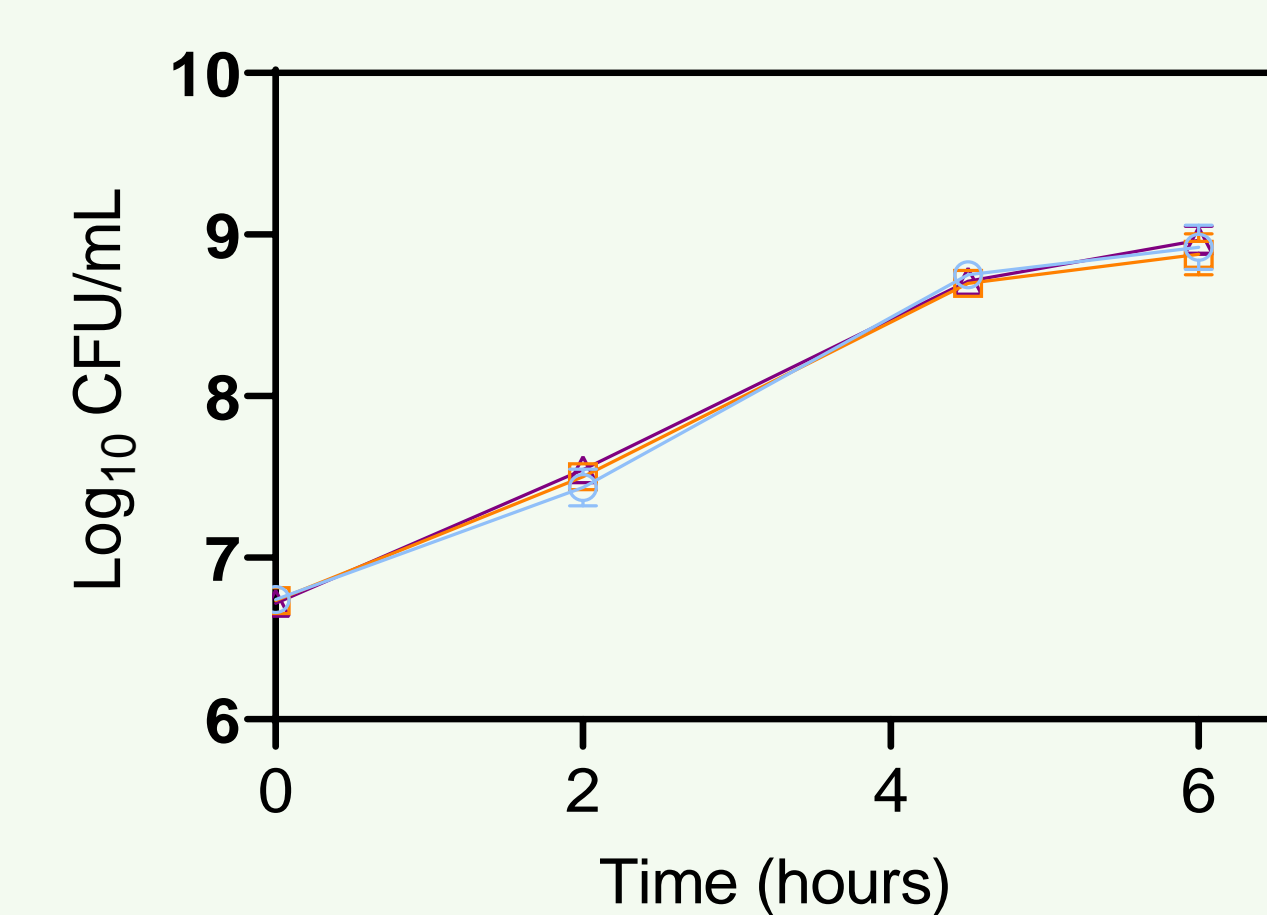
After 4,5 hours, both neurotransmitters decrease % of depolarized *E. faecalis*



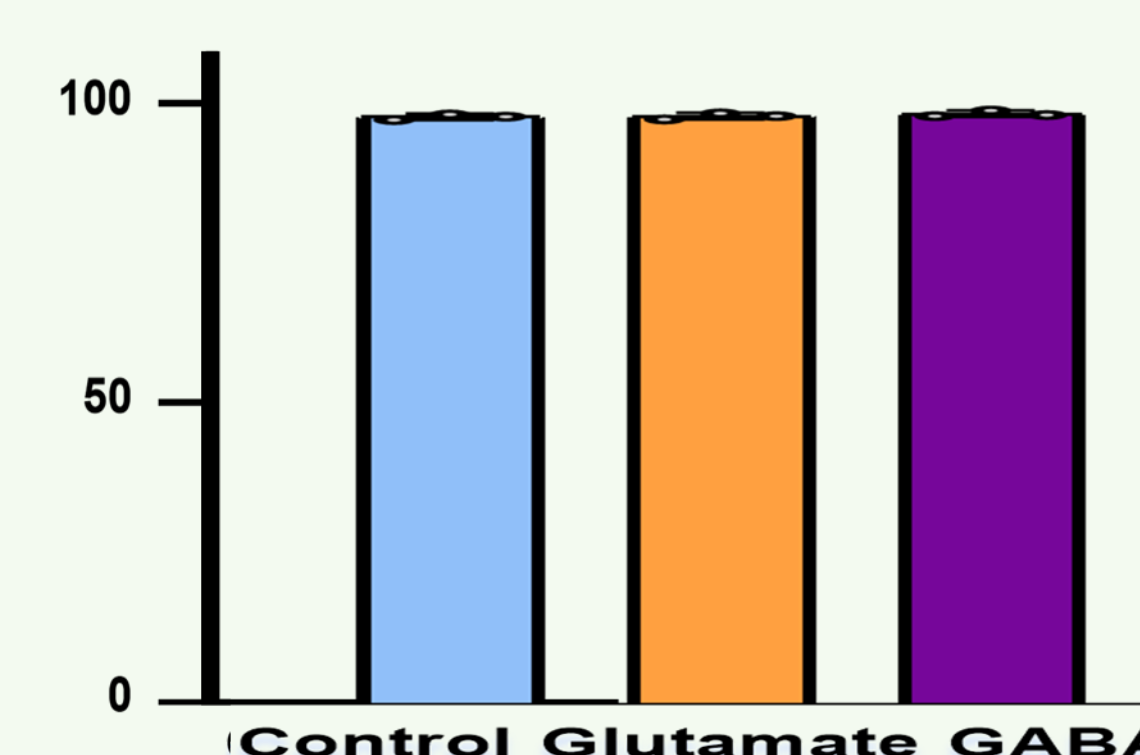
Depolarization threshold: 96 a.u



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



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



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

Conclusions

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

Neurotransmitters do not affect bacterial viability or cultivability, so changes in V_{mem} 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