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Brain Research 993 (2003) 222–226
BRAIN RESEARCH
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Short communication

The effect of repetitive transcranial magnetic stimulation on long-term potentiation in rat hippocampus depends on stimulus intensity

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Accepted 8 September 2003

Abstract

We investigated the effect of repetitive transcranial magnetic stimulation (rTMS) on long-term potentiation (LTP) in the rat hippocampus. Rats were magnetically stimulated at a rate of 1000 pulses/day for 7 days by a round coil, in which the peak magnetic fields at the center of the coil were 0.75 and 1.00 T. LTP enhancement was observed only in the 0.75-T rTMS group, while no change was observed in the 1.00-T rTMS group. These results suggest that the effect of rTMS on LTP depends on the stimulus intensity.

Theme: Neural basis of behavior
Topic: Neural plasticity

Keywords: Repetitive transcranial magnetic stimulation (rTMS); Hippocampus; Long-term potentiation (LTP); Eddy current

Transcranial magnetic stimulation (TMS) is a non-invasive technique to stimulate the brain by magnetically induced eddy currents through a coil positioned on the surface of the head [2,25]. TMS has been widely used for functional brain mapping [8,26]. Recently, repetitive TMS (rTMS) has become an increasingly important therapeutic tool for the potential treatment of neurological and psychiatric disorders such as depression and Parkinson's disease [9,14]. Many studies have reported that gene expressions, such as c-*fos*, glial fibrillary acidic protein (GFAP), and brain derived neurotrophic factor (BDNF), were enhanced in the rat brain by rTMS [7,10,17]. The effect of rTMS on brain function must still be clarified.

Long-term potentiation (LTP) is a long-lasting increase in synaptic efficacy resulting from the high-frequency stimulation of afferent fibers [5]. LTP in the hippocampus is thought to be a typical model of synaptic plasticity related to learning and memory [15]. rTMS-related effects in the hippocampus have been previously investigated, e.g., monoamine release, neurogenesis, and memory function [6,12,21]. To clarify the mechanisms underlying the effects of rTMS on the hippocampus, an electrophysiological approach was adopted. Our previous study reported that LTP was not affected by 0.50 T rTMS (< motor threshold, same stimulus condition as this study), while LTP was significantly suppressed by 1.25 T rTMS (>motor threshold) [18], suggesting that rTMS affects hippocampal function. To maximize efficacy and reduce the risk of rTMS, the effect of rTMS on the synaptic plasticity in the hippocampus at different intensities must be clarified. In this study, we investigated the effects of 0.75 T (< motor threshold) and 1.00 T (>motor threshold) rTMS on the LTP in the rat hippocampal CA1, and clarified the dependence of the stimulus intensity in rTMS.

All experimental procedures performed in this study were approved by the Animal Ethics Committee of the University of Tokyo. Male Wistar rats (4 weeks old, 60–80 g, Saitama Experimental Animals Supply) were used. Pairs of rats (one stimulated and one sham control) were housed in individual cages with free access to food and water at room temperature. Rats were magnetically stimulated by a round coil (inner diameter = 15 mm, outer diameter = 75 mm, thickness = 10 mm) positioned over the rat's head (Fig. 1B). Rats were held by the nape of the neck beneath the coil in a wakeful state during the stimulation delivery. The stimulator (NIHON KOHDEN) delivered

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doi:10.1016/j.brainres.2003.09.009

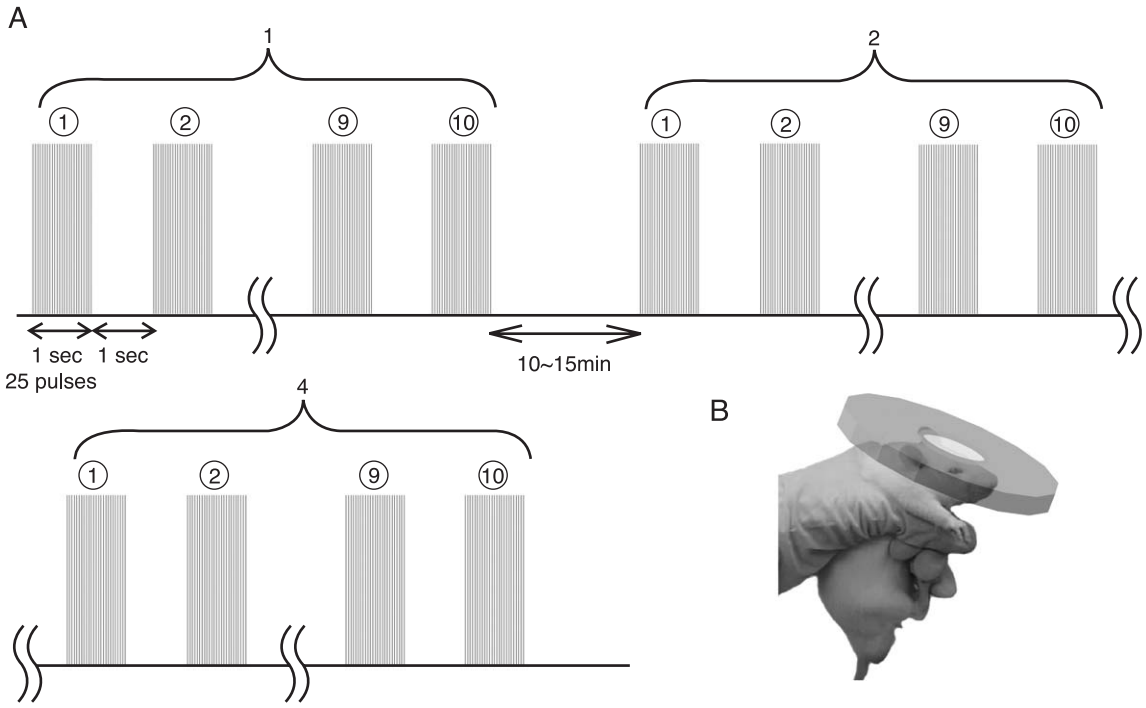

Fig. 1. (A) Stimulus pattern for one day. Ten 1-s trains of 25 pulses/s with a 1-s intertrain interval were applied to the rats four times per day for 7 days. (B) Magnetic stimulation of a rat.

biphasic cosine current pulses for 238 μs. The peak magnetic fields were set to 0.75 T (<motor threshold) and 1.00 T (>motor threshold) at the center of the coil. The motor threshold was determined based on previously reported methods [17]. The motor-evoked potentials (MEP) at the hindlimb biceps femoris muscle were measured in response to the TMS intensity. The motor threshold was determined as the TMS intensity when the MEP peak was greater than 5% of the maximum peak of the MEP. Since determining the motor threshold in each individual rat is a stressful and invasive procedure, six different rats of the same age, weight, and sex as the rats used in the rTMS experiment were used solely to determine the motor threshold to exclude any possible brain damage in the experimental rats. The average motor threshold was approximately 0.93 T.

Ten 1-s trains of 25 pulses/s with a 1-s intertrain interval were applied to the rats four times per day for 7 days (Fig. 1A). During the intervals between the four stimulations, the coil was cooled down. Rats of the sham control were treated with a sham coil (i.e., nonstimulated) and exposed to the same noise produced during the stimulation.

The eddy current induced in the rat brain was calculated using a rat head model constructed from the scalp, the skull and the brain based on MR images (Fig. 2A–C). The conductivities of the brain, the skull, and the scalp were set to 0.20, 0.015, and 0.43 S/m, respectively [19]. There is a strong spatial inhomogeneity of the electrical characteristics of brain tissue [12]. For simplicity, we adopted the previously reported method using a human head model constructed from the scalp, the skull and the brain [22]. Electric currents applied to the coil were 4.2 kHz continuous sinusoidal waves, and the current density applied to the coil in this calculation was 8.75×10^7 A/m^2, which produced a peak magnetic field of approximately 0.75 T at the center of the coil. The model was constructed and calculated using a computer program (PHOTO-Series). When the peak magnetic field at the center of the coil was 0.75 T, the maximum eddy current in the brain was approximately 9 A/m^2 (Fig. 2D). The eddy current density is proportional to the changing rate of the magnetic field or the peak magnetic field. Therefore, when the peak magnetic field at the center of the coil is 1.00 T, the eddy current in the brain is approximately 12 A/m^2.

Approximately 15 h after the final stimulation, the rats were anesthetized with diethyl ether and decapitated. The brain was quickly removed from the skull and placed on an ice-cold filter paper damped with artificial cerebrospinal fluid (ACSF) containing (in mM) NaCl 125, KCl 3, NaH_2PO_4 1.2, $NaHCO_3$ 26, $CaCl_2$ 2.0, $MgCl_2$ 1.0, and glucose 10. The hippocampus was dissected, and transverse slice sections (400 μm) were obtained with a microslicer. The slices were incubated and allowed to recover in ACSF bubbled with 95% O_2/5% CO_2 (pH 7.4) at room temperature for a minimum of 1 h before recording. The slices were then transferred to a recording chamber and continuously perfused (approximately 2 ml/min) with ACSF at 30 °C. Field excitatory postsynaptic potentials (fEPSP) were recorded using a tungsten electrode from the dendrites of CA1 pyramidal cells by stimulating Schaffer collaterals with a tungsten bipolar stimulating electrode. A single stimulus

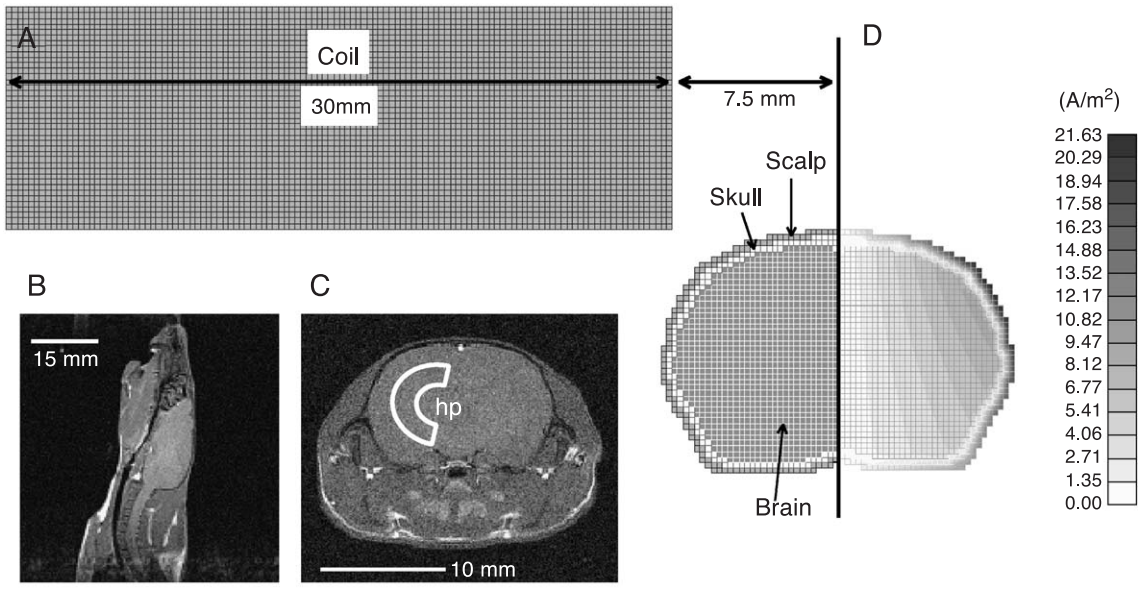

Fig. 2. Modeling of the rat head and calculation of the eddy current. (A) Positional relationship between the coil and the rat head. The rat head model was constructed from the scalp, skull and brain. (B) Sagittal MR image of a rat head. (C) Coronal MR image of a rat head. hp: area of hippocampus. (D) Estimated eddy current when the peak magnetic field was set to 0.75 T at the center of the coil.

was administered at 20-s intervals. The stimulus intensity was set to generate a fEPSP with a slope that was approximately 30% of the maximum determined from the input–output curve. After obtaining stable fEPSP recordings for 20 min, LTP was induced by tetanus stimulation (100 Hz for 1 s, 0.1-ms duration). fEPSP recordings were then continuously obtained for 60 min after tetanus stimulation and subsequently analyzed with pCLAMP software (Axon Instrument). Each slice was used for only one experiment and then discarded. LTP data were obtained from 10 sham rats (1–5 LTPs from each rat for a total of 26 LTPs) and 10 stimulated rats (1–4 LTPs from each rat for a total of 26 LTPs) of the 0.75-T rTMS group, and from 8 sham rats (1–4 LTPs from each rat for a total of 15 LTPs) and 8 stimulated rats (2–4 LTPs from each rat for a total of 21 LTPs) of the 1.00-T rTMS group. All the LTP data for each group were averaged and statistically analyzed by repeated measures of ANOVA. Data were expressed as the mean ± standard error (S.E.). A probability level of less than 0.05 was considered to be statistically significant.

LTPs were observed in both the 0.75-T stimulated and sham control groups, as shown in Fig. 3. The induction phase of LTP (first 10 min after tetanus stimulation) of the 0.75-T stimulated group was enhanced compared with that of the sham control group. The maintenance phases of LTP (from 10 min after tetanus stimulation to 60 min) of the stimulated group (267 ± 26%) was significantly enhanced compared with the sham control group (212 ± 10%) ($F_{1,50} = 4.410$, $p = 0.0408$). LTPs were also observed in both the 1.00-T stimulated and sham control groups, as shown in Fig. 4. The induction phase of LTP of the 1.00 T stimulated group was also enhanced compared with that of the sham control group. There were no significant differences ($F_{1,34} = 1.749$, $p = 0.1948$), however, between the maintenance phases of LTP of the 1.00-T stimulated group (223 ± 13%) and the sham control group (199 ± 13%).

According to our data, LTP is not significantly affected by 1.00 T rTMS, but enhanced by 0.75 T rTMS, suggesting that 0.75 T rTMS possibly activates hippocampal function. Two possible mechanisms for the enhancement of LTP by 0.75 T rTMS are as follows: (1) LTP induction is affected by rTMS directly. Previously reported studies revealed that the expression of c-*fos*, glial fibrillary acidic protein (GFAP) and brain derived neurotrophic factor (BDNF) were en-

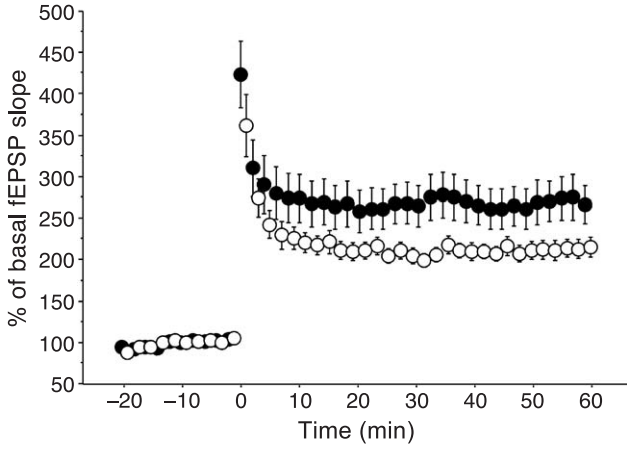

Fig. 3. LTPs of 0.75 T stimulated and sham control groups. LTPs were observed in both the stimulated and sham control groups. The maintenance phase of the LTP of the stimulated group (267 ± 26%) (black circle) was significantly enhanced compared with the sham control group (212 ± 10%) (white circle) ($p = 0.0408$). Each circle represents the average of six successive responses (for 2 min). Rat $N = 10$ for each group. Error bar = ± 1 S.E.

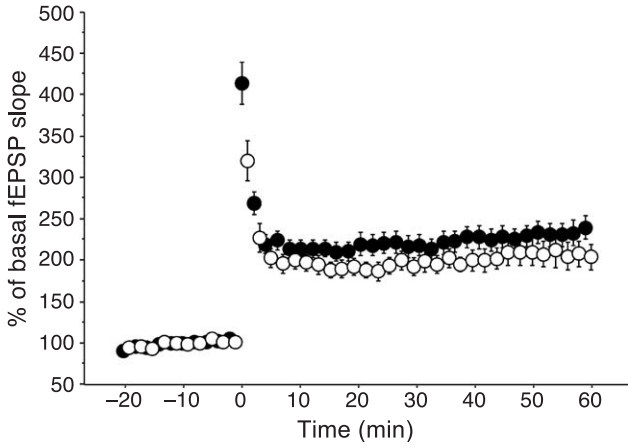

Fig. 4. LTPs of 1.00-T stimulated and sham control groups. LTPs were observed in both the stimulated and sham control groups. There was no significant difference ($p = 0.1948$) between the maintenance phase of the LTP of the stimulated group (225 ± 14%) (black circle) and the sham control group (199 ± 13%) (white circle). Each circle represents the average of six successive responses (for 2 min). Rat $N = 8$ for each group. Error bar = ± 1 S.E.

hanced in the dentate gyrus and hippocampal CA3 by TMS [7,10,17]. It is also reported that GFAP, which is intimately associated with LTP [16,27], is one of the genes that is strongly upregulated by intense neuronal activity in the hippocampal dentate gyrus [23,24]. There are many mechanisms associated with LTP induction, for example, the enhancement of transmitter release, the activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors, and changes in the number of synaptic-spine contacts and in the shape of the spine heads [3,4,13]. Therefore, 0.75 T rTMS possibly induces the gene expression in the hippocampus, and affects some of the mechanisms associated with LTP induction, resulting in LTP enhancement. (2) LTP in the hippocampus is indirectly affected by 0.75 T rTMS via gene expression in brain regions other than the hippocampus because the peripheral brain regions are exposed to stronger eddy currents than the hippocampus (Fig. 2D). It is reported that TMS induces the expression of c-*fos* in the cingulate gyrus, frontal cortex and parietal cortex [10,11]. In general, information is transferred from the cingulate cortex, temporal lobe cortex, amygdala, orbital cortex, and olfactory bulb to the hippocampus [1]. Therefore, there is a possibility that brain regions other than the hippocampus are affected by 0.75 T rTMS, and hippocampal function is activated indirectly. Further studies are needed to clarify these two mechanisms.

In our previous study, we reported that 0.50 T rTMS (< motor threshold) had no effect on LTP in the rat hippocampus, while 1.25 T rTMS (>motor threshold) suppressed LTP [18]. In summary, we conclude that rTMS of 0.50 T (< motor threshold) and 1.00 T (>motor threshold) have no effect on hippocampal function, rTMS of 0.75 T (< motor threshold) may potentially activate hippocampal function, and rTMS of 1.25 T (>motor threshold) may potentially impair hippocampal function. It is reported that the effect of rTMS depends on individual parameters (e.g., frequency, intensity) [20]. Our results suggest that the effect of rTMS depends on the stimulus intensity, and rTMS administered at the appropriate stimulus intensity may potentially activate hippocampal function.

Acknowledgements

This work was supported partly by a Grant-in-Aid for Specially Promoted Research (No. 12002002) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to S.U.), and partly by a grant in aid from the Magnetic Health Science Foundation (to M.O.). The authors would like to thank Dr. H. Funamizu, Mr. T. Tsurugizawa, Mr. N. Yasumatsu, Dr. N. Takata, and Ms. E. Rosenberg for their help.

References

[1] D.G. Amaral, M.P. Witter, Hippocampal formation, in: G. Paxinos (Ed.), The Rat Nervous System, Academic Press, San Diego, 1995, pp. 443–493.
[2] A.T. Barker, R.I. Jalinous, L. Freeston, Non-invasive magnetic stimulation of human motor cortex, Lancet 1 (1985) 1106–1107.
[3] J.M. Bekkers, C.F. Stevens, Presynaptic mechanism for long-term potentiation in the hippocampus, Nature 346 (1990) 724–729.
[4] T.V. Bliss, G.L. Collingridge, A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361 (1993) 31–39.
[5] T.V. Bliss, T. Lomo, Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path, J. Physiol. 232 (1973) 357–374.
[6] B. Czeh, T. Welt, A.K. Fischer, A. Erhardt, W. Schmitt, M.B. Muller, N. Toschi, E. Fuchs, M.E. Keck, Chronic psychosocial stress and concomitant repetitive transcranial magnetic stimulation: effects on stress hormone levels and adult hippocampal neurogenesis, Biol. Psychiatry 52 (2002) 1057–1065.
[7] M. Fujiki, O. Steward, High frequency transcranial magnetic stimulation mimics the effects of ECS in upregulating astroglial gene expression in the murine CNS, Brain Res. Mol. Brain Res. 44 (1997) 301–308.
[8] J.R. Gates, Transcranial magnetic stimulation, Neuroimaging Clin. N. Am. 5 (1995) 711–720.
[9] M.S. George, E.M. Wassermann, W.A. Williams, A. Callahan, T.A. Ketter, P. Basser, M. Hallett, R.M. Post, Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression, NeuroReport 6 (1995) 1853–1856.
[10] A. Hausmann, C. Weis, J. Marksteiner, H. Hinterhuber, C. Humpel, Chronic repetitive transcranial magnetic stimulation enhances c-*fos* in the parietal cortex and hippocampus, Brain Res. Mol. Brain Res. 76 (2000) 355–362.
[11] R.R. Ji, T.E. Schlaepfer, C.D. Aizenman, C.M. Epstein, D. Qiu, J.C. Huang, F. Rupp, Repetitive transcranial magnetic stimulation activates specific regions in rat brain, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 15635–15640.
[12] M.E. Keck, T. Welt, A. Post, M.B. Muller, N. Toschi, A. Wigger, R. Landgraf, F. Holsboer, M. Engelmann, Neuroendocrine and behavioral effects of repetitive transcranial magnetic stimulation in a psychopathological animal model are suggestive of antidepressant-like effects, Neuropsychopharmacology 24 (2001) 337–349.

[13] K.S. Lee, F. Schottler, M. Oliver, G. Lynch, Brief bursts of high-frequency stimulation produce two types of structural change in rat hippocampus, J. Neurophysiol. 44 (1980) 247–257.
[14] J. Mally, T.W. Stone, Improvement in Parkinsonian symptoms after repetitive transcranial magnetic stimulation, J. Neurol. Sci. 162 (1999) 179–184.
[15] S. Maren, M. Baudry, Properties and mechanisms of long-term synaptic plasticity in the mammalian brain: relationships to learning and memory, Neurobiol. Learn. Mem. 63 (1995) 1–18.
[16] M.A. McCall, R.G. Gregg, R.R. Behringer, M. Brenner, C.L. Delaney, E.J. Galbreath, C.L. Zhang, R.A. Pearce, S.Y. Chiu, A. Messing, Targeted deletion in astrocyte intermediate filament (Gfap) alters neuronal physiology, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 6361–6366.
[17] M.B. Muller, N. Toschi, A.E. Kresse, A. Post, M.E. Keck, Long-term repetitive transcranial magnetic stimulation increases the expression of brain-derived neurotrophic factor and cholecystokinin mRNA, but not neuropeptide tyrosine mRNA in specific areas of rat brain, Neuropsychopharmacology 23 (2000) 205–215.
[18] M. Ogiue-Ikeda, S. Kawato, S. Ueno, The effect of transcranial magnetic stimulation on long-term potentiation in rat hippocampus, IEEE Trans. Magn. (2003) 3390–3392.
[19] T.F. Oostendorp, J. Delbeke, D.F. Stegeman, The conductivity of the human skull: results of in vivo and in vitro measurements, IEEE Trans. Biomed. Eng. 47 (2000) 1487–1492.
[20] A. Pascual-Leone, C.M. Houser, K. Reese, L.I. Shotland, J. Grafman, S. Sato, J. Valls-Sole, J.P. Brasil-Neto, E.M. Wassermann, L.G. Cohen, Safety of rapid-rate transcranial magnetic stimulation in normal volunteers, Electroencephalogr. Clin. Neurophysiol. 89 (1993) 120–130.
[21] A. Post, M.B. Muller, M. Engelmann, M.E. Keck, Repetitive transcranial magnetic stimulation in rats: evidence for a neuroprotective effect in vitro and in vivo, Eur. J. Neurosci. 11 (1999) 3247–3254.
[22] M. Sekino, S. Ueno, Comparison of current distributions in electroconvulsive therapy and transcranial magnetic stimulation, J. Appl. Phys. 91 (2002) 8730–8732.
[23] O. Steward, Electroconvulsive seizures upregulate astroglial gene expression selectively in the dentate gyrus, Brain Res. Mol. Brain Res. 25 (1994) 217–224.
[24] O. Steward, E.R. Torre, R. Tomasulo, E. Lothman, Neuronal activity up-regulates astroglial gene expression, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 6819–6823.
[25] S. Ueno, T. Tashiro, K. Harada, Localized stimulation of neural tissues in the brain by means of paired configuration of time-varying magnetic fields, J. Appl. Phys. 64 (1988) 5862–5864.
[26] S. Ueno, T. Matsuda, M. Fujiki, Functional mapping of the human motor cortex obtained by focal and vectorial magnetic stimulation of the brain, IEEE Trans. Magn. 26 (1990) 1539–1544.
[27] J. Wenzel, G. Lammert, U. Meyer, M. Krug, The influence of long-term potentiation on the spatial relationship between astrocyte processes and potentiated synapses in the dentate gyrus neuropil of rat brain, Brain Res. 560 (1991) 122–131.