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EISC Processor Core Application for Transcranial Magnetic Stimulation on the Occurrence of BAX in Cerebral of a Rat with Transient Global Ischemia

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In studying application of magnetic stimulation device for SD mouse brain's killer cell caused by cerebral ischemia induction with Transcranial Magnetic Stimulation (TMS) applying EISC Processor Core gives the block of cell death. It is a drug-dependent application of stimulation for early ischemic brain injury that validated the effect of stimulation in the ischemic state in relation to cell apoptosis with Bax, Caspase-3, immunoreactivity cells at 12 and 24 hours after ischemia. C-Fos rapidly induced the early gene and its transcription factor at CA1 site after ischemic injury, and continuous c-Fos induction caused promotion of neuronal death after ischemic injury check. In this study, to investigate the influence of brain ischemia-induced in rats on Bax, c-Fos expression in cerebrum after low frequency magnetic stimulation, low frequency magnetic stimulation was added to ST 36, We studied more aggressive approaches from the viewpoint of magnetic stimulation on neuronal death caused by early ischemic brain injury by examining changes in nerve cell Bax, c-Fos protein at the cerebral site.

Keywords : EISC processor, TMS, BAX, cerebral, rat, trasient global ischemia

1. Introduction

In the case of ischemia, neuronal cells are blocked from oxygen supply of cells, depleted of mitochondrial ATP, and cells die from preformed metabolism. Exogenous pathway activated Caspase 8 and Caspase 3 in which Fas or TNF was activated by membrane receptors, and cell apoptosis occurred. Caspases 3 is the main effector caspases that destroys various substrates. Caspase 1, 4, 5, 11, 12, 13, 14 of Group 1 play roles mainly as cytokine porters, Caspases 2, 8, 9, 10 of Group 3 go through the signaling system of initiator caspases.

Effector Caspase has 3, 6, 7 of Group 2, that play significant roles in intracellular matrix. Cerebral ischemia is strong stimulation of IEGs and the most sensitive of the hippocampus DG, CA1, CA3 vertebral body neurons and neocortex. Cerebellar purkinje celis significantly increases expression of C-Fos, and c-jun, Mrna. COX, usually known as prostaglandin H synthase, acts as a key regulatory enzyme that converts cell membrane phospholipids to prostaglandins (PG).

PG is generated from oxidative ring formation of unsaturated fatty acid having 20 carbons typified by Arachidonic acid (AA), and COX plays a role of AA to synthesize PG. It is a substance that induces expression of Cox-2 and is representative of inflammatory cytokines such as growth factors, tumor promoting factors, bacterial cell byproducts, IL-1β, and TNF-αIL-6. Cytokines variously regulate inflammation and regeneration processes of neurobiochemical substances secreted by cells in the acute phase of brain injury, IL-6 in cytokine hitherto known has severe brain injury. Involvement in metabolism and dioxide action is acute in patients.

IL-6 is produced by astrocyte cells and microglia cells of the brain and play significant roles in formation of collagen after brain injury, activation of endothelial function, formation of blood vessels, astrocyte cell proliferation, cerebral edema and recovery of brain inflammation. Poly (ADP-ribose) polymerase (PARP) is an enzyme existing in cell nucleus of brain-containing organs and is effective if DNA is damaged. It repairs broken DNA, and is involved in cell differentiation and gene expression.

PARP enzyme is a nuclear enzyme activated by cleavage of DNA by oxygen flow or peroxynitrite reaction. While ischemia occurs, that has been revealed by widespread activation of PARP is active from necrotic cell

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death and is responsible for pathological process of cell apoptosis. C-Fos and c-jun belong to early expressed genes, and early expressed genes are induced by numerous factors of the central nervous system. Long-term potentiation and

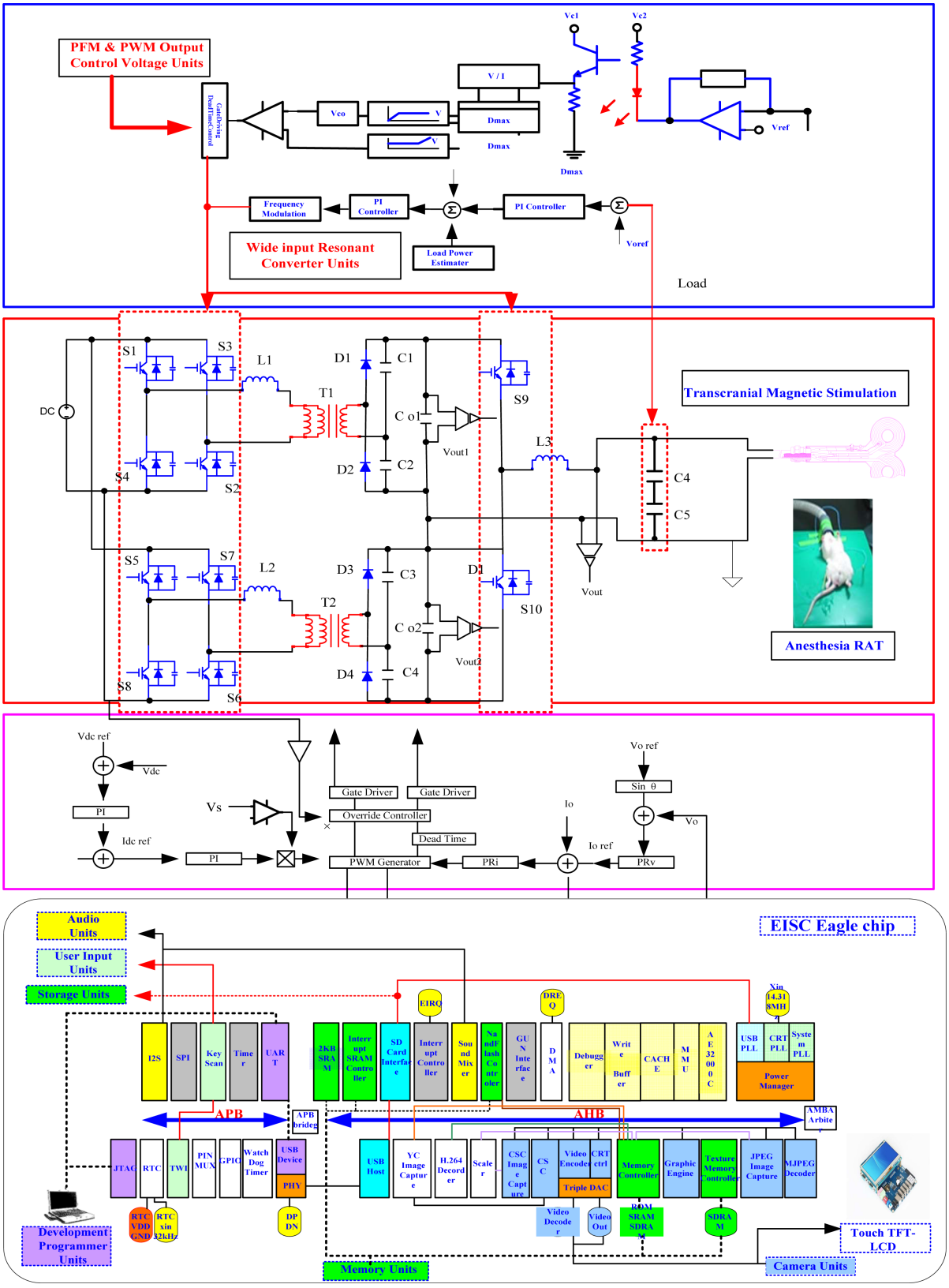

Fig. 1. (Color online) Diagram of the proposed transcranial magnetic-stimulation device.

pain sense during expression are induced. Expression of genes may be observed in epilepsy during epilepsy and ischemia (Fig. 1).

In this study, to investigate how it affects cerebral c-Fos expression after magnetic stimulation in cerebral ischemia-induced rats, low-frequency magnetic stimulation was added to ST36, and neural cells by studying change of c-Fos protein. We are seeking a more active approach with a magnetic stimulation device for neuronal death from early ischemic brain injury. In this study, investigating application of magnetic stimulation device for cell death of SD mouse's brain generated by cerebral ischemia induction gives the block of cell killing, it is possible to treat ischemic brain injury with application of stimulation studies relative to effects of magnetic stimulation of Bax, Caspase-3, immunoreactive cells in the ischemic state.

2. Methods

2.1. 16bits EISC Processor Core

Demand for embedded system diffuses, debugging method using JTAG is critical while installing JTAG interface of embedded system. For remote debugging method, GDB supports only serial communication. Also, to provide a USB-JTAG interface, embedded systems equipped with EISC processors must communicate using GDB with USB. The target board started in debug mode is in a state waiting for the command of the JTAG module, and executes the command if the command is passed.

After executing the TDI command, the target board configures the execution result as a TDO (Test Data Output) packet and transmits it to the JTAG module via JTAG, and the JTAG module stores the resulting data in a form that can be analyzed by GDB. After changing to a packet, it transmits via USB. To use the USB-JTAG interface, GDB loads the USB device library. The USB device library is implemented in a DLL (Dynamic Link Library) and includes a USB I/O function. Finally, upon termination of GDB, the close function is called connection with the EISC processor is terminated.

The USB remote structure contains a function for remote communication that is provided from GDB and a function for using the USB interface. After connecting the USB I/O function to the wrapper function, GDB calls the Open Device function to start using the USB device. At this time, when receiving Resume TDI input (100111), execute the program until the value of breakpoint registers and PC value are equal, or until Break TDI input is received.

The EISC processor can read and write values of up to 10 consecutive registers via JTAG.

In the case of Register Write, the TDI packet consists of 11101/register number/the number of consecutive registers/register data 00001, and if register writing is completed, (–22 + number of registers X 32) - zeros are transmitted as TDO. The debugging environment using the USB-JTAG interface may be used more quickly and easily than using serial communication.

2.2. Experimental mammals

Figure 2 experimental mammals used in this experiment were five 8-week-old age-specific pathogen free (SPF)

Fig. 2. (Color online) Induction of global ischemia through common carotid artery occlusion after induction of anesthesia.

male Sprague Dawley (SD) rats with body weight of 500 g or more and adapted to laboratory environment for two weeks or more. Temperature and humidity, and a clean state was kept constant. Water and feed were supplied adequately, laboratory temperature was maintained at 24° C and humidity was maintained at approximately 45-65 percent.

2.3. Ischemia model and stimulation of magnetic stimulation device

Experimental mammals were kept anesthetized with 4 percent isoflurane. We carefully incised 3 cm of common carotid artery on both sides from neck midline, then separated the vagus nerve and exposed the common carotid artery and common carotid artery was completely ligated and held for five minutes after which the suture was removed and blood could be perfused (Fig. 3). A

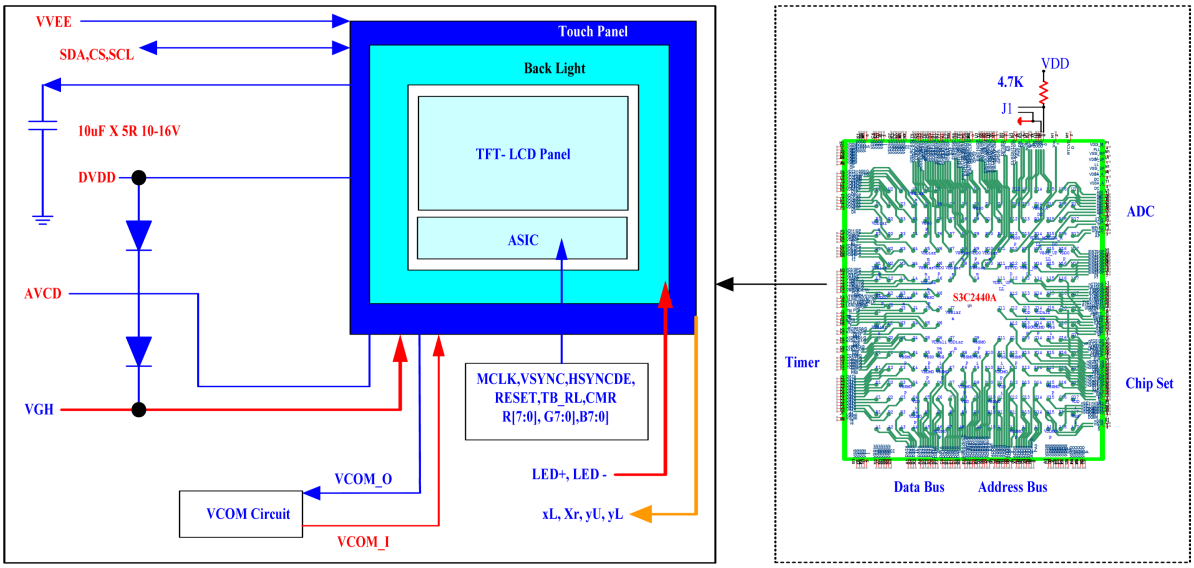

Fig. 3. (Color online) 64-Bit-EISC Processor-Core microprocessor to control and operate the energy storing condenser recharging method that employs a two-stem type converter.

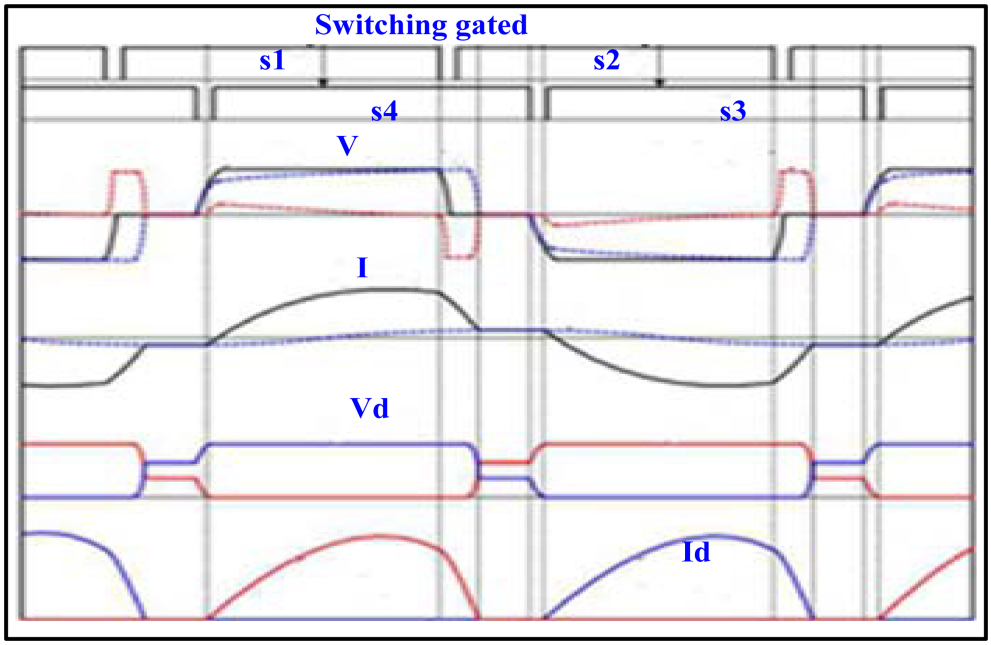

Fig. 4. (Color online) Induction of anesthesia and fixation of experiment animal using 2 % isoflurane with two-tank resonant converter.

control group that did not induce common carotid artery closure and a global ischemia that induced common carotid artery occlusion were designated after triggering the group (GI group), GI, a self stimulating apparatus (Trancular Magnetic Stimulation; TMS). We classified into TMS groups that were enforced and studied using three mice for each group (Fig. 4).

The GI group and TMS group had common carotid artery closure after 12 hours, 24 hours and 48 hours. The GI group had only 3 percent anesthesia using isoflurane was allowed to recover, TMS group was concurrent with 3 percent isoflurane anesthesia and the magnetic stimulation device was used for ST36 to the left and right of each mouse for 30 minutes. Stimulation of the magnetic stimulation device was conducted with two tangles of AC, and intensity of the muscle around the stimulation point of the rat was adjusted according to movement.

2.4. Converter

Figure 5 in case of a dual converter, the secondary side of the transformer was configured with a voltage doubler in to reduce the number of kites of the transformer and increase efficiency.

In MODE I (t 0 to t 1), the transformer primary side current I pri increases and energy were transmitted to the secondary side. In MODE II (t 1 to t 2), if the switch S1 was off at the beginning, output capacitor of S1 was charged, output capacitor of S2 was discharged, and voltage across the two capacitors became the same as input voltage Vin. At this time, voltage on the secondary side of the transformer was maintained at VC 1, Vpri became nVC 1.

In MODE III (t 2 to t 3), loss occurred from impedance of the circuit, so this was considered when designing magnetizing inductance ILm. In MODE IV (t 3 to t 4), if S4 was off, output capacitor of S4 was charged with the leakage inductance voltage Vlkg, and residual energy caused current to flow to the station parallel diode of the switch S3. Regarding leakage inductance and voltage doubler capacitor for ZVS output capacitor of a switch in a dual converter design used the leakage inductance component of the high frequency transformer for charging and discharging.

Current flowing in the magnetizing inductance must flow sufficient current to charge and discharge the output capacitor of the lagging-leg switch. The voltage doubler rectifier raises the step-up ratio by doubling voltage and constituting two capacitors of Cr1 and Cr2 in series. It has several advantages of resonating at a constant frequency with transformer primary leakage inductance Llkg at the time of designing capacitance value of the rectifier.

It is necessary to design impedance of the filter to 3 percent or less of load impedance, so impedance inductor must be designed and inductance must be calculated. In the dual converter, the magnetization current on the primary side of the transformer decreased in reflux mode section. If voltage across the switch was “0”, switch S4 was turned on and ZVS turn-on was conducted. Lagging-leg indicated ZVS operation of switch S3. If the switch S4 was off, the output capacitor of S4 was charged to the

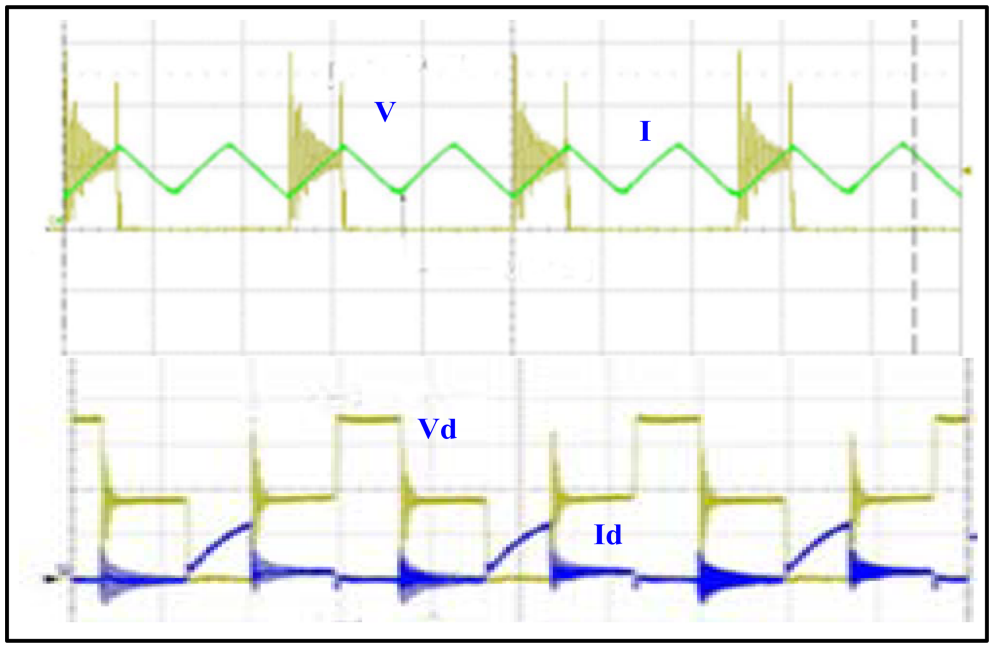

Fig. 5. (Color online) Waveform of the converter and operating waveform of the Resoant Converter (Input voltage).

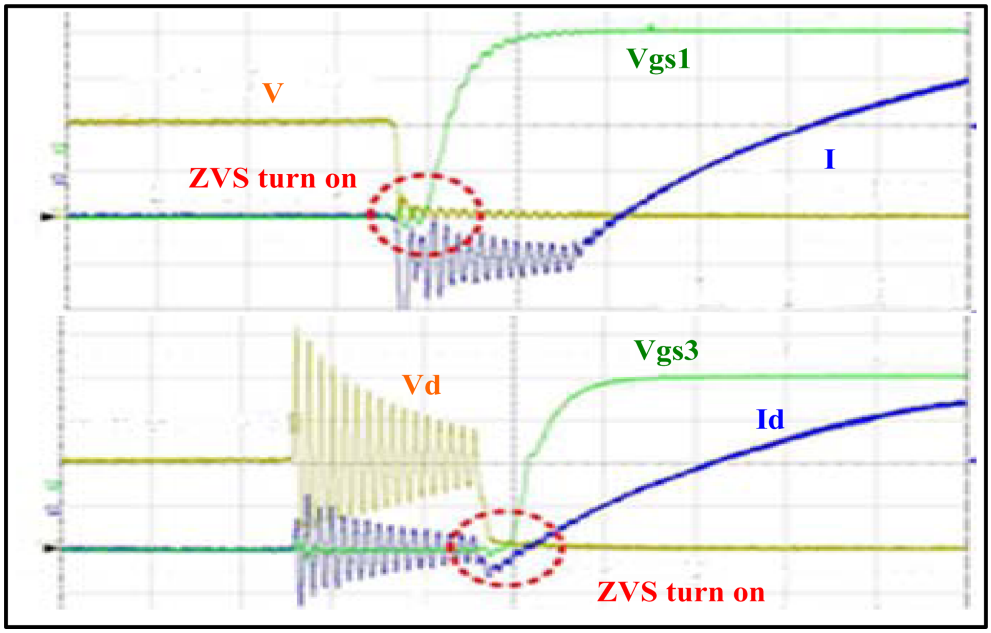

Fig. 6. (Color online) Experimental Waveform of the Boost converter and operating waveform of the Resoant Converter voltage.

leakage inductance voltage Vlkg and current flows to the station parallel diode of the switch S3 from residual energy. S3 became ZVS turn-on.

2.5. Immune system

Using GI-induced model by common carotid artery closure after choosing cerebral site of the rat, a sample was prepared by cutting on the right section and immunohistochemical examination was conducted. The brain was fixed at 5 percent paraformaldehyde, was frozen and sectioned, free-floating method was used, and Vectastain ABC Kit was used. Sliced tissue was washed with 1 M PBS for five minutes and then blocked with 1 percent H 2 0 2 (peroxidase blocking), 1 M PBS and left at room temperature for 50 minutes with 2 percent norma 3 goat serum. Anti-c-Fos was used as primary antibody, and primary antibody was allowed to react at 5 °C. for five days, followed by washing with 0.1 MPBS for seven minutes four times, and reacting the secondary antibody at 4 °C for two days.

After rinsing with 0.1 M PBS for five minutes three times, Avidin-Biotin Peroxidase complex was reacted at room temperature for three hours and washed with 1 M PBS three times for five minutes. After DAB 4 color development, the tissue was placed on a slide with two percent gelatin solution, then dried in a drier for approximately two hours, dehydrated and transparent process was allowed to permpount 5 solution.

2.6. Western blotting

Mix the lysis buffer of the quantified protein and sample buffer (60 mM tris; pH 6.8, 10 percent glycerol, 2 percent SDS, 01 percent bromophenol blue), make the amount of protein the same, then heat at 100 °C heat block boil for 10 minutes, centrifuge and collect samples (Fig. 7).

After preparing separating gel (12-15 percent) by mixing 30 percent polyacrylamide mix and tertiary distilled water, 1.5 M tris (pH 8.8), 10 percent SDS, 10 percent ammonium persulfate produced on the day, TEMED, and then solution was poured onto an electrophoresis glass plate cleaned and assembled, and gel solidified.

Stacking gel was prepared by mixing 30 percent polyacrylamidemix with tertiary distilled water, 1 M tris (pH 6.8), 10 percent SDS, 10 percent ammonium persulfate produced on the day, TEMED and poured onto separating gel to form a complete gel. Electrophoresis for running buffer, 30.0 g of tris base, 144 g of glycine and 10 g of SDS was dissolved in 1 liter to make 10 × stock. Protein was added in an amount of 10 to 20 μl and flowed at 100 V for approximately one hour. Electrophoresed gel was transferred to a nitrocellulose rose membrane 100 V for one hour. Composition of transfer buffer was made with 1 liter of tris-base 3.03 g glycine 14.63 g, methanol 200 kg and stored at 4 °C and used. This thin membrane was washed with a TTBS (pH 7.5) solution prepared with 200 mM tris-base, 1.54 M NaCl, tertiary distilled water, tween 20, and placed in 5 percent skim milk and spent two days at 4 °C. After removing blocking solution, dilute the

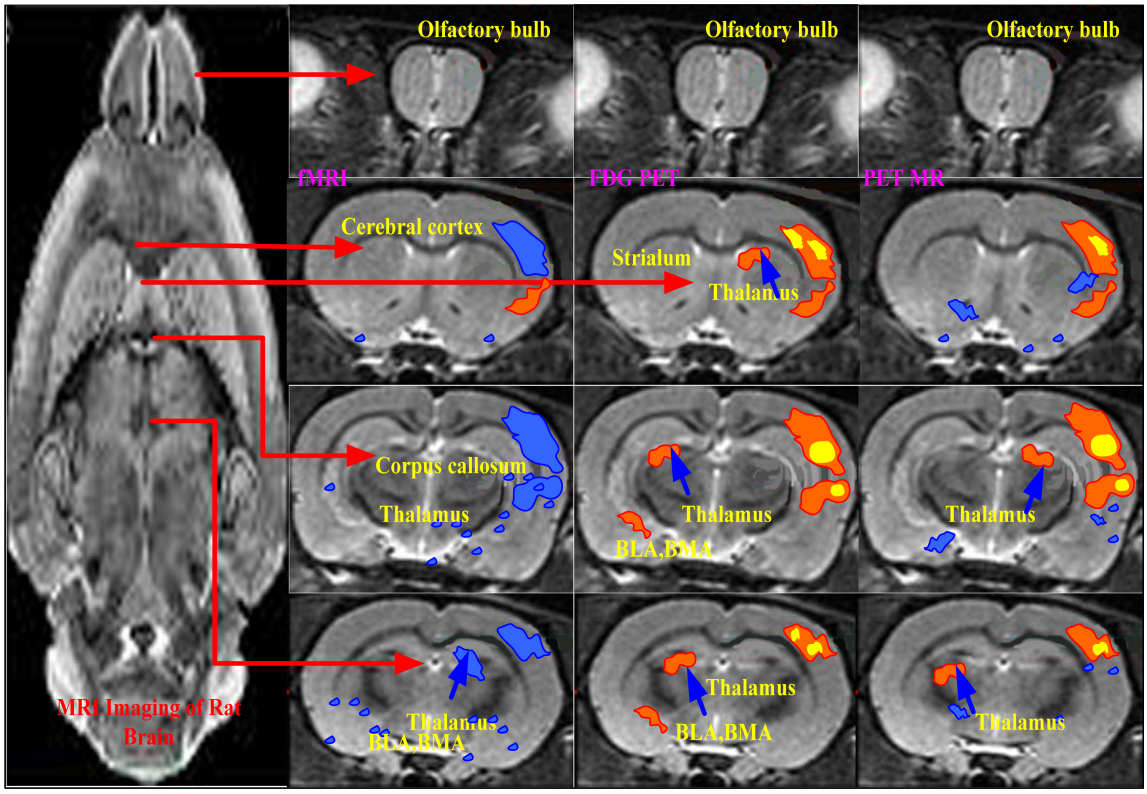

Fig. 7. (Color online) After Stimulating pulse from fMRI-FDG PET-RAT.

primary antibody same as the antibody used for immunostaining by 1,200 times with 5 percent skim milk and apply a thin membrane to the prepared solution and react for one hour, then incubate for 10 minutes with TTBS solution. Three washes were conducted and a thin membrane was added to solution prepared by diluting the secondary antibody by 1,000 times with 5 percent skim milk and reaction was induced for one hour. After removal of solution, it was washed with TTBS three times for 10 minutes each.

3. Results

ECL kit Solution A and B were well mixed at 40 : 1, immersed in a thin film and allowed to react for two minutes, then a thin film was placed on the cassette and exposed to X-ray film. After exposure for a certain period, it was expressed and the band was validated.

Changes in Bax and Caspase-3 after six hours comparative analysis of the number of reactive cells of Bax and Caspase-3 in each group revealed that expression of Bax increased. Caspase-3 revealed statistically no significant difference in expression levels between the groups.

After 12 hours, Bax and Caspase-3 were changed 12 hours later, the numbers of reactive cells of Bax and

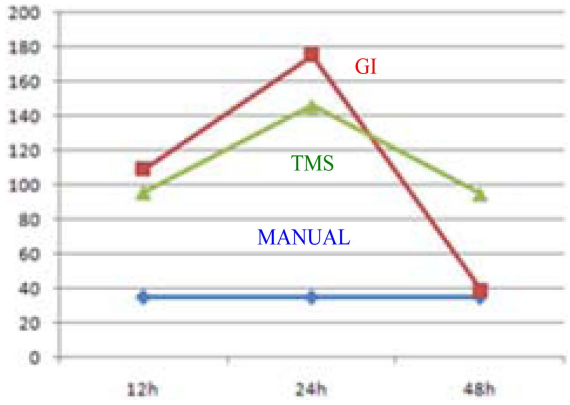

Fig. 8. (Color online) BAX reaction in the Cerebellum.

Caspase-3 were compared and analyzed. Bax expression was most frequently observed in the untreated group and observed at the same. In addition, expression levels between groups revealed statistically significant differences ($p < 0.05$, $p < 0.001$) (Fig. 8).

4. Discussion

Expression level of Caspase-3 was verified to confirm self-destructive private gas-cell death, but expression of

Caspase 3 was most frequently expressed in the untreated group at six hours after induction of ischemia. However, regarding expression of Bax similarly, it was not statistically significant compared with the applied group. It could not affect expression of Bax and Caspase 3 within a short time. Expression level of Caspase-3 also appeared most frequently in the untreated group after 12 hours, and statistically lower expression was observed in the Valley group compared with the untreated group. It is thought that expression of Bax and Caspase-3 associated with cell apoptosis caused by magnetic stimulation decreased. Expression of Bax and Caspase-3, that is an indicator of electroencephal cell death in the initial stage after induction of ischemia, is inferred by observation in which occurred a decrease or delay in expression. It was observed in the group to which magnetic stimulation was applied, and there was an effect of decreasing cell apoptosis. Of the mechanisms of cell death, since we are studying only the cell jamyulosa part, it will be necessary to study it as a factor displayed in other cellular mechanisms.

5. Conclusion

Cerebral ischemia was induced by ligating the common carotid artery of male SD rats, holding for five minutes, then removing the suture and perfusing blood. After left and right of rat ischemia-induced, magnetic stimulation was performed for 30 minutes at 12 hours, 24 hours, and 48 hours after to foot. Cerebral neurons were examined for expression of proteins in immune reactive cells reacting to c-Fos antibody and Western blotting.

As a result of immunohistochemistry, the number of c-Fos reactive cells revealed significant decrease in the TMS group compared with the control group (GI) in which magnetic stimulation was not performed in 24 hours. As a result of Western blotting, c-Fos expression decreased significantly in the TMS group as compared with the control group (GI) not subjected to magnetic stimulation after 24 hours induction of ischemia. It is considered that the magnetic stimulation may play a significant role in early treatment after ischemic stroke, because TMS is displayed most effectively 24 hours after ischemia.

Although degree of expression of Bax and Caspase-3 was observed slightly after six hours after ischemia, there was no statistically significant difference. Expression of Bax and Caspase 12 hours after ischemia was least expressed. Based on the above content, it is thought that it may be effectively used as basic material for researching therapeutic methods that may reduce degree and size of early injury of damaged cerebral cells from ischemia.

All the collected data were analyzed by computerized statistical processing program (SPSS 17.0/PC) after coding. Frequency, mean and standard deviation were calculated using Descriptive Statistics to investigate the distribution of the characteristics of the study subjects.

Acknowledgement

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