ISSN (Print) 1226-1750
ISSN (Online) 2233-6656
Journal of Magnetics 23(3), 465-472 (2018) https://doi.org/10.4283/JMAG.2018.23.3.465

Processor Application for the Magnetic Stimulation in the Cerebellum of Rat with Ischemia and its Effect on the c-Fos

Jun-Il Kim[1], Jun-Hyeung Kim[1], and Whi-Young Kim[2]*

[1]Department of Free Nursing, Pierce College Fort Steilacom 9401 Farwest Drive SW Lakewood, Washington 98498, USA
[2]Department of Biomedical Engineering, Dongju College University, Pierce College (Research of Year), Fort Steilacom 9401 Farwest Drive SW Lakewood, Washington 98498, USA

(Received 29 May 2018, Received in final form 4 September 2018, Accepted 5 September 2018)

Instead of relying on traditional medical electrotherapy, we seek to determine a more positive approach to early ischemic brain injury by researching the effect of applying a magnetic stimulation device in an SD mouse's brain to stop apoptosis, where a 64-Bit-EISC Processor Core delivers transcranial magnetic stimulation (TMS). We determined the change of the post-ischemic stimulatory effects on the Bax, caspase-3, and immune-reactive perikarya over time by stimulating the mouse's brain. c-Fos and Cox-2 were used to find a crucial determining factor regarding inflammation-related cytotoxicity. The cerebral ischemia caused a biochemical change in the brain tissue and increased the neuronal genes within a few minutes. The genes showed that these very fast reactions involve an early gene. Next, we found an approach that is more favorable than electrotherapy for the apoptosis that is caused by early ischemic brain injury by researching the c-Fos protein that changes large-brain neurons; this was achieved after we stimulated the ischemic mouse brain using a two-tank LLC resonant converter as part of the TMS experimental equipment.

Keywords : EISC, transcranial magnetic stimulation, c-Fos, trasient global ischemia

1. Introduction

Moderate-impact neuronal apoptosis can occur repeatedly without causing brain-tissue damage. Necrosis occurs in the directly impacted areas due to secondary causes such as an increased intracranial pressure, hypoxia, and ischemia. In the extrinsic pathway, apoptosis occurs with the activation of the Fas or the tumor necrosis factor (TNF) that is due to the activation of the membrane receptor, caspase-8, or caspase-3 [1-3].

The action mechanism of neuronal apoptosis can be explained using the relationships between the caspases, Bax, and Bcl-2. Bax is used as an index whether neuronal damage occurs or not, while the effector-caspases 3, 6, and 7 of group 2 play crucial roles in cytosol. c-Fos, c-Jun, and IEGs are caused by various factors in the central nervous system [4, 5]. Cytokines such as TNF-α and interleukin interact as a sleep regulator in the hypothalamus, and Cox-2 is known as a transcriptional regulatory factor [6, 7]. Inflammatory cytokines such as the growth factor, TNF, bacterial-cell by-product, IL-1β, TNF-α, and IL-6 are typically the causative substances of Cox-2. IL-6 is produced by the astrocyte and microglia in the brain, and it plays important roles in the production of collagen and blood vessels, the activation of the endothelial function, the proliferation of astrocytes, and the recovery from cerebral edema [8, 9].

An over-activated PARP pathway causes cell necrosis by increasing the poly(ADP-ribose) and amide, decreasing the cell nicotin-amide adenine dinucleotide (NAD^+)/adenosine triphosphate (ATP), and causing a cell-energy deficiency [10]. In this research, a more beneficial approach to early ischemic brain injury is sought out by researching the effect of the application of a magnetic-stimulation device in the brains of Sprague Dawley (SD) mice to stop apoptosis, instead of traditional electrotherapy, where the changes of the c-Fos protein in large-brain neurons are analyzed. Figure 1 Diagram of the proposed transcranial magnetic-stimulation device.

*Corresponding author: Tel: +82-51-200-3449
Fax: +82-51-200-3449, e-mail: neurondyag@gmail.com

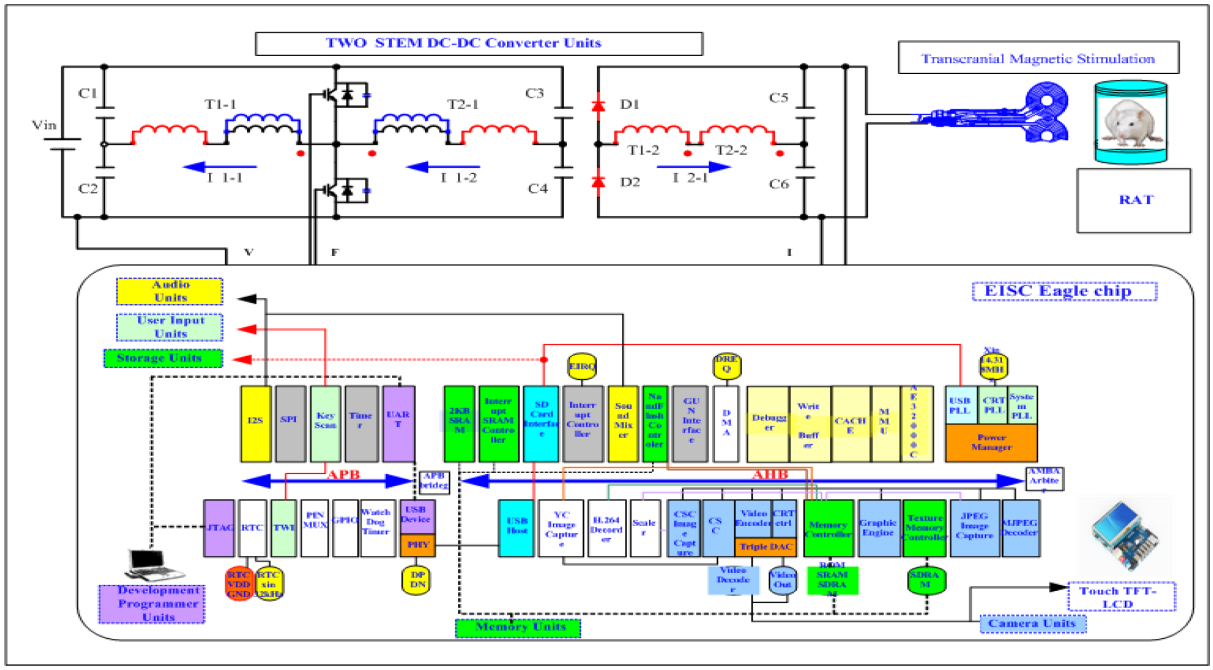

Fig. 1. (Color online) Diagram of the proposed transcranial magnetic-stimulation device.

2. Methods

2.1. AE32000C Processor Core

In the microcontroller, the EISC (Extension Instruction Set Computer) was used to control the transcranial magnetic stimulation (TMS) and a two-tank LLC resonant converter. A Bluetooth communication-module chip was used for the serial peripheral interface (SPI) interface to connect with the microcontroller unit (MCU). To verify the result of the analog-to-digital-converter (ADC) transformation, the transformed heart-rate (HR) data were sent to the computer via the RS-232 port. To verify the communication function, the RSC-232 port or the Bluetooth chip was used to send the data to the computer for a comparison of the data.

The EISC-architecture-based embedded system includes the Joint Test Action Group (JTAG) interface, and the interface was used for the debugging application. The GNU debugger (GDB) program translated the user command into a packet form and sent it to the JTAG module via the USB interface. The target board that was booted as the debugging mode waited for the command of the JTAG module, and the command was performed when it had been delivered. In the GDB, the incorporated USB interface was used with the USB device, and it explains the JTAG test-data-in (TDI)/test-data-out (TDO) packet of the EISC processor. The TDI packet of the JTAG module, following the GDB command, was subsequently formed. To use the USB–JTAG interface, the GDB loads the USB-device library. The USB-device library was implemented with the dynamic-link library (DLL) and includes the USB input/output (I/O) functions [5-7].

The GDB and the EISC processor communicate via the USB, and the GDB packet used the incoming and outgoing data with the read/write functions. If the EISC processor is connected to the USB interface, the usb remote open functions are operated [8-10]. The EISC processor can read or write up to 10 connected register values through the JTAG. For the present paper, the USB interfacing was provided, and the packet JTAG that is used in the GDB was analyzed.

2.2. Experimental animals

Six male SD mice of an approximate weight of 300 g and an age of 8 weeks were adjusted to the laboratory environment for more than one week and specific pathogen free (SPF) experimental animals were used. A moderate temperature, humidity, air conditioning, food, and water were provided; the laboratory temperature was fixed as 20.2 °C, and the humidity was maintained between 45 % and 50 %. Figure 2 64 Bit EISC Processor Core microprocessor to control and operate the energy storing condenser recharging method that employs a two-stem type converter.

2.3. Ischemia-causing model and magnetic-stimulation device

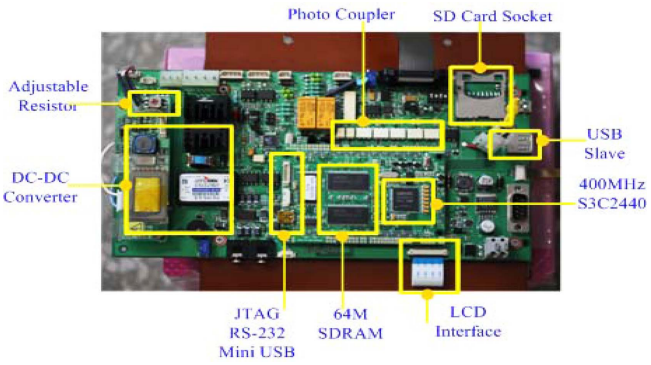

Fig. 2. (Color online) 64-Bit-EISC Processor-Core microprocessor to control and operate the energy storing condenser recharging method that employs a two-stem type converter.

The experimental animals were placed under anesthesia with 3 % isoflurane1, and a 2-cm incision was made at both of the side common carotid arteries while the vagus nerve was isolated. The common carotid arteries were exposed, and a suture was maintained using a non-absorbable suture for 5 min. Next, the suture was removed to facilitate a blood reperfusion [12].

The mice were divided into three groups. The closure of the common carotid arteries was not implemented for the control group, and the experimental groups are the global ischemia (GI) group for which the common-carotid arteries were closed and the TMS group that received the post-GI magnetic stimulation. The research was conducted with two mice in each group. After 12, 24, and 48 hr, the GI group recovered from the anesthesia with the 3 % iso-fluranne, and the magnetic stimulation was applied to the left and right acupuncture points of the TMS group with the 3 % iso-flurane anesthetic. The magnetic stimulation was performed using 10 Hz, and the intensity was controlled during the muscle movement around the stimulated points [13].

2.4. Power Converter

The two-tank LLC resonant converter was used to compensate for the defect of the traditional single transformer that is typically applied to the LLC resonant converter. To form a slim converter, the two transformers each comprise a resonant circuit, and the input current is delivered regardless of the on/off of the main switching element (Q1). The reductions of the capacity and volume of the input capacitor that are due to the reductions of the maximum input current and the ripple current are advantageous here. The converter is also suitable for integration and a high power density due to the minimization of the heat that is due to the dimidiated effective current on the transformer.

Regarding Loop 1 in the point of t0, if the main switching element is inactive, the output capacitance of the switching element Q2 is charged with a voltage through the magnetizing of the current IM1, the IM2 flow on the magnetizing inductance Lm1, and the Lm2. The current flows in the antiparallel diode of Q1 after the main switching element of the Q1 output capacitor is discharged as a 0 voltage. The voltage polarity of the transformer 1,2

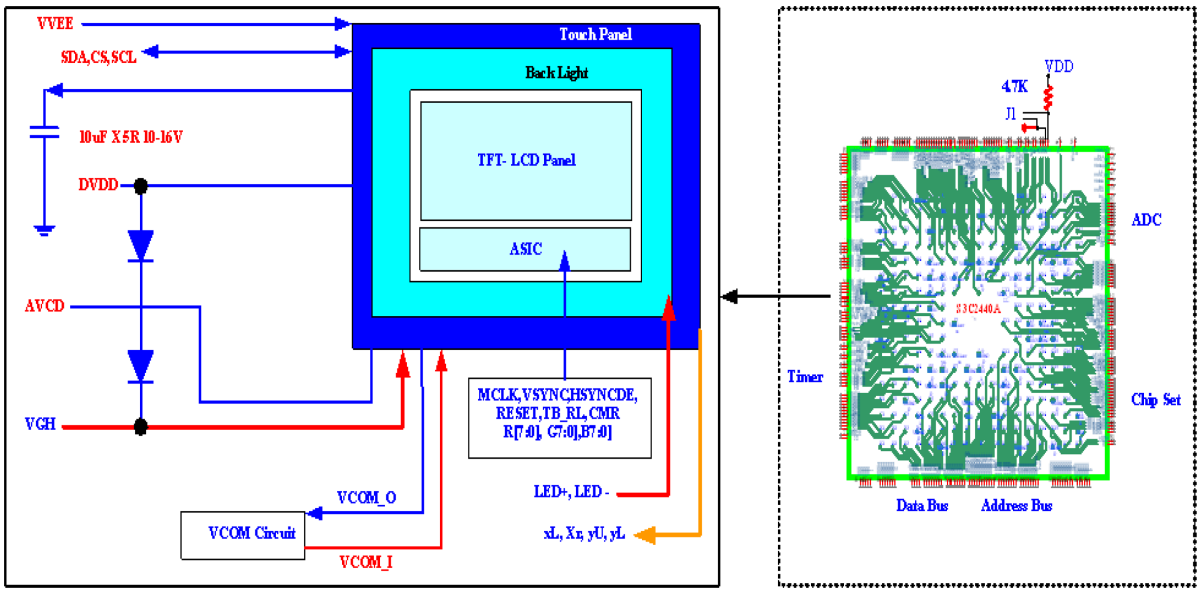

Fig. 3. (Color online) Program that generates a display signal by the 64-Bit-EISC Processor-Core receiving the keyboard input from the control circuit. The EISC Processor-Core then generates a timing signal from this, which is displayed on the 64-Bit- Processor-Core.

is simultaneously changed and the resonant current begins to flow.

For Loop 2 in mode 2, as a resonant-mode section, the main switching element is active, and the two separated resonant currents are flowing. The resonant circuit comprises its own resonant-tank circuit by flowing the resonant current that consists of the discharge-current and charge-current flows on the resonant capacitor, leakage inductance, and magnetizing inductance when the main switching element Q1 is activated.

For Loop 3 in mode 3, under the condition of the activated switching element Q1, the resonant current does not flow to the load, and only the exiting current flows through the magnetizing inductance of the transformers 1 and 2 in this section.

For Loop 4 in mode 4, at the beginning point of the negative half-cycle, the output capacitor of the main switching element Q1 is charged with the input voltage within a brief time frame when Q1 is deactivated. The main switching element Q2 becomes the zero-voltage switching (ZVS) when the output capacitor of the main switching element Q2 is discharged as a 0 voltage, the current flows through the antiparallel diode of the switching element Q2, and the main switching element Q2 is activated. Figure 3 Program that generates a display signal by the 64-Bit-EISC Processor-Core receiving the keyboard input from the control circuit. The EISC Processor-Core then generates a timing signal from this, which is displayed on the 64-Bit- Processor-Core. Figure 4 Induction of global ischemia through common carotid artery occlusion after induction of anesthesia.

2.5. Immunohistochemistry

The immunohistochemistry was operated using the forebrain of the closed common carotid arteries of a GI-model mouse. The fixed brain was frozen sectioned in 4 % paraformaldehyde using the free-floating method for which the Vecta-stain ABC Ki3 was used. Cut tissue was placed under room temperature for 50 min with 3 % of normal goat serum after a 5-min flushing was applied 5 times with a 0.1 M PBS and a blocking was applied with 1 % H_2O_2 (peroxidase blocking, 0.1 M PBS).

The anti-c-Fos was used as the primary antibody after the reaction from 5- and 10-time flushings with the 0.1 M PBS for 5 min each time that were performed over 5 days. The secondary antibody was reacted for 3 days using the 5-time flushing process; it was then flushed 3 times for 5 min each time with the 0.1 M PBS. The avidin-biotin-peroxidase (ABP) complex reacted at room temperature for 3 hr, and it was flushed 3 times for 5 min. After the DAB4 antibody was developed, the tissue was dried with a 3 % gelatin solution for 3 hr in a dryer, and it was placed into Permount-5 solution after a drying and transparency process.

2.6. Western blotting

A centrifugation only obtained the supernatant. For the amount of protein, a protein assay kit (Bio-Rad, USA) was used and the absorbance was determined at 850 nm. Lysis and sample buffers (60 mm tris, pH 6.8, 10 % glycerol, 2 % sodium dodecyl sulfate, .01 % bromophenol blue) were added onto the quantitative protein and were boiled in a 100 °C heat block for 5 min, and the centrifu-

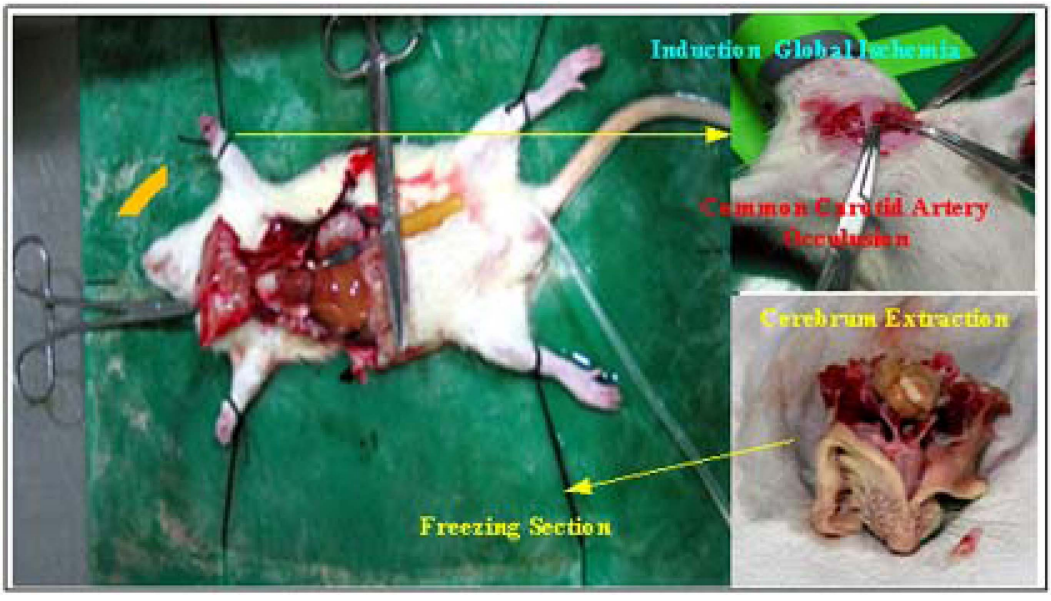

Fig. 4. (Color online) Induction of global ischemia through common carotid artery occlusion after induction of anesthesia.

gation was performed using the same amount of the mixed protein with each buffer [6, 13].

For the stacking gel, 30 % polyacry-lami-demix, one third of distilled water, 1 M tris (pH 6.8), 10 % sodium dodecyl sulfate (SDS), a 10 % ammonium persulfate that was made on the day, and tetra-methyl-ethylene-diamine (TEMED) were mixed and poured on a separating gel to form a complete gel. The electrophoresis running buffer was made with 30 g of a melted tris base, 144 g of glycine, and 10 g of the SDS on 2 ℓ to make a 10× stock. The quantitative protein was stimulated with 10-30 μl and 220 V for 2 hr. The electrophoresis gel was transferred onto a nitrocellulose membrane using 220 V for 2 hr.

The transfer buffer was made with 3.03 g of a tris base, 14.63 g of glycine, and 200 ml of methanol with 1 ℓ, and it was used after it was stored in 4 ℃. After it was washed with a tri-buffered saline solution (pH 7.5) consisting of a 200 mm tris base, 1.54 M sodium chloride (NaCl), one third of distilled water, and Tween 20, the transferred thin membrane was stored in 5 % skim milk on 5 ℃ for

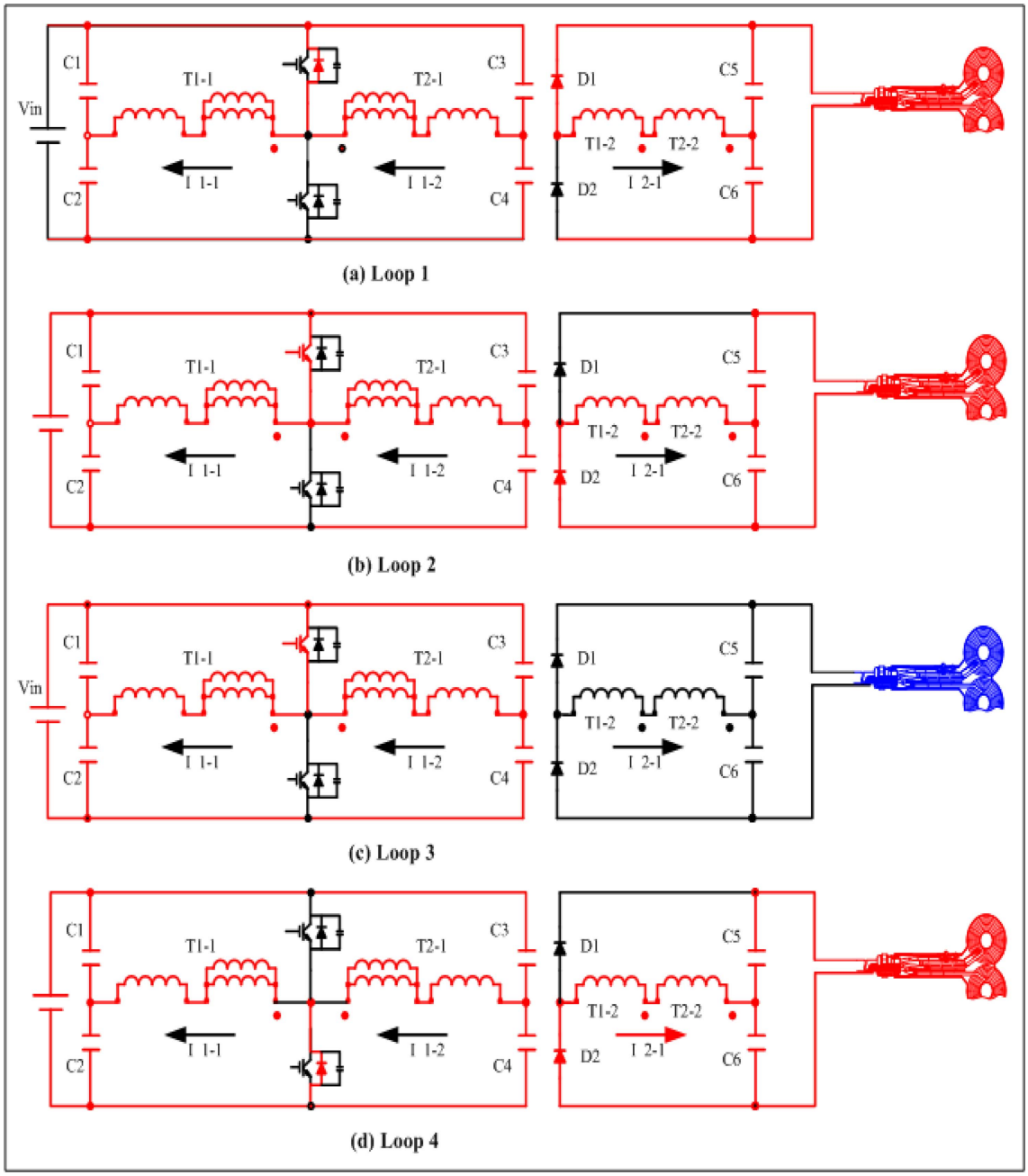

Fig. 5. (Color online) Induction of anesthesia and fixation of experiment animal using 2 % isoflurane with two-tank LLC resonant converter.

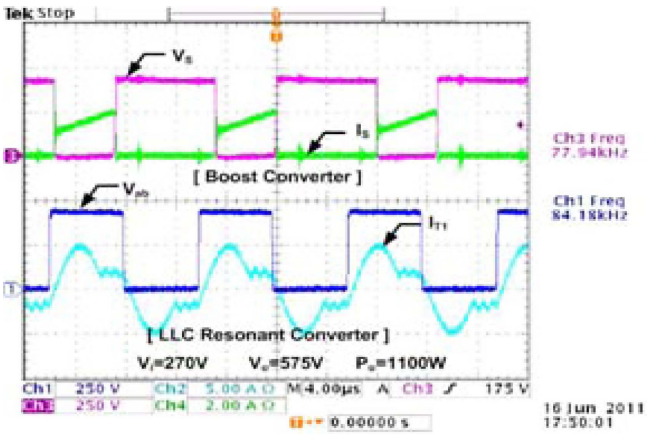

Fig. 6. (Color online) Waveform of the Boost converter and operating waveform of the LLC Resoant Converter (Input voltage = 270 V).

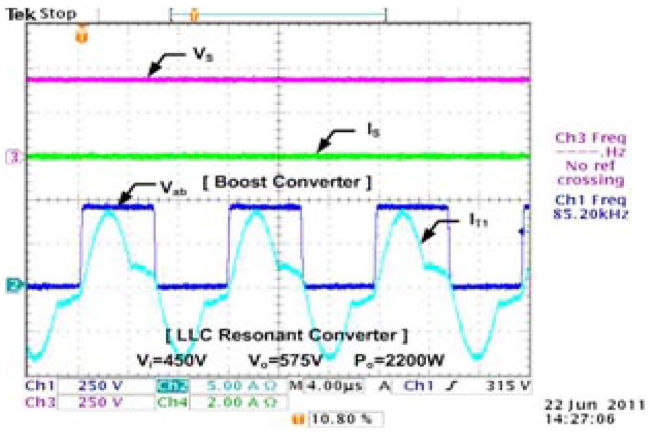

Fig. 7. (Color online) Bioelectro Magnetics Experimental Waveform of the Boost converter and operating waveform of the LLC Resoant Converter (Input voltage = 450 V).

one day.

After the removal of the blocking solution, the thin membrane of the first antibody was reacted with a 1000-times-diluted 5 % skim-milk solution for 2 hr, and it was then washed 5 times with a TBS solution for 20 min each time. The thin membrane of the second antibody reacted with a 100-times-diluted 5 % skim-milk solution for 1 hr, and it was then washed 5 times with the TBS solution for 10 min each time Fig. 5 Induction of anesthesia and fixation of experiment animal using 2 % isoflurane with two-tank LLC resonant converter.

Figure 6 Waveform of the Boost converter and operating waveform of the LLC Resoant Converter (Input voltage = 270 V). Figure 7 Experimental Waveform of the Boost converter and operating waveform of the LLC Resoant Converter (Input voltage = 450 V). Figure 8 After Stimu-

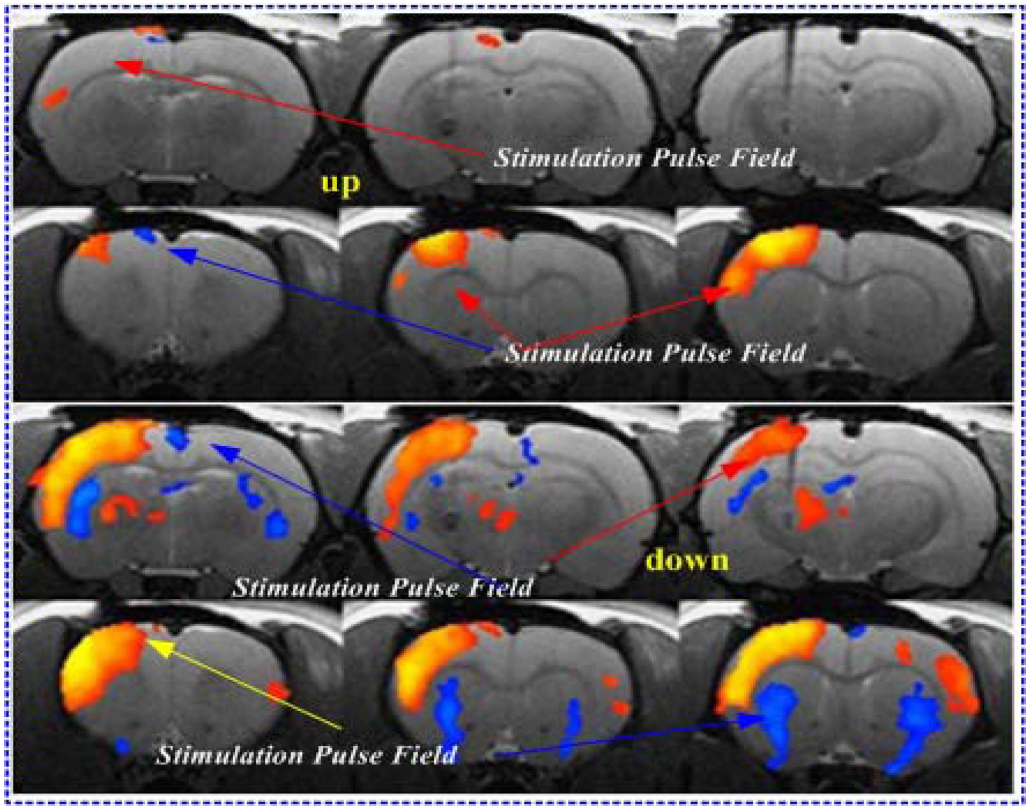

Fig. 8. (Color online) After Stimulating pulse from fMRI-FDG PET-RAT.

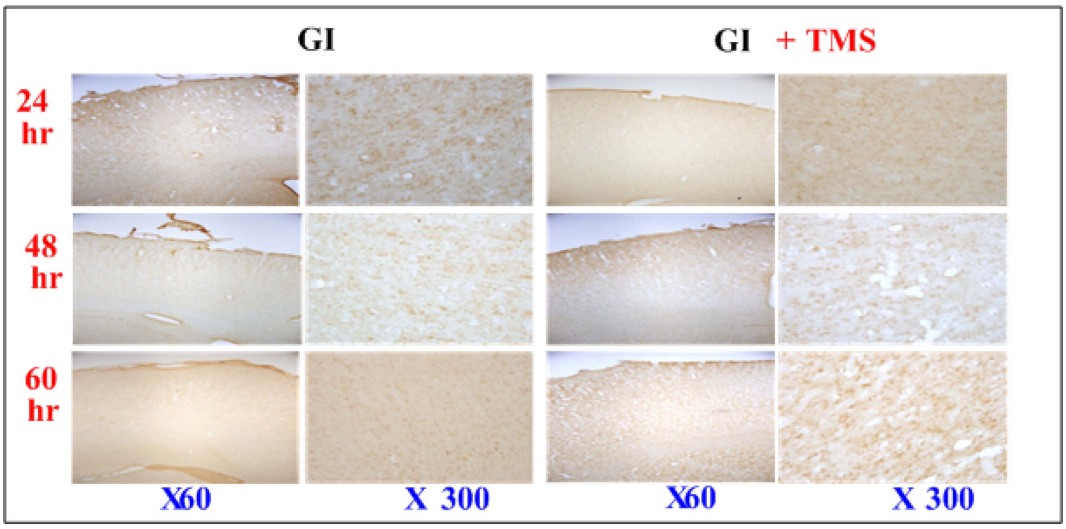

Fig. 9. (Color online) c-Fos reaction in the Cerebellum.

lating pulse from fMRI-FDG PET-RAT. Figure 9 C-Fos reaction in the Cerebellum.

3. Results

3.1. The number of c-Fos-reacted cell changes in the large brain

After the ischemia was induced on the mouse, the numbers of c-Fos-reacted cells on the large brain after 12 hr are 35.00 ± 7.00 in the control group, 109.33 ± 15.31 in the GI group, and 96.00 ± 12.77 in the TMS group, which showed an increase compared with the control group.

At 24 hr after the ischemia inducement, the number of the c-Fos-reacted cells of the control group is 36.33?, and it is 140.33? for the GI group. In the TMS group, the number of c-Fos-reacted cells, 102.33? ($p < .05$), shows a significant reduction compared with the GI group. At 48 hr after the ischemia inducement, the number of the c-Fos-reacted cells of the GI group is 48.67 ± 8.50, and it is 59.33 for the TMS group. Both groups showed a reduction compared with 24 hr after the inducement, but a significant change is not evident. Figure 9 After Stimulating pulse from fMRI-FDG PET-RAT. Figure 10 C-Fos reaction in the Cerebellum.

3.2. c-Fos protein expression change of the large brain

In the large mouse brain at 12 hr after the ischemia inducement, the c-Fos in the control group is 111.02, while it is 197.99 in the GI group and 184.78 in the TMS group; in both groups, the number of c-Fos reacted cells increased compared with the control group. At 24 hr, the c-Fos numbers are 113.67 for the control group, 200.59 for the GI group, and 178.26 for the TMS group, which is significantly reduced compared with the GI group.

4. Discussion

Magnetic stimulation is used to increase the functional recovery from various diseases including cerebral infarction (stroke), and one of the strongest effects of magnetic-stimulation therapy is an early recovery from stroke complications. In the present study, for ischemia-induced apoptosis, the number of c-Fos reacted cells increased in the forebrain in the results of the immunohistochemistry and Western-blot analyses. This finding is the same as a previous research result for which the renal cortex was studied.

As a result of the immunohistochemistry analysis that was conducted after the magnetic stimulation, at 12 hr after the ischemia was induced, the numbers of c-Fos reacted cells in the GI and TMS groups were increased compared with the control group, and the GI-group number is greater than that of the TMS group. At 24 hr after the ischemia inducement, the numbers of c-Fos reacted cells in the GI and TMS groups were increased compared with the control group, and the TMS group (102.33 ± 1.53) is significantly decreased compared with the GI group (140.33 ± 15.95). At 48 hr after the ischemia inducement, the numbers of the c-Fos reacted cells in the GI and TMS groups are decreased compared with those at 24 hr, but significant changes are not evident. At 24 hr, the presented c-Fos is 200.59 for the GI group and 178.263 for the TMS group, which is a significant reduction compared with the GI group. The numbers of the c-Fos reacted cells in the GI and TMS groups at 24 hr after the ischemia inducement are higher than those at 12 hr, and it

seems that this is a result of the inflammation caused by the peripheral cells.

The result of the Western-blot analysis shows that at 12 hr after the ischemia inducement, the presented c-Fos numbers of the GI and TMS groups are increased compared with the control group, and the GI group shows a greater increase than the TMS group. At 24 hr after the ischemia inducement, the number of c-Fos in the GI group is 200.59, while the TMS-group number is 178.26, which is a significant reduction compared with the GI group. The TMS-group number decreased more than that of the GI group at 48 hr after the ischemia inducement, but a significant change is not evident.

In both the immunohistochemistry and Western-blot analyses, the application of the TMS caused significant changes of the c-Fos numbers of the GI large brain at 24 hr after the ischemia inducement. At 12 hr after the ischemia was induced, the TMS group showed a lesser increase than the GI group, but significant changes are not evident.

According to the research results of this section, magnetic stimulation can delay the presentation of c-Fos and inhibits apoptosis. Caution and systematic research are required in the application of these animal-experiment research results in the treatment of a brain-vessel-damaged patient.

5. Conclusion

Stresses such as those of the synapse, depolarization, and a calcium influx can lead to biochemical intracellular changes, and the immediate early genes (IEGs) quickly adjust to such biochemical changes. The stroke-causing ischemia-induced inflammation and the neuronal apoptosis exert many effects on a functional system.

This research was conducted to study the presentation of c-Fos in a large brain after the application of low-frequency magnetic stimulation on the ST 36 of an ischemia-induced mouse. To induce the brain ischemia, the common carotid arteries of a male SD mouse were sutured with a non-absorbable suture and it was maintained for 5 min. Then, the suture was removed to allow for a blood reperfusion. The magnetic stimulation was conducted on the left and right sides of the ST 36, 12, and 24 acupuncture points 48 hr after the ischemia inducement. The immune-cyte that reacted with the c-Fos antibody in the neurons and the Western-blot analysis were used to research the presentations of these proteins.

(1) In the immunohistochemistry result 24 hr after the ischemia inducement, the number of the c-Fos reacted cells in the TMS group is significantly decreased compared with the GI group for which the low-frequency magnetic stimulation was not applied.

(2) In the result of the Western-blot analysis 24 hr after the ischemia inducement, the presentation of the c-Fos in the GI group is significantly decreased compared with the TMS group.

Therefore, the role of magnetic stimulation in the early treatment of brain stroke is now considered as important due to the observation that the TMS. Effect is the strongest 24 hr after an ischemia inducement. The limitations of this research are the difficulty regarding a direct application of the research results to humans, and it might be necessary to account for differences in the ischemia levels of the experimental animals.

Acknowledgement

This work was supported by the Dong-ju College Research Fund.

References

[1] R. S. J. Frackowiak, K. J. Friston, and C. Frith, Human brain function, 2nd ed., Academic Press, San Diego, (2003).
[2] Nicole A. Lazar, The Statiscal Analysis of Functional MRI Data. Springer, Berlin (2008).
[3] E. Wassermann, Oxford University Press, Oxford (2007).
[4] Orrin Devinsky and Aleksandar Beric, Eectrical and magnetic stimulation of the brain and spinal cord, Raven Press, New York (1993).
[5] B. W. Wilson, K. Caputa, M. A. Stuchly, J. D. Saffer, K. C. Davis, C. E. Washam, L. G. Washam, G. R. Washam, and M. A. Wilson, Bioelectromagnetics **15**, 563 (1994).
[6] R. S. J. Frackowiak, K. J. Friston, and C. Frith, Human Brain Function. 2nd ed., Academic Press, San Diego (2003).
[7] S. S. Choi, J. Magn. **16**, 246 (2011).
[8] Richard S. J. Frackowiak, John T. Ashburner, William D. Penny, Semir Zeki, Karl J. Friston, Christopher D. Frith, Raymond J. Dolan, and Cathy J. Price, Human Brain Function, Second Edition (2004).
[9] S. S. Choi, J. Magn. **15**, 210 (2010).
[10] C. Plewnia, M. Bartels, L. Cohen, and C. Gerloff, Neurosci. Lett. **307**, 41 (2001).
[11] S. Mark and M. D. George, Transcranial Magnetic Stimulation in Clinical Psychiatry, American Psychiatric Publishing, Inc. Arlington, VA (2006).
[12] M. Inghilleri, A. Berardelli, P. Marchetti, and M. Manfredi, Exp. Brain. Res. **109**, 467 (1996).
[13] J. H. Kim, J. Magn. **22**, 617 (2017).