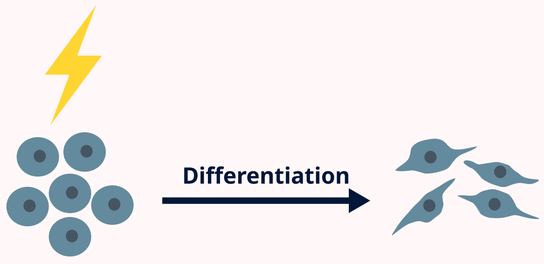
Electrical stimulation induces IL-6 secretion in N2a cells

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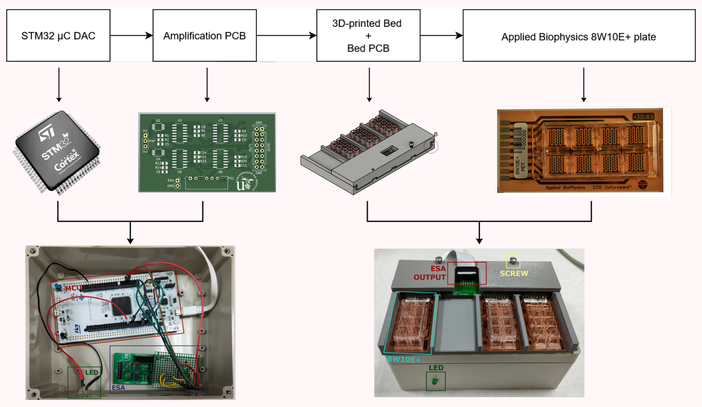
Electrical Stimulation

Electrical stimulation (ES) is a technique that consists of applying an electric field (voltage, current or charge) to living tissue, biological entities, or cell cultures. ES has been shown to promote specific cell responses, including adhesion, proliferation, **differentiation**, directional migration, and cell division.



ES System

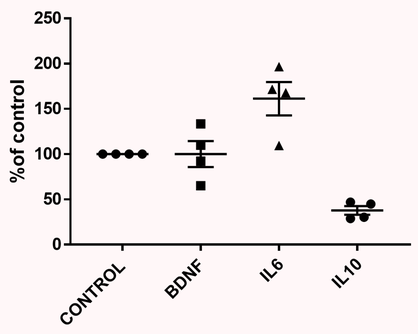
The system is based on an STM32 microcontroller and an analog amplification stage. Cells were cultured on 8W10E+ plates from Applied Biophysics The system is based on an STM32 microcontroller and an analog amplification stage. Cells were cultured on 8W10E+ plates from Applied Biophysics.TM32 microcontroller and an analog amplification stage. Cells were cultured on 8W10E+ plates from Applied Biophysics



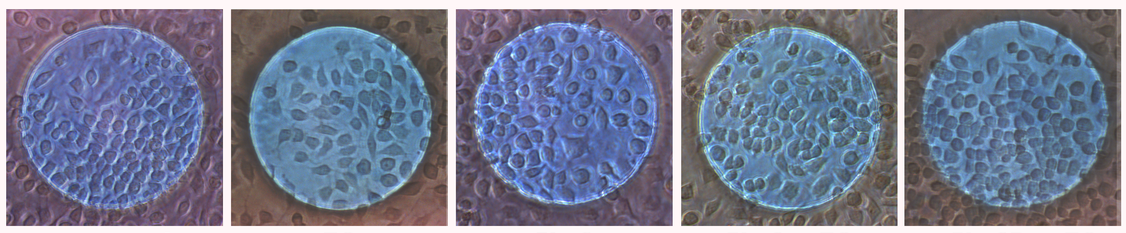
ES Induces Bystander Effect

ES induces the bystander effect on N2a cells, as the stimulated medium promotes the differentiation of the cells.

ES promotes de production of interleukin-6 (IL-6) as detected by RT-qPCR analysis.

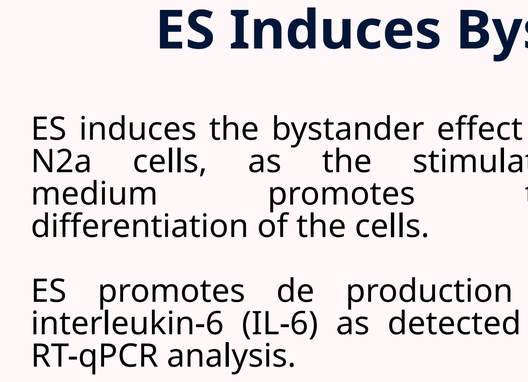


ES Bystander Effect is mediated by IL-6



Control **ES** **IL-6** **IL-6 + ES** **αIL-6R + ES**

Differentiation Percentage



Differentiation Percentage

ES cells display a more differentiated morphology, with a polygonal shape, longer extensions (neurites) and less density.

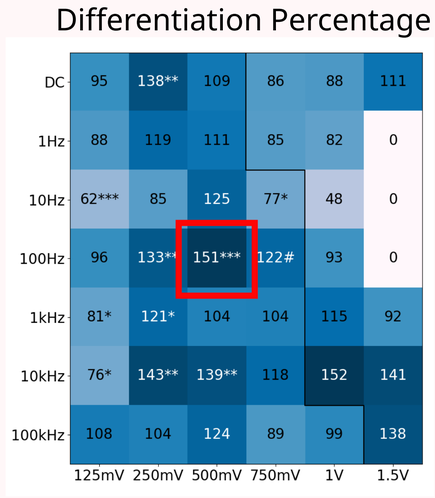
This effect is found in cells treated with IL-6 although silightly restrained and the combined treatment did not show any difference with IL-6 alone.

Neutralization of IL-6 receptor (αIL-6R) suppressed the effect of ES .

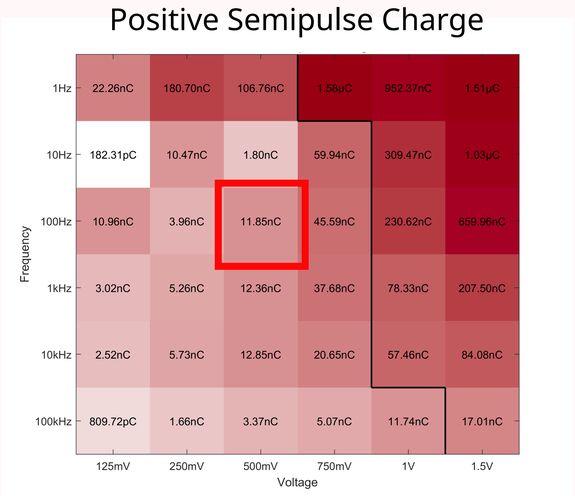
What are the physical variables involved in ES success?

- AC / DC
- **Frequency**
- **Voltage**
- Current
- **Charge**

Differentiation Percentage

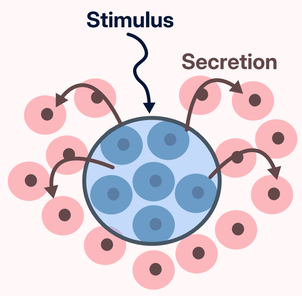


Positive Semipulse Charge



The best conditions are: 500mV/mm at 100Hz (biphasic)

Bystander Effect

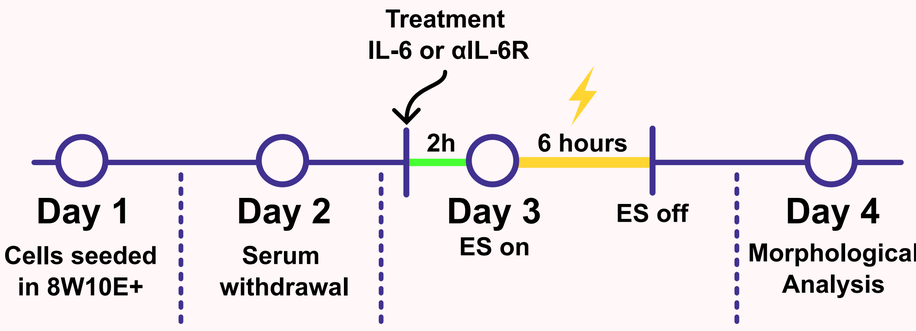


The **Bystander Effect** is a phenomenon where **non stimulated cells** experience a similar effect to stimulated ones.

Stimulated cells segregate signaling molecules that are sensed by non stimulated cells.

Experimental Protocol

10000 N2a cells were seeded on each well of an 8W10E+ plate. After 24 hours, the culture medium was replaced with serum-free medium to promote differentiation. Twenty-four hours later, ES was applied for 6 hours. The following day, at the same time ES had been initiated, images were acquired using an inverted microscope with a 20X objective (Leica, DMi1).



Conclusions

- ES promoted de production of interleukin-6 (IL-6) as detected by RT-qPCR analysis.
- Functional tests implicated IL-6 as a principal mediator of ES bystander effect, as recombinant dosing reproduced morphological outcomes and IL-6 receptor neutralization attenuated the effects of ES, clarifying IL-6's contribution within the ES-conditioned secretome.

Acknowledgments

This publication is part of the project PID2021-1225290B-100, funded by MICIU/ AEI/ 10.13039/501100011033 and by ERDF/EU and by grant USECHIP (TSI-069100-2023-001), project funded by the Secretary of State for Telecommunications and Digital Infrastructure, Ministry for Digital Transformation and Civil Service and by the European Union–NextGenerationEU/PRTR.

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