ANTICANCER RESEARCH *40*: 5151-5158 (2020)
doi:10.21873/anticanres.14518

Static Magnetic Stimulation Induces Cell-type Specific Alterations in the Viability of SH-SY5Y Neuroblastoma Cell Line

HELOUISE R. MEDEIROS[1,2,3], JOSÉ A. F. ASSUMPCAO[1,2], LICIANE F. MEDEIROS[2], MARTINA STAPENHORST[3], LARA NUNES[3], NICOLE A.C. HENCKES[3], CAROLINA URIBE CRUZ[4], FELIPE FREGNI[5], PAULO R.S. SANCHES[6], FERNANDA S.O. OLIVEIRA[3], WOLNEI CAUMO[1,2], ELIZABETH O. CIRNE-LIMA[3] and IRACI L.S. TORRES[1,2*]

[1]PostGraduate Program in Medicine: Medical Sciences, Faculty of Medicine, Universidade do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil;
[2]Translational Nucleus: Pain Pharmacology and Neuromodulation, Experimental Research Center, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil;
[3]Embryology and Cell Differentiation Laboratory, Experimental Research Center, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil;
[4]Experimental Laboratory of Hepatology and Gastroenterology, Experimental Research Center, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil;
[5]Laboratory of Neuromodulation, Department of Physical Medicine & Rehabilitation, Spaulding Rehabilitation Hospital & Massachusetts General Hospital Harvard Medical School and Center for Non-invasive Brain Stimulation, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, U.S.A.;
[6]Biomedical Engineering Laboratory, Research and Post Graduate Group, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil

Abstract. *Background/Aim: Magnetic stimulation is used in the treatment of a diversity of diseases, but a complete understanding of the underlying mechanisms of action requires further investigation. We examined the effect of static magnetic stimulation (SMS) in different cell lines. Materials and Methods: A culture plate holder with attached NeFeB magnets was developed. Different magnetic field intensities and periods were tested in tumoral and non-tumoral cell lines. To verify the cellular responses to SMS, cell viability, cell death, cell cycle and BDNF expression were evaluated. Results: Exposure of SH-SY5Y cells to SMS for 24 hours led to a decrease in cell viability. Analysis 24 h after stimulation revealed a decrease in apoptotic and double-positive cells, associated with an increase in the number of necrotic cells. Conclusion: The effects of SMS on cell viability are cell type-specific, inducing a decrease in cell viability in SH-SY5Y cells. This suggests that SMS may be a potential tool in the treatment of neuronal tumors.*

Correspondence to: Iraci L. S. Torres (ORCID: 0000-0002-3081-115X), Laboratory of Pain Pharmacology and Neuromodulation: Pre-clinical Research, Center of Experimental Research, Hospital de Clínicas de Porto Alegre - Porto Alegre, RS, 90035-007, Brazil. Tel: +55 5133598937, e-mail: iltorres@hcpa.edu.br

Key Words: Static magnetic stimulation, neuroblastoma, cell viability, cell death, cell cycle.

Over the years, several electrophysiological studies have expanded the understanding of normal brain activity and its pathological conditions. Technological advances have been an important part of the improvement of therapies and research in several areas such as neurology, psychology, and psychiatry (1). Brain stimulation is a tool for modulating brain function, allowing the association of activity patterns and cognitive function to establish cause-consequence relations (2). Brain stimulation techniques are usually divided into two different types: invasive and non-invasive (NIBS) techniques. Whereas invasive techniques involve greater risk for patients, as demonstrated by studies that compare the impact of different procedures (3, 4), non-invasive techniques have shown favorable results together with lower risks (3, 5). Indeed, NIBS's application has been described in different scenarios: 1. Cognitive improvement on depression (6, 7); 2. Improvement in post-stroke recovery (8); 3. Improvement of the memory and the quality of life of patients with Alzheimer's disease (9, 10); 4. Relief of

chronic pain (11, 12). These findings, alongside a reduction in induced adverse effects, set NIBS as promising alternatives for the treatment of several diseases and neurological disorders (13).

Recently, *in vitro* studies reporting stimulation using non-invasive techniques have been reported. Potential uses and effects of magnetic stimulation over cellular processes have been described, particularly using static magnetic stimulation (SMS). Apart from physiological and homeostatic events, such as wound healing (14), SMS has shown effects in cancer models of glioblastoma (15), adenocarcinoma (16) and leukemia (17), where it has been shown to control the cell cycle, reduce drug resistance to cisplatin and enhance natural killer cell cytotoxicity against tumor cells, respectively.

SMS, unlike repetitive transcranial magnetic stimulation (rTMS) – in which changes in the magnetic field create an electric current through electromagnetic induction - does not induce electric currents; however, it has been shown to influence a variety of biological systems (18). Transcranial stimulation with a static magnetic field applied in humans reduces the excitability of the motor cortex for a few minutes after the end of the stimulation (19). Few studies have explained the effects of SMS on nervous cells. A comparison between renal cells and cortical astrocytes in rats showed that SMS decreases proliferation and increases apoptosis and necrosis in renal treated cells, while the opposite effect was seen in cortical astrocytes; stimulated cells showed more proliferation and less cell death (20). These results suggest that different cell types can respond differently to SMS.

Immortalized cell lines are widely used models for *in vitro* studies, for their ease of maintenance, high proliferative rates, highly homogenous and reproducible results (21). In this context, the human neuroblastoma cell line SH-SY5Y is often used for neuronal cell studies, since SH-SY5Y cells can be differentiated in dopaminergic neurons (22).

This study aimed to establish a method for *in vitro* SMS, to investigate its effects on cell viability, cell death and the cell cycle of different cell lines, and determine whether the responses are cell type specific.

Materials and Methods

Cell culture and differentiation. Adipose-derived mesenchymal stem cells, human vaginal malignant melanoma HMVII cell line and human neuroblastoma SH-SY5Y cell line, were obtained from the Banco de Células do Estado do Rio de Janeiro (Rio de Janeiro, Brazil). Mesenchymal stem cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 20% heat-inactivated Fetal Bovine Serum (FBS) (GIBCO) and 1% Penicillin/Streptomycin (GIBCO) at 37˚C and 5% CO_2. HMVII cells were maintained in Roswell Park Memorial Institute Medium (RPMI) 1640 (GIBCO) supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin (GIBCO) at 37˚C and 5% CO_2. SH-SY5Y cells were maintained in 1:1 Ham's F12 and DMEM Low Glucose (GIBCO) supplemented with 10% heat-inactivated FBS (GIBCO), and 1% penicillin/streptomycin (GIBCO) at 37˚C and 5% CO_2. Cells were passaged at 80-90% confluency. Cells were seeded in 24-well plates (using only 6 wells per plate, according to Figure 1) at a density of 1×10^6 cells per well and kept at 37˚C and 5% CO_2. Differentiation was induced 24 h after plating using 1:1 Ham's F12 and DMEM Low Glucose (GIBCO) supplemented with 1% heat-inactivated FBS (GIBCO), 1% penicillin/streptomycin (GIBCO) and 10 μM Retinoic Acid (RA). The RA-containing culture medium was replaced every three days until day 10. Evaluation of cell morphology and differentiation was done using phase-contrast light microscopy.

Static magnetic stimulation (SMS). Stimulation with SMS was done using a specially designed stand for attachment of standard 24 well plates. Each stand contains six NdFeB (neodymium-iron-boron) magnets with cylindrical shape (12 mm diameter and 6 mm height), spaced out so that the magnetic fields do not interact (Figures 1 and 2). The distribution of the magnets is made so that they are coupled exactly to 6 wells of a 24-well plate. Each 24-well plate, therefore, is seeded in only 6 wells. There is an adjustment for the distance between the culture plate and the stand to guarantee the strength of the magnetic field. The magnetic field traversed a layer or several layers of cells, in the same way, *i.e.*, cell grouping, or density does not change the field's intensity. The adjustment of the magnetic field was performed with a customized screw, and the measurement of the magnetic field was done using a Hall Effect Gaussmeter (Wuntronic GmbH, Germany), available at the HCPA Biomedical Engineering Laboratory. Stimulation for the initial MTT assay using only the SH-SY5Y cell line was done using intensities of 0.1 T, 0.2 T and 0.3 T (±2% tolerance), for 60 min. The remaining stimulations were performed for 24 h from plating, with an intensity of 0.3 T. Control groups did not receive stimulation. The 48-h group received stimulation during the first 24 h only.

MTT assay. The MTT assay 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Aldrich, Brazil) is a colorimetric assay that reflects cell viability. Immediately and 24 h after SMS exposure, cells were incubated with MTT in saline (132 mM NaCl, 4 mM KCI, 1 mM $CaCl_2$, 6 mM glucose, 10 mM HEPES, pH 7.4). Without removing the medium from the cells, 0.75 mg/ml MTT was added, incubated for 1 h at 37˚C and dimethyl sulfoxide (DMSO) was added for cell disruption. The absorbance was determined at a wavelength of 570 nm, using a wavelength of 620 nm as a reference in a spectrophotometer. Cell viability was expressed as a percentage relative to the absorbance determined in the control cells.

Cell death (PI/Hoechst staining). Viable and dying cells were identified after staining of the nuclei with Propidium Iodide (PI) (Thermo Fischer, Carlsbad, CA, USA) and Hoechst 33342 (HO) (Sigma Aldrich, Willow Creek Road, Eugene, EUA). The cells were incubated in a solution containing PI and HO 5 mg/ml for 15 min and visualized by fluorescence microscopy. To quantify the number of dead/alive cells, ten photos per well were taken, randomly chosen. Images were analyzed using ImageJ software.

Cell death (Annexin-V/PI staining). Annexin-V/PI staining was performed to obtain a more detailed profiling of SH-SY5Y cell death. Apoptotic cells lose the asymmetric disposition of membrane

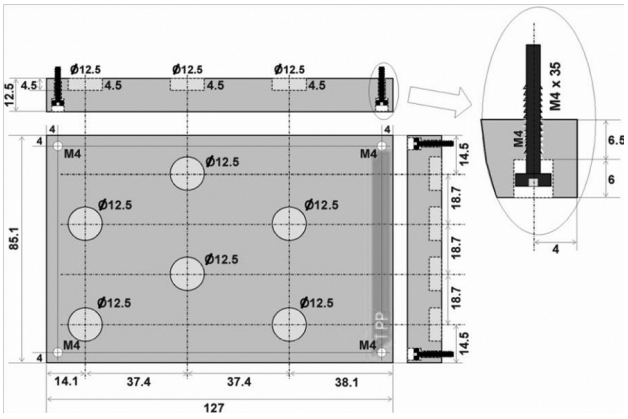

Figure 1. *Schematic for the static magnetic stimulation device.*

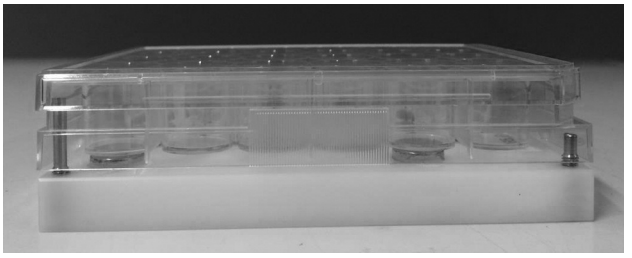

Figure 2. *Cell culture plate placed on SMS device.*

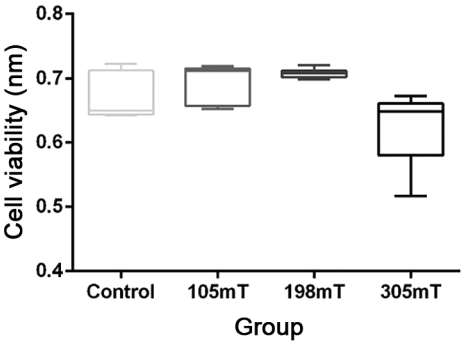

Figure 3. *Cell viability of SH-SY5Y cells exposed to different intensities of SMS. Cells were exposed to 0.1 T, 0.2 T, 0.3 T SMS for 60 min and analyzed using MTT. Results are presented in nm. Results are presented in nm. Data are expressed as medians (interquartile 25; interquartile 75) (Kruskal–Wallis, p>0.05).*

components and proteins, such as phosphatidylserine residues, usually found on the inside sheet of the plasma membrane. Upon entering apoptosis, these proteins are exposed to the outer sheet of the plasma membrane, where it is made available for Annexin-V-FITC staining. On the other hand, necrotic cells lose membrane integrity and are, therefore, positively stained using PI. In this manner, the different events surrounding cell death can be distinguished from one another according to different staining profiles. Double-positive cells do not have a clear phenotype. These cells can be either late-stage apoptotic or necrotic cells. Apoptotic cells (Annexin-V+/PI–), necrotic cells (Annexin-V–/PI+), and alive cells (Annexin-V–/PI–) are quantified through flow cytometry. After treatments, the samples were washed with PBS, resuspended in 100 µl of Annexin-V Binding Buffer 1X and incubated with 2.5 µl of Annexin-V FITC for 15 min, at room temperature, protected from light. The samples were incubated in Propidium Iodide solution (2 µg/ml), an additional fluorescent marker, in Annexin-V Binding Buffer 1X for 5 min at 4˚C protected from light. Alive cells show membrane integrity, which prevents PI from entering the cell and staining nucleic acids (RNA and/or DNA). The samples were immediately analyzed by flow cytometry using the Attune® Acoustic Focusing Cytometer (Applied Biosystem- Life-Thermo). As an experimental control, apoptosis was induced using 20% DMSO for 15 min and necrotic cells were obtained by heating the cells at 70˚C for 15 min.

Cell cycle. Treated cells were resuspended in 100 µl of PBS and 900 µl of ice-cold 70% ethanol were added and incubated for 1 h at 4˚C. Samples were centrifuged at 5000 rpm for 10 min and the pellet was washed three times in PBS 1X and resuspended in a standard staining solution (0.1% Triton X-100, 100 µg/ml PI and 50 µg/ml DNAse-free RNAse) for 15 min at 37˚C, protected from light. Samples were resuspended in PBS for immediate flow cytometry analysis using the Attune® Acoustic Focusing Cytometer (Applied Biosystem- Life-Thermo).

BDNF expression. Total RNA was extracted as recommended by the manufacturer using the RNeasy Mini Kit (Qiagen, Austin, TX, USA). Complementary DNA was synthesized from 1 µg RNA using SuperScriptVILOtm (Invitrogen, Brazil). PCR reactions were prepared using MasterMix TaqMan (Applied Biosystems, Germantown, MD, USA) and StepOne™ Real-Time PCR System (Applied Biosystems). Real-time PCR was optimized to run under the initial incubation conditions of 95˚C for 2 min, denaturation at 95˚C for 15 s, annealing at 60˚C for 1 min, for 45 cycles. The expression of the BDNF gene was normalized with the Glyceraldyehyde-3-phosphate Dehydrogenase (GAPDH) with ΔΔCT correlation.

Statistical analysis. The first analyses were normality and lognormality to choose between a parametric or non-parametric test. For parametric samples, data are presented as mean±SD and analyzed using Student's *t*-test. For non-parametric samples, data are presented as median (interquartile 25; interquartile 75) and analyzed using Kruskal–Wallis followed by Mann–Whitney test. Values of $p<0.05$ were considered significant. All analyses were performed using the statistical software GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA).

Results

Cell viability and cell death. MTT was performed to evaluate the SMS effects on the cell viability (Figure 3). SH-SY5Y cells were stimulated with SMS for 60 min in three different intensities (0.1 T, 0.2 T and 0.3 T). There was no significant

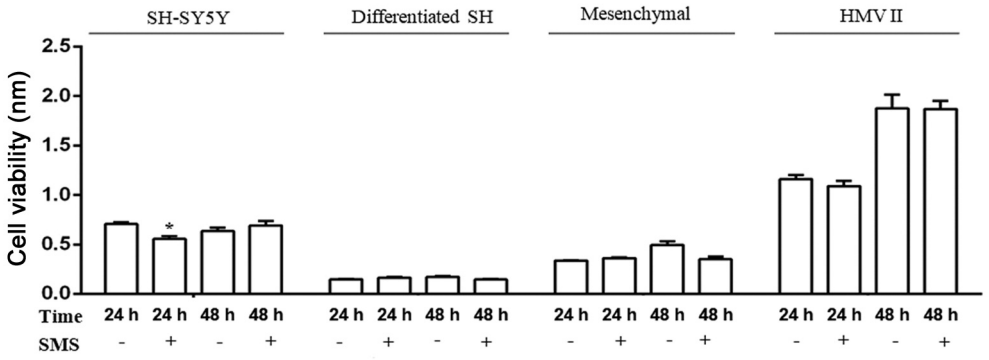

Figure 4. *Cell viability of SH-SY5Y, differentiated SH-SY5Y, HMV II and MSCh cells exposed to 0.3 T (305 mT) SMS. MTT analysis was done immediately after SMS exposure (24 h) and 24 h after SMS exposure (48 h). Results are presented as nm. In the MSCh, SH Dif and SH groups, data are expressed as mean±SD. In the HMV II group, data are expressed as medians (interquartile 25; interquartile 75). *Indicates significant difference when compared to the 24 h (-SMS) group (Student's t-test, p<0.05).*

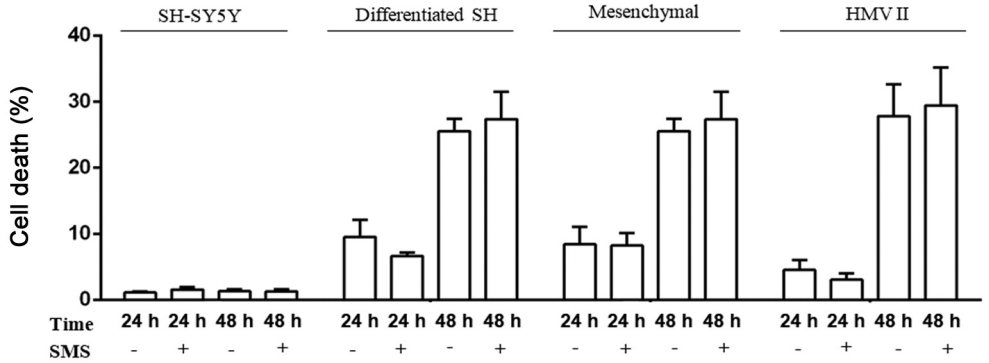

Figure 5. *Cell death of SH-SY5Y, differentiated SH-SY5Y, HMVII and MSCh cells evaluated by PI/HO staining, immediately after SMS exposure (24 h) or 24 h after SMS exposure (48 h). Results are presented as percentages. In all groups, data are expressed as mean±SD. There was no difference between groups (Student's t-test, p>0.05).*

difference between control cells and cells exposed to the three different SMS intensities (0.1 T, 0.2 T and 0.3 T) (Kruskal–Wallis, $p>0.05$). Based on these results, the highest intensity (0.3 T) and longer exposure time (24 h) were chosen for further analysis of cell viability (Figure 4). SH-SY5Y cells, evaluated immediately after 24 h of SMS exposure, presented a significant decrease in viability when compared to the control group (Student's *t*-test, $p<0.05$). Stimulated SH-SY5Y cells evaluated 24 h after SMS exposure did not present a significant difference in viability when compared to the control group (Kruskal–Wallis $p>0.05$). In differentiated SH-SY5Y, adipose-derived mesenchymal and HMVII cells, no difference in viability was found after SMS exposure for both evaluated periods (immediately and after 24 h of SMS exposure) (Student's *t*-test or Kruskal–Wallis, $p>0.05$).

In SH-SY5Y cells, PI/HO analysis of cell death (Figure 5) showed no significant difference after SMS exposure, suggesting there was no increase in cell death in these cells (Student's *t*-test or Kruskal–Wallis, $p>0.05$). Similarly, in differentiated SH-SY5Y no difference was found (Student's *t*-test or Kruskal–Wallis $p>0.05$). Annexin-V/PI evaluation (Figure 6) showed a decrease in apoptotic (Annex+/PI) (1.594% to 0.004%, Figure 6B) and double-positive (Annex+/PI+) cells (0.086% to 0.190%, Figure 6D), and an increase in necrotic (Annex-/PI+) (0.359% to 1.580%, Figure 6C) SH-SY5Y cells exposed to SMS for 24 h and analyzed 24 h after the stimulation (48 h) (Kruskal–Wallis, $p<0.05$, Figure 6). In groups evaluated immediately after 24 h of exposure to SMS (24 h), there was a decrease in double-positive (Annex+/PI+) cells (0.150% to 0.130%, Figure 6D).

Cell cycle (PI staining). Cell cycle analysis (Figure 7) of SH-SY5Y cells showed no difference in the percentage of cells in sub-G1, G1, S, G2 and >4N phases in both periods analyzed (Student's t-test, $p>0.05$), suggesting that exposure to SMS does not alter cell cycle distribution.

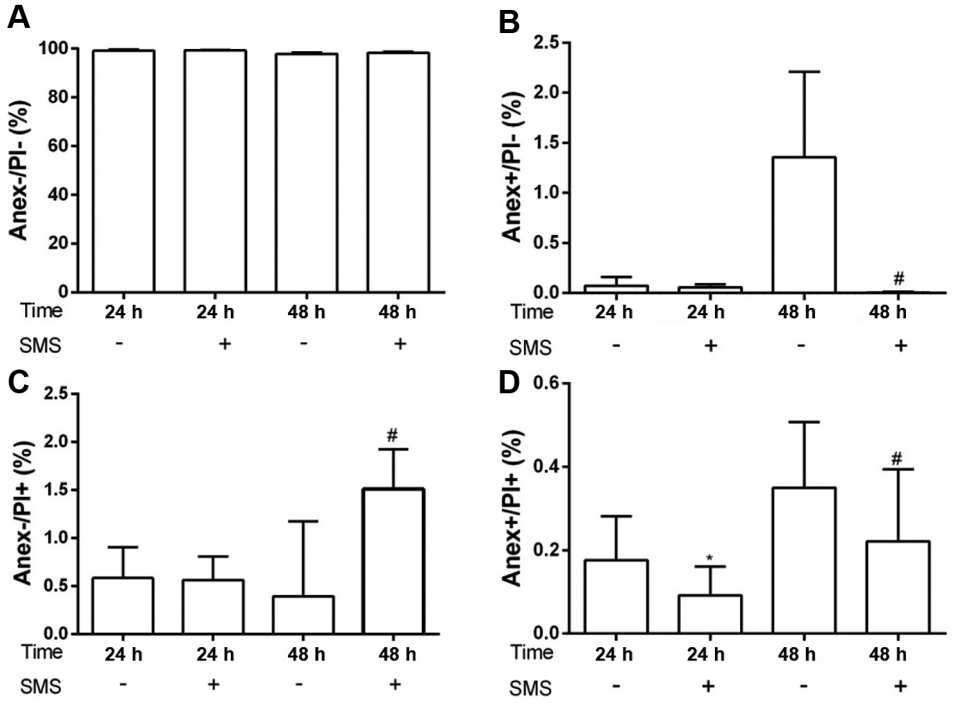

Figure 6. *Cell death of SH-SY5Y cells evaluated by Annexin-V/PI staining, immediately after SMS exposure (24 h) or 24 h after SMS exposure (48 h). A) Live cells (Annex-/PI–). B) Apoptotic cells (Annex+/PI–). C) Necrotic cells (Annex-/PI+). D) Double-positive cells (Annex+/PI+). *Indicates significant difference when compared to the 24 h (-SMS) group (Kruskal–Wallis, p<0.05). #Indicates significant difference when compared to the 48 h (-SMS) group (Student's t-test, p<0.05). Data are expressed as mean±SD/Data as expressed as median (interquartile 25; interquartile 75).*

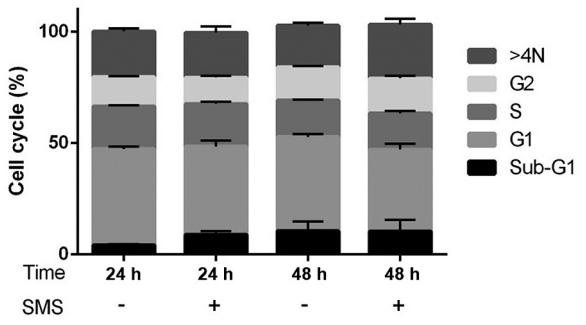

Figure 7. *Cell cycle analysis of SH-SY5Y cells evaluated by PI staining, immediately after SMS exposure (24 h) or 24 h after SMS exposure (48 h). Results are presented as percentages. Data are expressed as mean±SD. There was no difference between the different times (Student's t-test, p>0.05).*

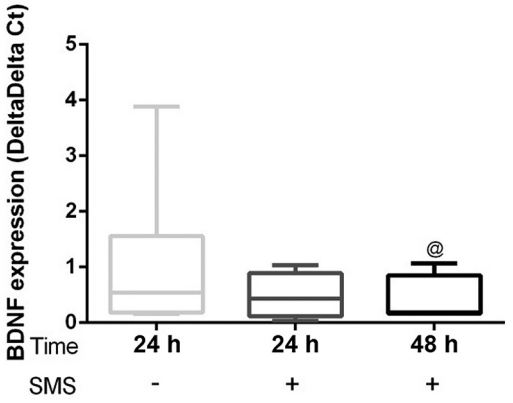

Figure 8. *Gene expression analysis of BDNF gene in SH-SY5Y cells after SMS exposure (24 h) or 24 h after SMS exposure (48 h). Data are expressed as median (interquartile 25; interquartile 75). @Indicates significant difference between cells exposed to 24 h of SMS and cells exposed to 48 h of SMS (Kruskal–Wallis, p<0.05).*

BDNF expression. In SH-SY5Y cells, even though detection of BDNF was successful (Figure 8), there was no difference in cells exposed to SMS for both periods when compared to control groups (Kruskal–Wallis, $p>0.05$). Although there was no difference in BDNF expression between the stimulated cells and the control, there was a difference between the stimulated groups, which is expected, due to analysis in different periods.

Discussion

Our results demonstrated that SH-SY5Y cells exposed to SMS for 24 h show a decrease in cell viability immediately after the exposure (24 h). A previous study using glioblastoma cells submitted to SMS for 24 h corroborates these findings regarding cell viability (15). This effect on cell viability, however, was not long-lasting, since 24 h after exposure treated groups were not different from the control group. SH-SY5Y cells did not show alterations in cell viability after exposure to magnetic stimulation, emphasizing that magnetic stimulation has cell type-dependent effects on cell viability.

PI/HO and Annexin-V/PI staining were performed in order to evaluate cell death. Evaluation of cell death 24 h after SMS exposure showed small differences compared to control cells (showing a decrease in apoptotic and double-positive cells, associated with an increase in necrotic cells). Alongside cell death, cell cycle profiling was performed, which indicated no changes in cell cycle distribution.

Our results indicated that SMS effects may also extend beyond the modulation of neuronal proliferation and plasticity. Neurotrophins, such as the brain-derived growth factor (BDNF), regulate the plasticity of the nervous system and are overexpressed in several types of cancer (27, 28). In fact, BDNF was initially characterized in oncogenic neuroblastoma, a type of cancer in nervous tissue (28). Even though the effects of SMS on nerve cells and brain tissues have been extensively described (23, 29-37), we found no difference in BDNF expression in SH-SY5Y cells exposed to SMS with the exception of an expected difference from 24 to 48 h.

When comparing our findings on undifferentiated and differentiated nerve cell lines only undifferentiated SH-SY5Y cells were influenced by SMS. Both SH-SY5Y cell subsets show differences ranging from polarization, number and length of the processes to proliferation (21, 38, 39), which may be distinctively affected by SMS. The difference in cell viability responses to SMS may not be due to the selectivity of action upon excitability (23) or other membrane channel-related effects (18, 40, 41), but may also influence other cellular processes. Indeed, SMS induces alterations in the viability of SH-SY5Y cells in response to cisplatin (24), having a modulatory effect on the cell's response to several pharmacological treatments, (42-47). Other effects of exposure to SMS have already been described in other cellular functions, such as ROS production (24), modulation of redox-related enzymes (25), pro- and anti-inflammatory cytokine release (24) and improvement in the killing function of NK cell (17). Given the diversity of the processes affected by SMS, changes in cell viability probably involve processes in addition to cell death, cell cycle distribution and neurotrophin production. Future studies, using higher intensities, as well as a longer exposure times, are necessary to evaluate if this technique induces or inhibits cell death.

Conclusion

The different effects exerted by exposure to SMS provide valuable information regarding the application potential of SMS. This study demonstrated that, considering the analyzed parameters, SMS is a potentially safe technique, at least in the utilized protocol (0.3 T SMS/24 h). The decrease in SH-SY5Y cell viability also shows potential for treatment of neuronal tumors with SMS. Also, our results showed that the effect of SMS is cell type specific. It is important to note that this is one of the first studies showing SMS as a potential tool in the treatment of neuronal tumors. Further investigations in this area are still necessary to better understand the effects of SMS exposure on cultured cells and in vivo models.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

HRM, FSOO, EOCL, PRSS, FF, WC and ILST were responsible for the study concept and design. HRM, JA, MS, LN, NAACH and CUC contributed to the acquisition of the data. HRM, JA, CUC, FSOO, EOCL and ILST were responsible by data analysis. HRM, LFM, JA and ILST drafted the manuscript. All Authors revised and edited the manuscript and approved the final version.

Acknowledgements

The Authors were supported by the Brazilian's agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (Dra ILS Torres; Dr. W. Caumo, Dr. P. R. S. Sanches), Federal University of Rio Grande do Sul and Brazilian Federal Agency for Support and Evaluation of Graduate Education – CAPES (Helouise Medeiros). The Authors also want to thank the Engineering Division from the HCPA for having developed the SMS stimulator; the Graduate Research Group of the Hospital de Clínicas de Porto Alegre – GPPG (I.L.S. Torres, grant number 15-0567); MCT/FINEP – COENG/2013. PRONEM FAPERGS. The Authors are also thankful for Research Incentive Fund received from FIPE/HCPA.

References

1 Ziemann U: Thirty years of transcranial magnetic stimulation: where do we stand? Exp Brain Res *235(4)*: 973-984, 2017. PMID: 28120010. DOI: 10.1007/s00221-016-4865-4

2 Bergmann TO, Karabanov A, Hartwigsen G, Thielscher A and Siebner HR: Combining non-invasive transcranial brain stimulation with neuroimaging and electrophysiology: Current approaches and future perspectives. NeuroImage *140*: 4-19, 2016. PMID: 26883069. DOI: 10.1016/j.neuroimage.2016.02.012

3 Brittain J-S and Cagnan H: Recent trends in the use of electrical neuromodulation in Parkinson's disease. Curr Behav Neurosci Rep *5(2)*: 170-178, 2018. PMID: 29862163. DOI: 10.1007/s40473-018-0154-9

4 Hariz M: Deep brain stimulation: new techniques. Parkinsonism Relat Disord *20*: S192-196, 2014. PMID: 24262179. DOI: 10.1016/S1353-8020(13)70045-2

5 Lefaucheur J-P, Antal A, Ayache SS, Benninger DH, Brunelin J, Cogiamanian F, Cotelli M, Ridder D, Ferrucci R, Langguth B, Marangolo P, Mylius V, Nitsche MA, Padberg F, Palm U, Poulet E, Priori A, Rossi S, Schecklmann M, Vanneste S, Ziemann U, Garcia-Larrea L and Paulus W: Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). Clin Neurophysiol *128(1)*: 56-92, 2014. PMID: 27866120. DOI: 10.1016/j.clinph.2016.10.087

6 Martin DM, McClintock SM, Forster JJ, Lo TY and Loo CK: Cognitive enhancing effects of rTMS administered to the prefrontal cortex in patients with depression: A systematic review and meta-analysis of individual task effects. Depress Anxiety *34(11)*: 1029-1039, 2017. PMID: 28543994. DOI: 10.1002/da.22658

7 Blumberger DM, Vila-Rodriguez F, Thorpe KE, Feffer K, Noda Y, Giacobbe P, Knyahnytska Y, Kennedy SH, Lam RW, Daskalakis ZJ and Downar J: Effectiveness of theta burst versus high-frequency repetitive transcranial magnetic stimulation in patients with depression (THREE-D): a randomised non-inferiority trial. Lancet Lond Engl *391(10131)*: 1683-1692, 2018. PMID: 29726344. DOI: 10.1016/S0140-6736(18)30295-2

8 Kubis N: Non-invasive brain stimulation to enhance post-stroke recovery. Front Neural Circuits *10*: 56, 2016. PMID: 27512367. DOI: 10.3389/fncir.2016.00056

9 Marron EM, Viejo-Sobera R, Quintana M, Redolar-Ripoll D, Rodríguez D and Garolera M: Transcranial magnetic stimulation intervention in Alzheimer's disease: a research proposal for a randomized controlled trial. BMC Res Notes *11(1)*: 648, 2018. PMID: 30185210. DOI: 10.1186/s13104-018-3757-z

10 Rutherford G, Lithgow B and Moussavi Z: Short and long-term effects of rTMS treatment on Alzheimer's disease at different stages: A pilot study. J Exp Neurosci *9*: 43-51, 2015. PMID: 26064066. DOI: 10.4137/JEN.S24004

11 Galhardoni R, Correia GS, Araujo H, Yeng LT, Fernandes DT, Kaziyama HH, Marcolin MA, Bouhassira D, Teixeira MJ and Andrade DC: Repetitive transcranial magnetic stimulation in chronic pain: a review of the literature. Arch Phys Med Rehabil *96(4 Suppl)*: S156-172, 2015. PMID: 25437106. DOI: 10.1016/j.apmr.2014.11.010

12 Yılmaz B, Kesikburun S, Yaşar E and Tan AK: The effect of repetitive transcranial magnetic stimulation on refractory neuropathic pain in spinal cord injury. J Spinal Cord Med *37(4)*: 397-400, 2014. PMID: 24621025. DOI: 10.1179/2045772313Y.0000000172

13 Hallett M: Transcranial magnetic stimulation: A Primer. Neuron *55(2)*: 187-199, 2007. PMID: 17640522. DOI: 10.1016/j.neuron.2007.06.026

14 Ebrahimdamavandi S and Mobasheri H: Application of a static magnetic field as a complementary aid to healing in an *in vitro* wound model. J Wound Care *28(1)*: 40-52, 2019. PMID: 30625046. DOI: 10.12968/jowc.2019.28.1.40

15 Kim SC, Im W, Shim JY, Kim S-K and Kim BJ: Static magnetic field controls cell cycle in cultured human glioblastoma cells. Cytotechnology *68(6)*: 2745-2751, 2016. PMID: 27121019. DOI: 10.1007/s10616-016-9973-2

16 Jalali A, Zafari J, Jouni FJ, Abdolmaleki P, Shirazi FH and Khodayar MJ: Combination of static magnetic field and cisplatin in order to reduce drug resistance in cancer cell lines. Int J Radiat Biol *95(8)*: 1194-1201, 2019. PMID: 30822212. DOI: 10.1080/09553002.2019.1589012

17 Lin SL, Su YT, Feng S-W, Chang WJ, Fan KH and Huang HM: Enhancement of natural killer cell cytotoxicity by using static magnetic field to increase their viability. Electromagn Biol Med *38(2)*: 131142, 2019. PMID: 30889986. DOI: 10.1080/15368378.2019.1591439

18 Rosen AD: Mechanism of action of moderate-intensity static magnetic fields on biological systems. Cell Biochem Biophys *39(2)*: 163-173, 2003. PMID: 14515021. DOI: 10.1385/CBB:39:2:163

19 Oliviero A, Mordillo-Mateos L, Arias P, Panyavin I, Foffani G and Aguilar J: Transcranial static magnetic field stimulation of the human motor cortex. J Physiol *589(Pt 20)*: 4949-4958, 2011. PMID: 21807616. DOI: 10.1113/jphysiol.2011.211953

20 Buemi M, Marino D, Pasquale GD, Floccari F, Senatore M, Aloisi C, Grasso F, Mondio G, Perillo P, Frisina N and Corica F: Cell proliferation/cell death balance in renal cell cultures after exposure to a static magnetic field. Nephron *87(3)*: 269-273, 2001. PMID: 11287763. DOI: 10.1159/000045925

21 Kovalevich J and Langford D: Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology. Methods Mol Biol Clifton NJ *1078*: 9-21, 2013. PMID: 23975817. DOI: 10.1007/978-1-62703-640-5_2

22 Cheung YT, Lau WKW, Yu MS, Lai CSW, Yeung SC, So KF and Chang RCC: Effects of all-trans-retinoic acid on human SH-SY5Y neuroblastoma as *in vitro* model in neurotoxicity research. NeuroToxicology *30(1)*: 127-135, 2009. PMID: 19056420. DOI: 10.1016/j.neuro.2008.11.001

23 Klein RC, Goetz SM, Liedtke WB, Moore SD and Peterchev AV: Static magnetic field modulates excitatory activity in layer II/III pyramidal neurons of the rat motor cortex. Sixth International IEEE/EMBS Conference on Neural Engineering (NER), pp. 1190-1193, 2013.

24 Vergallo C, Ahmadi M, Mobasheri H and Dini L: Impact of inhomogeneous static magnetic field (31.7-232.0 mT) exposure on human neuroblastoma SH-SY5Y cells during cisplatin administration. PloS One *9(11)*: e113530, 2014. PMID: 25423171. DOI: 10.1371/journal.pone.0113530

25 Shokrollahi S, Ghanati F, Sajedi RH and Sharifi M: Possible role of iron containing proteins in physiological responses of soybean to static magnetic field. J Plant Physiol 226: 163-171, 2018. PMID: 29778670. DOI: 10.1016/j.jplph.2018.04.018

25 Lim WC, Kim H, Kim YJ, Park SH, Song JH, Lee KH, Lee YH, So KA, Choi KC and Ko H: Delphinidin inhibits BDNF-induced migration and invasion in SKOV3 ovarian cancer cells. Bioorg Med Chem Lett *27(23)*: 5337-5343, 2017. PMID: 29122484. DOI: 10.1016/j.bmcl.2017.09.024

27 Garrido MP, Torres I, Vega M and Romero C: Angiogenesis in gynecological cancers: Role of Neurotrophins. Front Oncol *9*: 913, 2019. PMID: 31608227. DOI: 10.3389/fonc.2019.00913

28 Nakagawara A, Azar CG, Scavarda NJ and Brodeur GM: Expression and function of TRK-B and BDNF in human neuroblastomas. Mol Cell Biol *14*: 759-767, 1994. PMID: 8264643. DOI: 10.1128/mcb.14.1.759

29 Silbert BI, Pevcic DD, Patterson HI, Windnagel KA and Thickbroom GW: Inverse correlation between resting motor threshold and corticomotor excitability after static magnetic stimulation of human motor cortex. Brain Stimul *6(5)*: 817-820, 2013. DOI: 10.1016/j.brs.2013.03.007

30 Kirimoto H, Asao A, Tamaki H and Onishi H: Non-invasive modulation of somatosensory evoked potentials by the application of static magnetic fields over the primary and supplementary motor cortices. Sci Rep *6*: 34509, 2016. DOI: 10.1038/srep34509

31 Gonzalez-Rosa JJ, Soto-Leon V, Real P, Carrasco-Lopez C, Foffani G, Strange BA and Oliviero A: Static magnetic field stimulation over the visual cortex increases alpha oscillations and slows visual search in humans. J Neurosci *35(24)*: 9182-9193, 2015. PMID: 26085640. DOI: 10.1523/JNEUROSCI.4232-14.2015

32 Aguila J, Cudeiro J and Rivadulla C: Effects of static magnetic fields on the visual cortex: reversible visual deficits and reduction of neuronal activity. Cereb Cortex *26(2)*: 628-638, 2016. PMID: 25260705. DOI: 10.1093/cercor/bhu228

33 Kirimoto H, Tamaki H, Matsumoto T, Sugawara K, Suzuki M, Oyama M and Onishi H: Effect of transcranial static magnetic field stimulation over the sensorimotor cortex on somatosensory evoked potentials in humans. Brain Stimul *7(6)*: 836-840, 2014. PMID: 25444588. DOI: 10.1016/j.brs.2014.09.016

34 Carrasco-Lopez C, Soto-Leon V, Cespedes V, Profice P, Strange BA, Foffani G and Oliviero A: Static magnetic field stimulation over parietal cortex enhances somatosensory detection in humans. J Neurosci *37(14)*: 3840-3847, 2017. PMID: 28280254. DOI: 10.1523/JNEUROSCI.2123-16.2017

35 Li G, Cheng L, Qiao X and Lin L: Characteristics of delayed rectifier potassium channels exposed to 3 mT static magnetic field. IEEE Trans Magn *46(7)*: 2635-2638, 2010. DOI: 10.1109/TMAG.2010.2045389

36 Giachello CNG, Scrutton NS, Jones AR and Baines RA: Magnetic fields modulate blue-light-dependent regulation of neuronal firing by cryptochrome. J Neurosci *36(42)*: 10742-10749, 2016. PMID: 27798129. DOI: 10.1523/JNEUROSCI.2140-16.2016

37 Shen JF, Chao YL and Du L: Effects of static magnetic fields on the voltage-gated potassium channel currents in trigeminal root ganglion neurons. Neurosci Lett *415(2)*: 164-168, 2007. PMID: 17289262. DOI: 10.1016/j.neulet.2007.01.015

38 Påhlman S, Hoehner JC, Nånberg E, Hedborg F, Fagerström S, Gestblom C, Johansson I, Larsson U, Lavenius E, Ortoft E and Söderholm H: Differentiation and survival influences of growth factors in human neuroblastoma. Eur J Cancer *31A(4)*: 453-458, 1995. DOI: 10.1016/0959-8049(95)00033-F

39 Gimenez-Cassina A, Lim F and Diaz-Nido J: Differentiation of a human neuroblastoma into neuron-like cells increases their susceptibility to transduction by herpesviral vectors. J Neurosci Res *84(4)*: 755-767, 2006. PMID: 16802347. DOI: 10.1002/jnr.20976

40 Balcavage WX, Alvager T, Swez J, Goff CW, Fox MT, Abdullyava S and King MW: A mechanism for action of extremely low frequency electromagnetic fields on biological systems. Biochem Biophys Res Commun *222*: 374-8, 1996. PMID: 8670212. DOI: 10.1006/bbrc.1996.0751

41 St Pierre TG and Dobson J: Theoretical evaluation of cell membrane ion channel activation by applied magnetic fields. Eur Biophys J *29*: 455-456, 2000. PMID: 11081406. DOI: 10.1007/s002490000090

42 Gray JR, Frith CH and Parker JD: In vivo enhancement of chemotherapy with static electric or magnetic fields. Bioelectromagnetics *21*: 575-583, 2000. DOI: 10.1002/1521-186X(200012)21:83.0.CO;2-F

43 Tofani S, Barone D, Berardelli M, Berno E, Cintorino M, LFoglia L, Ossola P, Ronchetto F, Toso E and Eandid M: Static and ELF magnetic fields enhance the in vivo anti-tumor efficacy of cis-platin against lewis lung carcinoma, but not of cyclophosphamide against B16 melanotic melanoma. Pharmacol Res *48*: 83-90, 2003. PMID: 12770519. DOI: 10.1016/S1043-6618(03)00062-8

44 Chen WF, Qi H, Sun RG, Liu Y, Zhang K and Liu J-Q: Static magnetic fields enhanced the potency of cisplatin on K562 cells. Cancer Biother Radiopharm *25*: 401-408, 2010. PMID: 20707721. DOI: 10.1089/cbr.2009.0743

45 Hao Q, Wenfang C, Xia A, Qiang W, Ying L, Kun Z and Runguang S: Effects of a moderate-intensity static magnetic field and adriamycin on K562 cells. Bioelectromagnetics *32*: 191-199, 2011. PMID: 21365663. DOI: 10.1002/bem.20625

46 Liu Y, Qi H, Sun RG and Chen WF: An investigation into the combined effect of static magnetic fields and different anticancer drugs on K562 cell membranes. Tumori *97*: 386-392, 2011. PMID: 21789021. DOI: 10.1700/912.10039

47 Sun RG, Chen WF, Qi H, Zhang K, Bu T, Liu Y and Wang S-R: Biologic effects of SMF and paclitaxel on K562 human leukemia cells. Gen Physiol Biophys *31*: 1-10, 2012. PMID: 22447825. DOI: 10.4149/gpb_2012_002

48 Vergallo C, Dini L, Szamosvölgyi Z, Tenuzzo BA, Carata E, Panzarini E and László JF: In vitro analysis of the anti-inflammatory effect of inhomogeneous static magnetic field-exposure on human macrophages and lymphocytes. PLoS One *8(8)*: e72374, 2013. PMID: 23991101. DOI: 10.1371/journal.pone.0072374

Received June 30, 2020
Revised July 17, 2020
Accepted July 20, 2020