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TMS Effect on Growth Rate of N27 Neuron Cells

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Transcranial Magnetic Stimulation (TMS)

- TMS is a non-invasive neuromodulation technique that uses time varying short pulses of magnetic field to induce an electric field in the conductive tissues of the brain thus, modulating the synaptic transmission of neurons.
- This technique can be used to excite or inhibit firing rate of neurons which can be used for treatment of various neurological disorders such as major depression disorder, Parkinson's disease, Posttraumatic stress disorder and migraine.
- US Food and Drug Administration (FDA) approved TMS as a treatment for depression in 2008. There has been less focus on in-vitro and animal studies in the literature compared to the in-vivo studies in human.



Method

- Cell Culture

The immortalized rat mesencephalic 1RB3AN27 cells, (N27) were used for the purpose of this study.

- Signal to generate the Magnetic Field

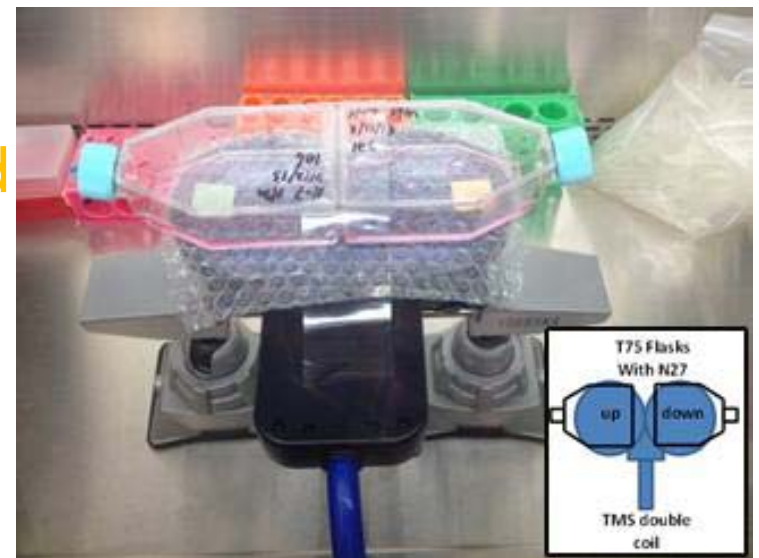
We used a monophasic stimulator which sent pulsed current of 5000 A with frequency of 2.5 kHz, six pulses with 4 seconds waiting time in between as one train and a waiting time of 10 seconds between each train with a total of 360 pulses were used.

- Cell Counting Method

Trypan blue: T 75 flask

MTS cell viability assay: 96 well

Cyquant cell viability assay: 24 well



Method

- Design of TMS Experiment

Exposure Time	Sample Description				Counting Time
0 hours	Incubator	Environmental	Field up	Field down	24 hours
12 hours	Incubator	Environmental	Field up	Field down	36 hours
24 hours	Incubator	Environmental	Field up	Field down	48 hours
36 hours	Incubator	Environmental	Field up	Field down	60 hours
48 hours	Incubator	Environmental	Field up	Field down	72 hours

- Incubator: Always kept in the incubator
- Environmental: Kept in the cabinet during the TMS treatment
- Field up: Received treatment with the orientation of magnetic field out of plane
- Field down: Received treatment with the orientation of magnetic field pointing into the plane

Do the cell counting 24 hours after the treatment

Results from Trypan Blue Cell Counting Method

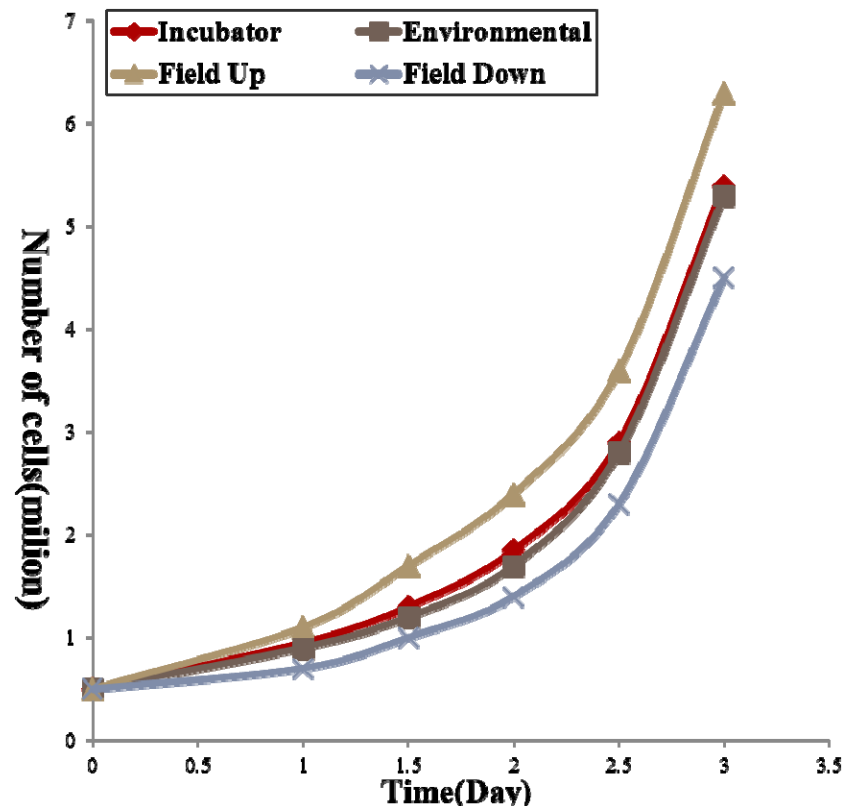


Fig.1 Result of TMS Experiment with seeding density of 0.5 million/flask

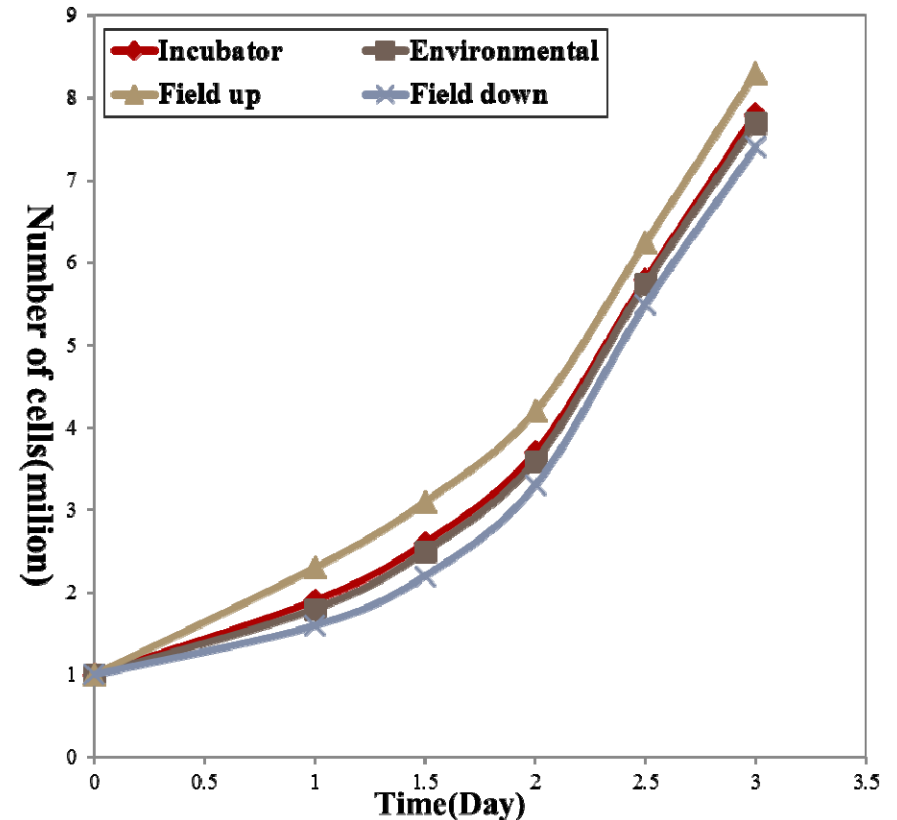


Fig.2 Result of TMS Experiment with seeding density of 1 million/flask

Results from MTS Cell Counting Method

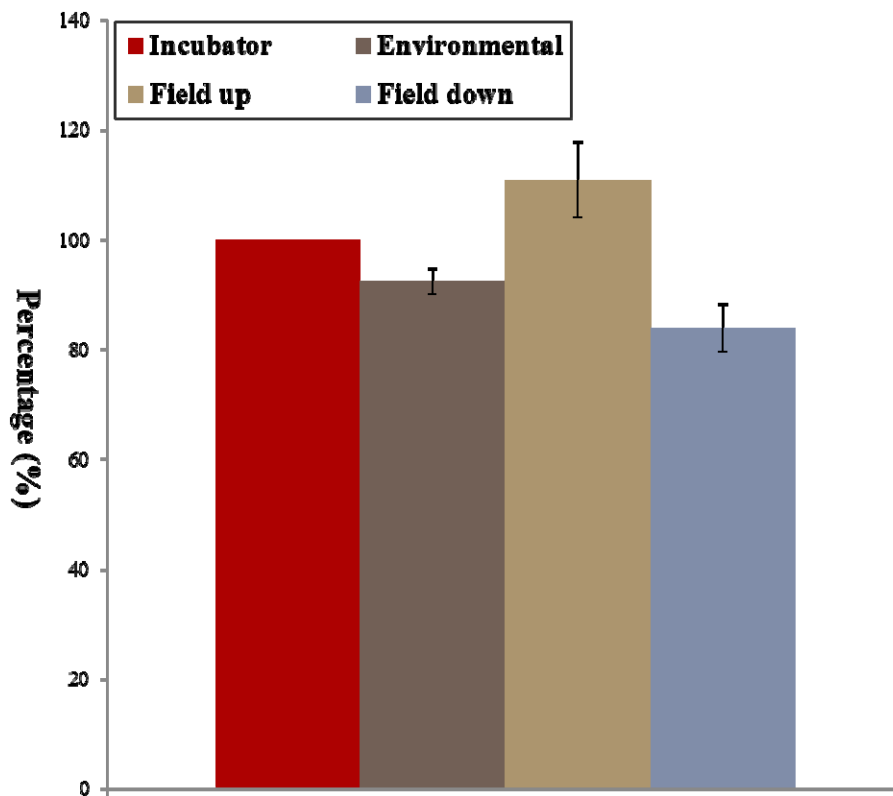


Fig.3 Result of TMS Experiment with seeding density of 20k/well

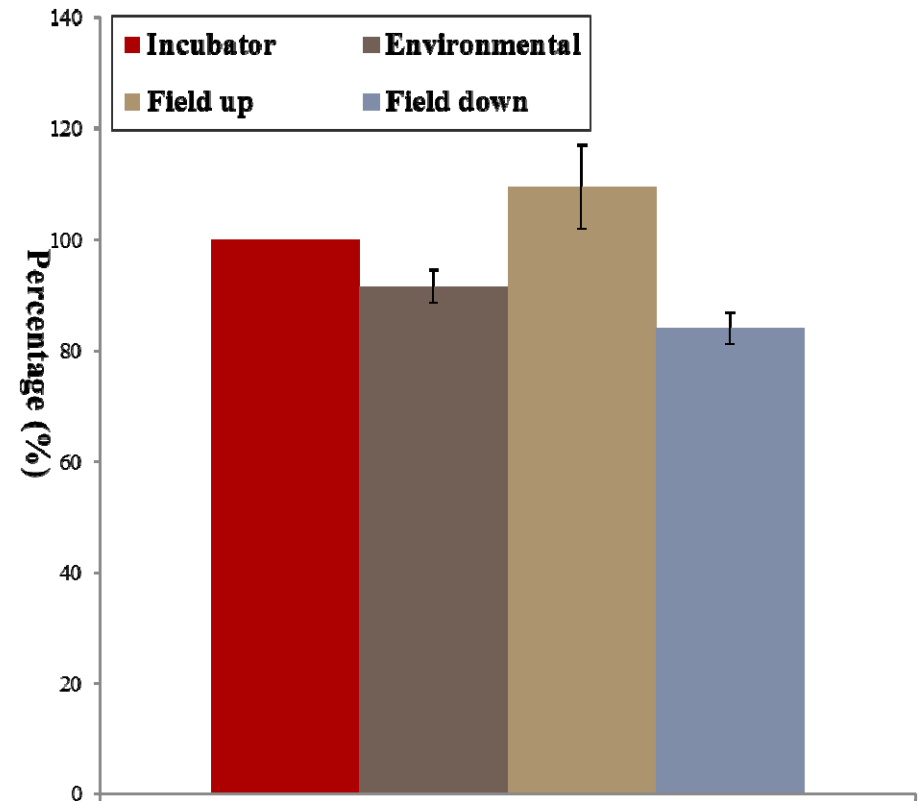


Fig.4 Result of TMS Experiment with seeding density of 20k/well

Results from Cyquant Cell Counting Method

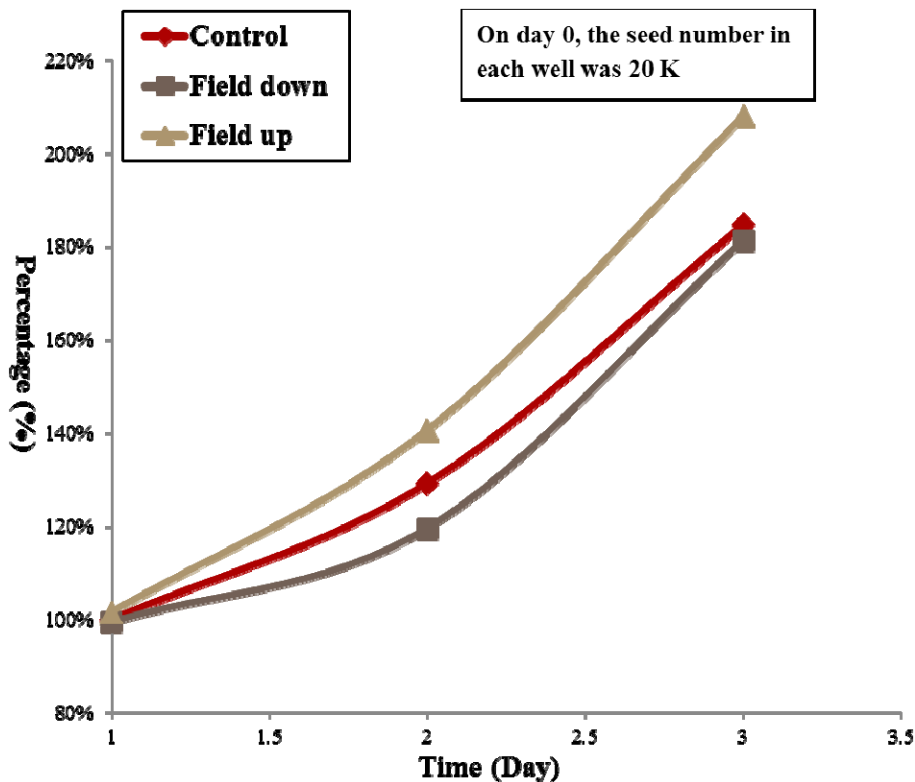


Fig.5 Result of TMS Experiment with seeding density of 20k/well

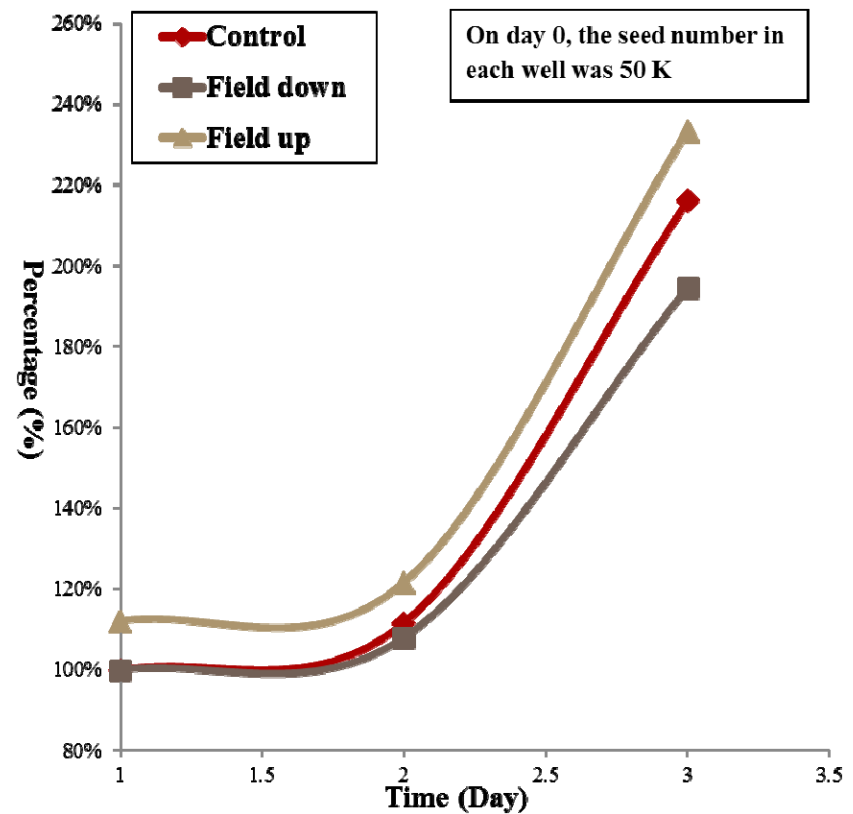


Fig.6 Result of TMS Experiment with seeding density of 50k/well

Results from Cyquant Cell Counting Method

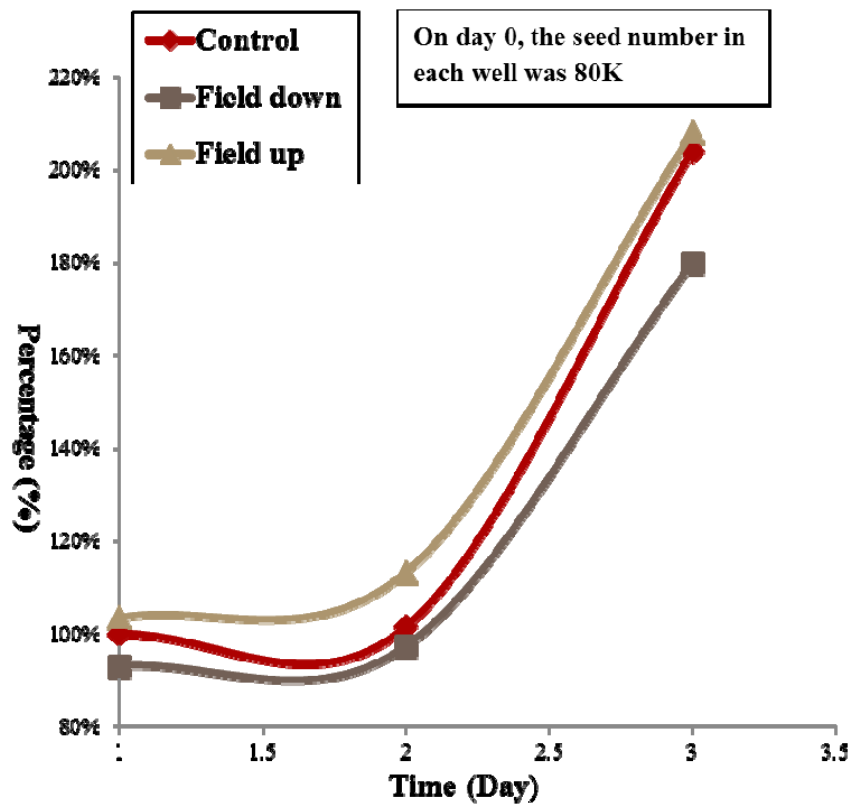


Fig.5 Result of TMS experiment with seeding density of 80k/well

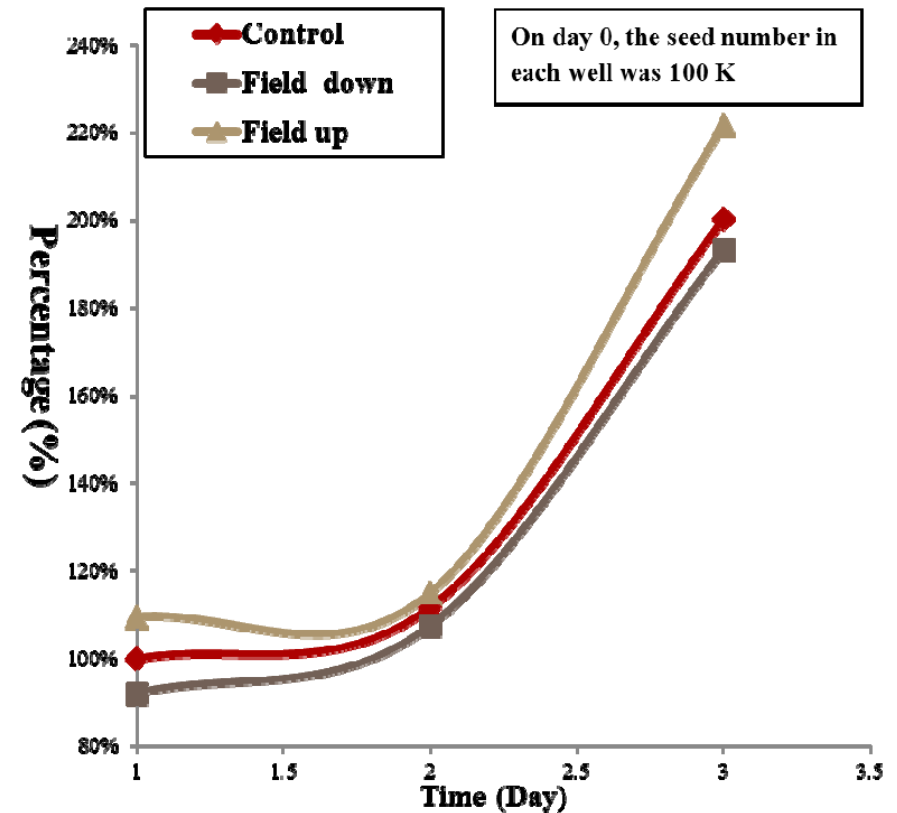


Fig.6 Result of TMS experiment with seeding density of 100k/well

Conclusions

- The growth rate of cells increased by exposing N27 cells with magnetic field out of the plane of neuronal growth compared with the control group
- The growth rate of cells decreased by exposing the magnetic field pointing into the plane of neuronal growth compared with the control group

With MTS Cell Counting Method:

- The number of cells in Field up group was $19.88 \% \pm 4.56$ (Mean \pm STD) higher than the Environmental Control group.
- The number of cells in Field down group was $9.3 \% \pm 2.10$ (Mean \pm STD) lower than the Environmental group. Thus,
- The total change of cell's growthrate was $+10.58 \%$.

Thank you!
Questions?