



Jong Yoon Chang, M.D. ¹, Mi Hee Kim², Ji Ae Kim, M.D. ¹,
Cho Rong Bae, M.D. ³, Eun Jae Ko M.D., Ph.D. ¹, Dae Yul Kim, M.D., Ph.D. ¹ (dykimsmart@gmail.com);
¹Department of Rehabilitation Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul
²Asan Institute for Life Sciences, Asan Medical Center, University of Ulsan College of Medicine, Seoul
³Department of Physical Medicine and Rehabilitation, Korea University Anam Hospital, Korea University College of Medicine, Seoul, Korea

Introduction

- **Transcranial direct current stimulation (tDCS)** has a variety of clinical applications and offers the advantages of safety, portability, and painlessness.
- Further basic research is necessary to maximize the effectiveness of tDCS and to utilize it as a treatment method.

Purpose

- Examine the neuroprotective effects of electrical stimulation in vitro
- Determine whether applying electrical stimulation to brain cells promotes cell viability and how it impacts the expression of **brain-derived neurotrophic factor (BDNF)**, **B-cell lymphoma-2 (bcl-2)**, **caspase-3**, and **relaxin-3**
- Ultimate objective is to facilitate future in vivo studies.

Methods

Design

- SH-SY5Y cells derived from the human neuroblastoma cell line
- Cells were categorized into four groups: control, control with electrical stimulation, oxygen and glucose deprivation/reperfusion (OGD/R), and OGD/R with electrical stimulation.

Electrical stimulation

- Intensity of 100 mV/mm
- One hour per day

Assessments

- Cell viability and protein expression were assessed on the 1st, 4th, and 7th days
- Cell viability : Water-soluble tetrazolium salt-1 (WST-1) assay
- Protein expression : Western blot and immunocytochemistry (ICC)

Results

- The **WST-1 assay** results indicated that application of electrical stimulation to SH-SY5Y cells contributes to **enhanced cell viability** (Figure 1).

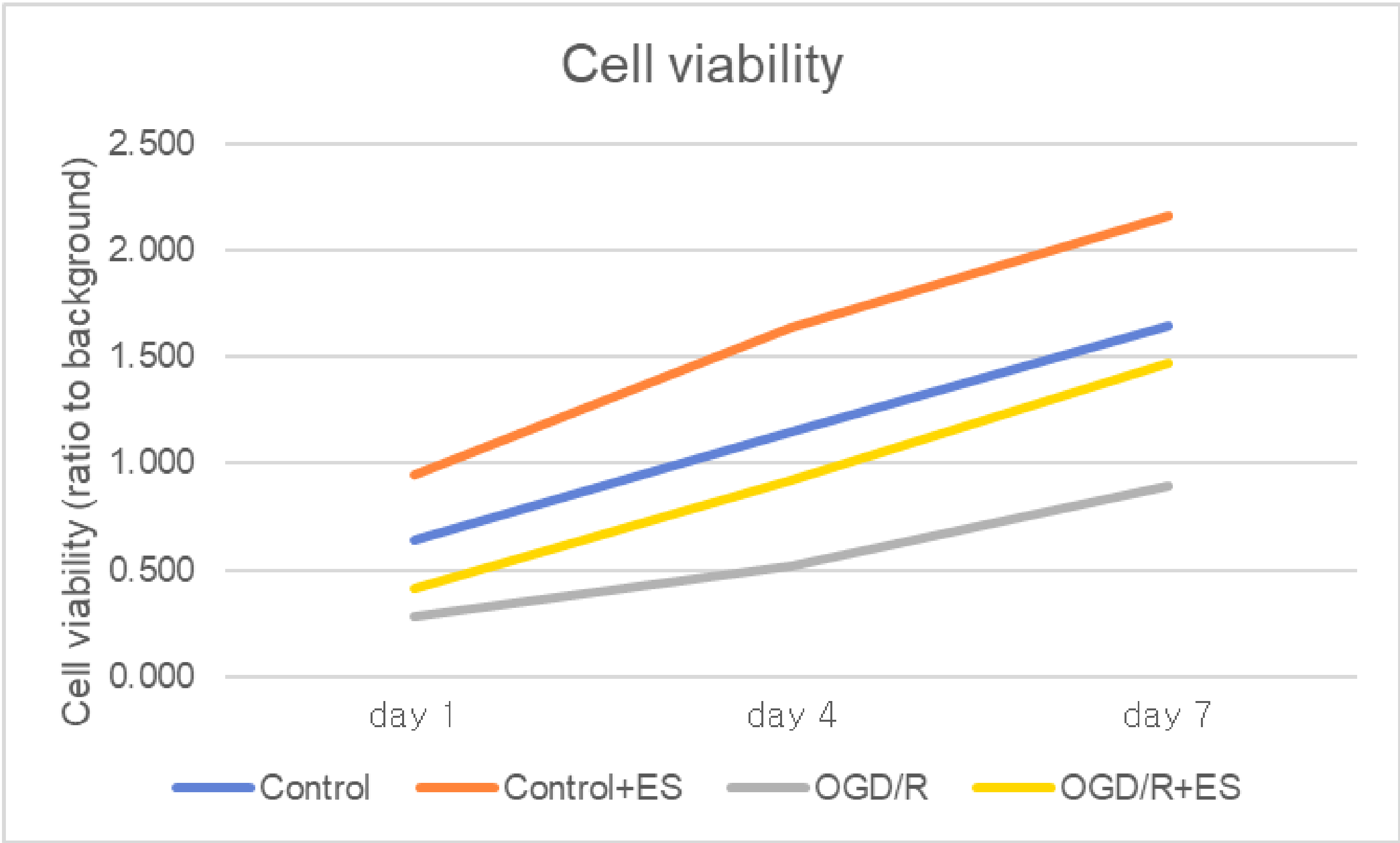


Figure 1. Cell viability assessed by water-soluble tetrazolium salt-1 (WST-1) assay.

- **Western blot** and **ICC** results demonstrated that the expression of BDNF, known for its neuroprotective effects, increased after electrical stimulation in the OGD/R group.
- Additionally, in the OGD/R group, both the anti-apoptotic marker bcl-2 and relaxin-3, a neuropeptide known for modulating essential functions, showed increased expression.
- However, caspase-3, an apoptosis-related marker, decreased following electrical stimulation in both the control and OGD/R groups (Figure 2, 3).

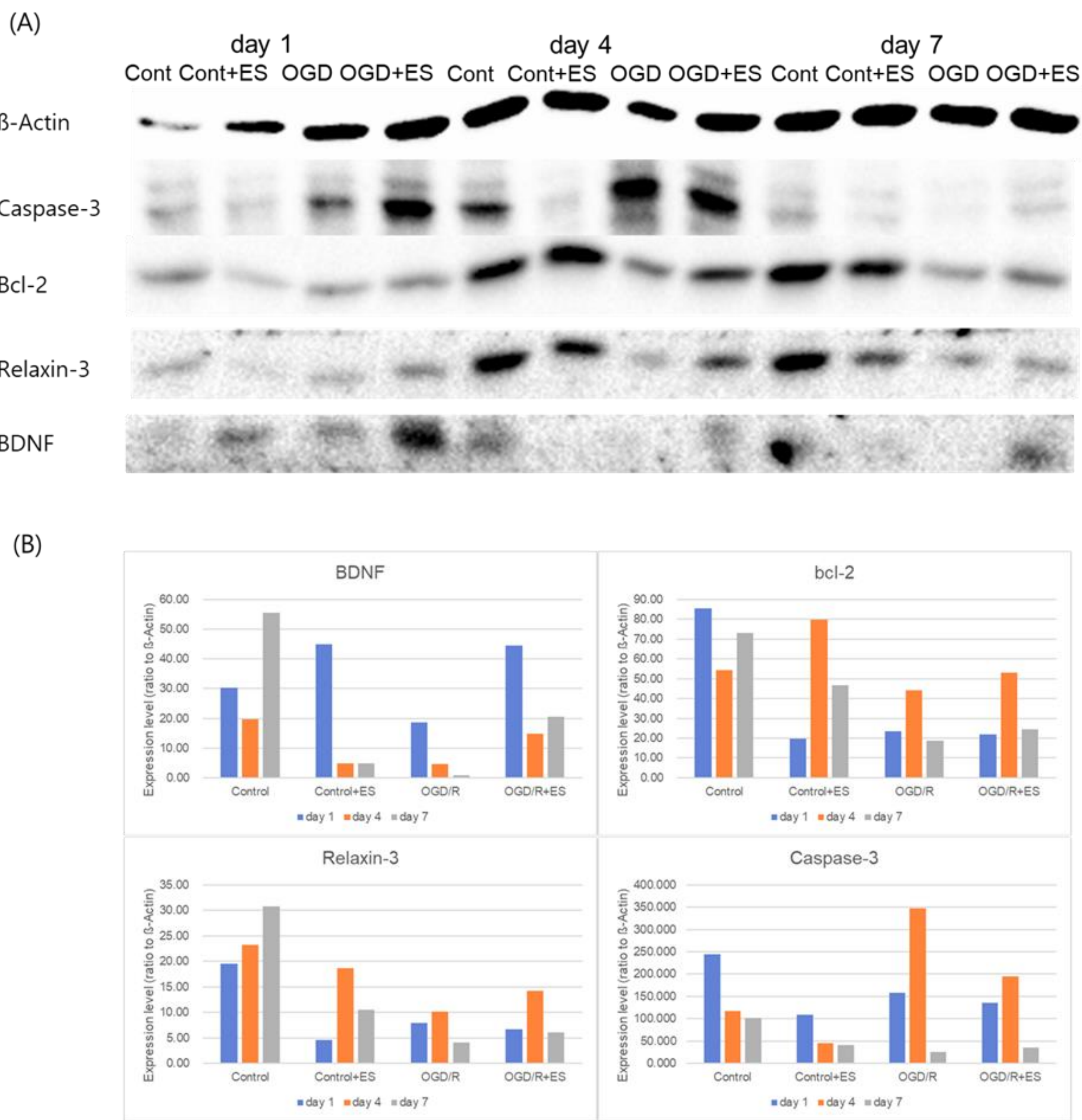


Figure 2. Western blot analysis of BDNF, bcl-2, relaxin-3, caspase-3 expression under control, control with electrical stimulation, OGD/R, OGD/R with electrical stimulation. (A) Representative bands of each group in the Western blot; (B) the expression level of BDNF, bcl-2, relaxin-3, caspase-3 expression in each group.

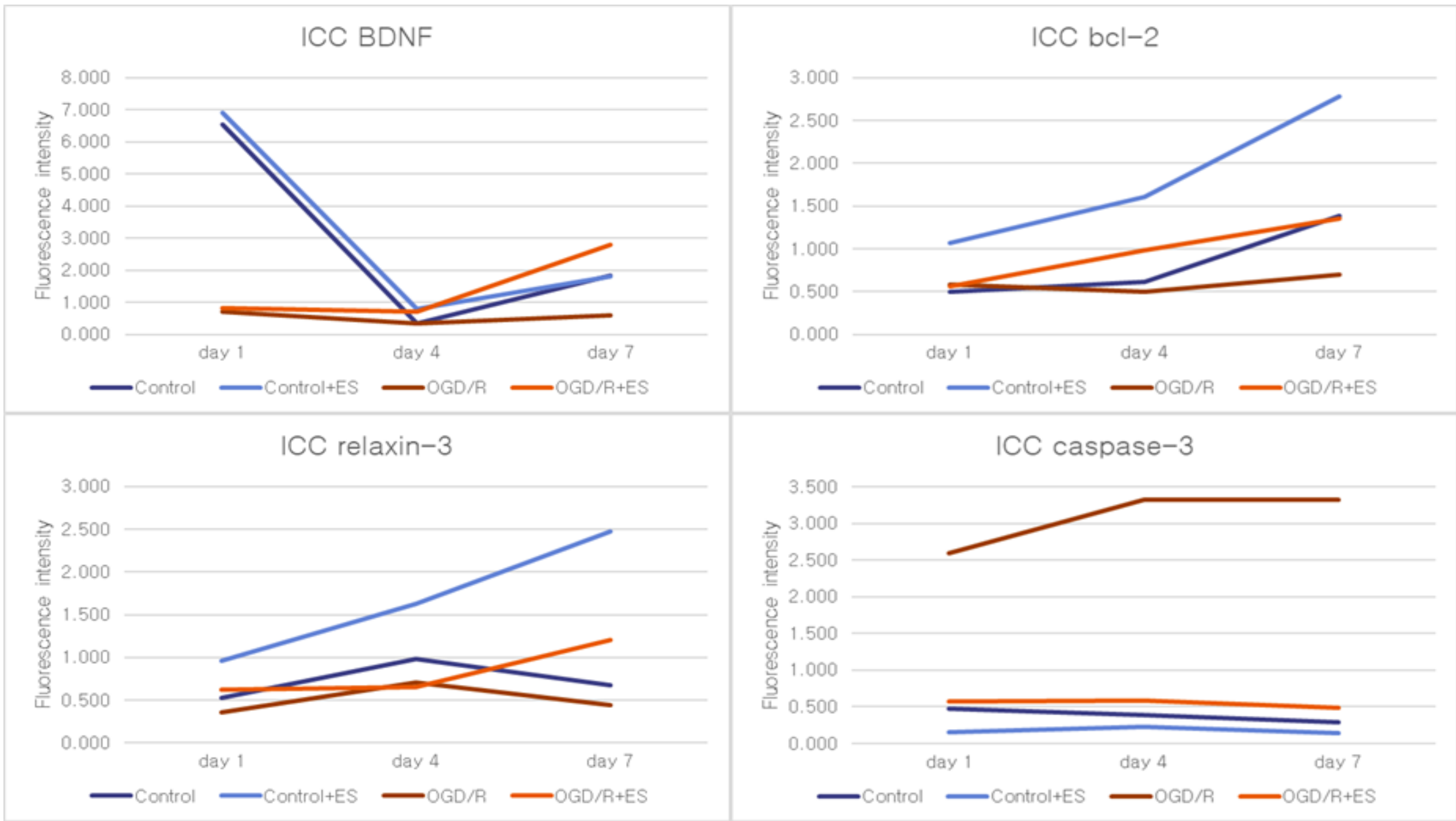


Figure 3. Immunocytochemistry findings of BDNF, bcl-2, Relaxin-3, caspase-3 expression levels under the conditions of control, control with electrical stimulation, OGD/R, OGD/R with electrical stimulation.

Conclusion

This study suggests that electrical stimulation applied to neuroblastoma cells exhibits neuroprotective effects, as demonstrated by enhanced cell viability and altered expression of multiple biomarkers. These findings could serve as a basis for forthcoming in vivo studies.

Acknowledgement

This study was supported by a grant (2022IE0010) from the Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea and the 2023 Research Grant from the Korean Society for Neurorehabilitation.