The Effect of Transcranial Magnetic Stimulation on Long-Term Potentiation in Rat Hippocampus

Mari Ogiue-Ikeda, Suguru Kawato, and Shoogo Ueno, *Fellow, IEEE*

***Abstract*—We investigated the effect of transcranial magnetic stimulation (TMS) on the brain by focusing on long-term potentiation (LTP) in the rat hippocampus. Male Wistar rats were magnetically stimulated by a round coil positioned over the rat's head. The stimulator delivered biphasic cosine current pulses 238 μs in duration. The peak magnetic fields were set to 0.50 T (<motor threshold) and 1.25 T (>motor threshold) at the center of the coil. Rats received 10 1 s trains of 25 pulses/s with a 1 s intertrain interval 4 times per day for 7 days. There was no significant difference between the LTP of the 0.50 T stimulated and sham control groups. The LTP of the 1.25 T stimulated group, however, was inhibited compared with the LTP of the sham control group, suggesting that the synaptic plasticity in the hippocampus was impaired by strong TMS. It is necessary to control the intensity of TMS for maximizing treatment efficacy and safety.**

***Index Terms*—Hippocampus, long-term potentiation (LTP), transcranial magnetic stimulation (TMS).**

I. Introduction

TRANSCRANIAL MAGNETIC STIMULATION (TMS) is a noninvasive technique to stimulate the brain by magnetically induced eddy currents through a coil positioned on the surface of the head [1], [2]. TMS has been widely used in neurology as a diagnostic tool and for functional brain mapping [3]. Recently, as an alternative to electroconvulsive therapy (ECT) which is associated with many side effects such as headaches and partial memory loss [4], TMS has become an increasingly important therapeutic tool for the potential treatment of neurological and psychiatric disorders such as depression and Parkinson's disease [5], [6]. However, the mechanisms underlying the therapeutic effects must be clarified to maximize efficacy and reduce risk.

Long-term potentiation (LTP) is a long-lasting increase in synaptic efficacy resulting from the high-frequency stimulation of afferent fibers [7]. LTP in the hippocampus is thought to be a typical model of synaptic plasticity related to learning and memory [8]. It is reported that TMS induces gene expression such as c-fos and GFAP in the rat hippocampus [9], [10]. Therefore, there is a possibility that TMS induces changes in synaptic plasticity in the hippocampus.

Manuscript received January 6, 2002. This work was supported by a Grant-in-Aid for Specially Promoted Research (Joint Research 12002002) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

M. Ogiue-Ikeda and S. Ueno are with the Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Tokyo, Japan (e-mail: ogiue@medes.m.u-tokyo.ac.jp; ueno@medes.m.u-tokyo.ac.jp).

S. Kawato is with the Department of Biophysics and Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan (e-mail: kawato@phys.c.u-tokyo.ac.jp).

Digital Object Identifier 10.1109/TMAG.2003.816160

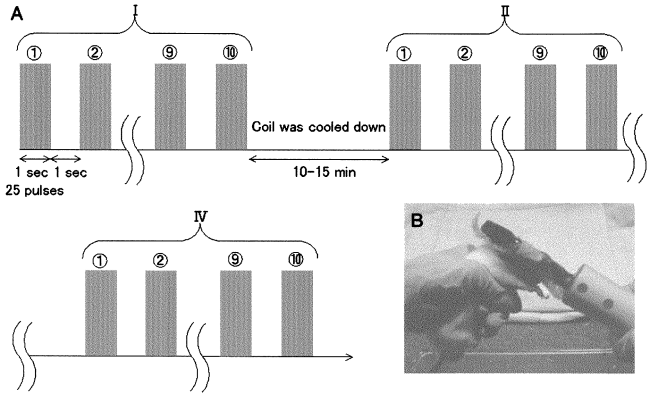

Fig. 1. (a) Stimulation pattern for one day. Rats received 10 1 s trains of 25 pulses/s with a 1 s intertrain interval four times per day for seven days. (b) Magnetic stimulation of a rat.

In this study, we investigated the effect of TMS on the brain by focusing on long-term potentiation in the rat hippocampus, which is relevant to safety aspects of TMS.

II. Materials and Methods

A. TMS

All experimental procedures performed in this study were approved by the Animal Ethics Committee of the University of Tokyo. Male Wistar rats (four weeks old, 60–80 g) 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 ($\text{inner diameter} = 1.5\ \text{cm}$, $\text{outer diameter} = 7.5\ \text{cm}$, $\text{thickness} = 1.0\ \text{cm}$) positioned over the rat's head [Fig. 1(b)]. To deliver the stimulation, rats were held by the nape of the neck beneath the coil in a wakeful state. The stimulator delivered biphasic cosine current pulses 238 μs in duration (NIHON KOHDEN). The peak magnetic fields were set to 0.50 T (<motor threshold, $n = 8$ for stimulated group and $n = 8$ for sham control group) and 1.25 T (>motor threshold, $n = 8$ for stimulated group and $n = 8$ for sham control group) at the center of the coil. The motor threshold was defined as the intensity when the hindlimbs of the rat moved as a result of the magnetic stimulation [Fig. 1(b)]. Rats received 10 1 s trains of 25 pulses/s with a 1 s intertrain interval four times per day for seven days [Fig. 1(a)]. During the intervals between the four stimulations, the coil was cooled down. Rats of the sham control were treated with a sham coil and exposed to the same noise produced during the stimulation.

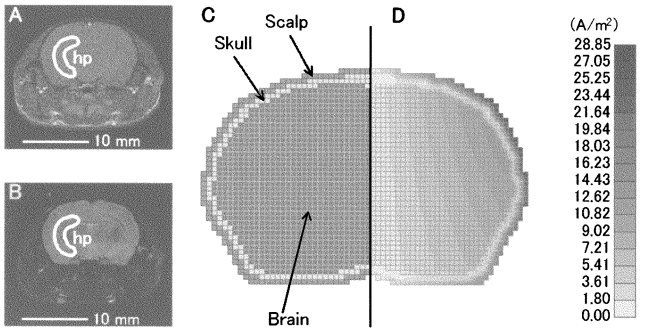

Fig. 2. Modeling of rat head and calculation of eddy current. (c) The rat head model was constructed with the scalp, skull and brain based on T1 (a) and T2 (b) MR images. (d) The estimated eddy current when the peak magnetic field was set to 1.00 T at the center of the coil. hp: area of hippocampus.

B. Eddy Current Calculation

The eddy currents induced in the rat brain were calculated using a rat head model constructed from the scalp, the skull and the brain based on MR images [Fig. 2(a)–(c)]. The conductivities of the brain, the skull, and the scalp were set to 0.20, 0.015, and 0.43 S/m, respectively [11]. Electric currents applied to the coil were continuous sinusoidal waves of 4.2 kHz in frequency that corresponded to a duration of 238 μs in a pulsed magnetic stimulation, and the current density applied to the coil in this calculation was $1.1 \times 10^8\ \mathrm{A/m^2}$, which produced a peak magnetic field of approximately 1.00 T at the center of the coil. The model was constructed using a computer program for finite element modeling and postprocessing (FEMAP, Structural Dynamics Research Corporation), and calculated using a computer program (PHOTO-Series, PHOTON) designed for electromagnetic field calculations.

C. Electrical Recording

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, $\mathrm{NaH_2PO_4}$ 1.2, $\mathrm{NaHCO_3}$ 26, $\mathrm{CaCl_2}$ 2.0, $\mathrm{MgCl_2}$ 1.0, and glucose 10. The hippocampus was dissected, and transverse slice sections (400 $\mu\mathrm{m}$) were obtained with a microslicer. The slices were incubated and allowed to recover in ACSF bubbled with 95% $\mathrm{O_2}/5\%$ $\mathrm{CO_2}$ (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 using a tungsten bipolar stimulating electrode. A single stimulus was administered at 20 s intervals. After obtaining stable fEPSP recordings for 20 min (60 stimuli), LTP was induced by tetanic stimulation (100 Hz for 1 s). fEPSP recordings continued for 60 min (180 stimuli) after tetanic stimulation. Records were filtered at 0.5–300 Hz, digitized at 10 kHz and stored on a computer. Data were analyzed with pCLAMP software (Axon Instrument). Approximately 3 to 4 LTP data were obtained from each rat. LTP data were statistically analyzed by repeated-measures of ANOVA. A probability level of less than 0.05 was considered to be statistically significant.

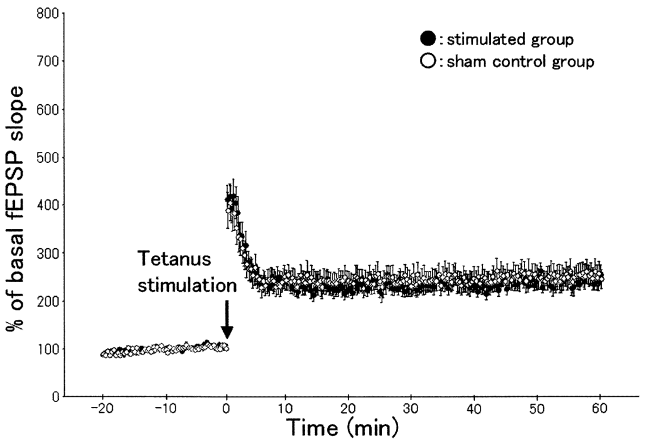

Fig. 3. LTPs of 0.50 T stimulated and sham control groups. There was no significant difference between the LTPs of the sham group and stimulated group ($p = 0.7883$). Rat $N = 8$ for each group. $\mathrm{Error\ bar} = \pm 1SE$.

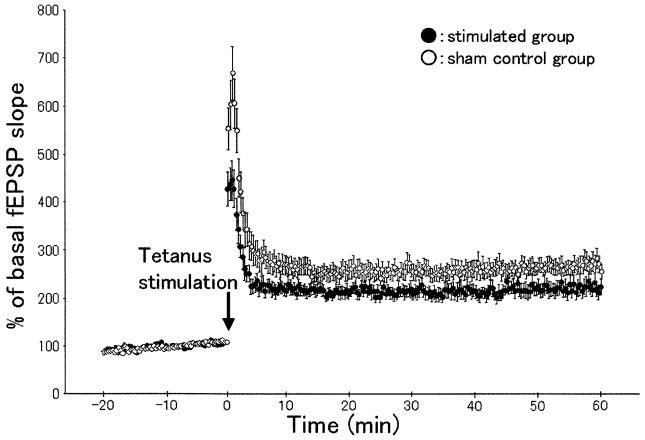

Fig. 4. LTPs of 1.25 T stimulated and sham control groups. The LTP of the stimulated group was inhibited compared with the LTP of the sham group ($p = 0.0232$). Rat $N = 8$ for each group. $\mathrm{Error\ bar} = \pm 1SE$.

III. Results and Discussion

When the peak magnetic field at the center of the coil was 1.00 T, the estimated eddy current around the hippocampus and the maximum eddy current in the brain were approximately 8 $\mathrm{A/m^2}$ and 12 $\mathrm{A/m^2}$, , respectively [Fig. 2(d)]. The eddy current density is proportional to the changing rate of the magnetic field or the peak magnetic field. Therefore, when the peak magnetic fields at the center of the coil are 0.50 T and 1.25 T, the estimated eddy currents around the hippocampus are approximately 4 $\mathrm{A/m^2}$ and 10 $\mathrm{A/m^2}$, respectively, and the maximum currents in the brain are approximately 6 $\mathrm{A/m^2}$ and 15 $\mathrm{A/m^2}$, respectively.

Fig. 3 shows LTPs of 0.50 T stimulated and sham control groups. LTPs were observed in both the stimulated and sham control groups. There was no significant difference between the LTPs of stimulated and sham control groups ($p = 0.7883$). Fig. 4 shows LTPs of 1.25 T stimulated and sham control groups. Although LTPs were observed in both groups, the LTP of the stimulated group was significantly inhibited compared with the

LTP of the sham control group ($p = 0.0232$). These results indicate that the synaptic plasticity in the hippocampus was not changed by weak TMS (<motor threshold), but was impaired by strong TMS (>motor threshold).

It is reported that when PC12 cells, model cells of neurons, were exposed to electrical stimulation of 10 Hz, 1800 V/m for 24 h (864 000 pulses), the cells were not damaged [12]. Compared with our stimulation conditions, 10 1 s trains of 25 pulses/s four times per day for seven days (=7000 pulses) and $75\,\mathrm{V/m}(= 15[\mathrm{A/m^2}]/0.2[\mathrm{S/m}])$ of the maximum eddy current in the brain, there is no possibility that the brain was damaged directly by the eddy current induced by 1.25 T TMS. There are three possible mechanisms for the degeneration of LTP by strong TMS:

1) direct effect on LTP induction by the activation of NMDA (N-Methyl-D-Aspartate) receptors and changes in the number of synaptic-spine contacts and in the shape of the spine heads [13], [14];
2) indirect effect on LTP induction via gene expression in the brain, TMS induces the expression of c-fos in the rat parietal cortex [9];
3) physical stress cascade mechanism, stress up-regulated glucocorticoids cause damage to the hippocampus by inhibiting the uptake of glucose into hippocampal neurons resulting in exacerbating numerous steps in the NMDA cascade when neuronal energy stores are diminished [15], [16].

All three proposed mechanisms may have caused the degeneration of LTP by strong TMS. For example, it is possible that other brain areas were affected by TMS and other brain areas affected the hippocampus. Also, since 1.25 T TMS is strong enough generate movement of the hindlimbs, it is possible that strong TMS causes stress to rats and induces hippocampal damage, resulting in LTP degeneration. Thus, it is necessary to control the intensity of TMS for maximizing treatment efficacy for brain diseases and reducing the risks of adverse effects.

ACKNOWLEDGMENT

The authors would like to thank Dr. H. Funamizu, T. Tsurugizawa, N. Yasumatsu, Dr. N. Takata, and E. Rosenberg, for their help.

REFERENCES

[1] A. T. Barker, R. Jalinous, and I. L. Freeston, "Non-invasive magnetic stimulation of human motor cortex," *Lancet*, vol. 1, pp. 1106–1107, May 1985.
[2] S. Ueno, T. Tashiro, and K. Harada, "Localized stimulation of neural tissues in the brain by means of paired configuration of time-varying magnetic fields," *J. Appl. Phys.*, vol. 64, pp. 5862–5864, 1988.
[3] J. R. Gates, "Transcranial magnetic stimulation," *Neuroimag. Clin. North Amer.*, vol. 5, pp. 711–720, Nov 1995.
[4] D. Gordon, "Electroconvulsive therapy," *J. Biomed. Eng.*, vol. 11, pp. 170–171, Mar 1989.
[5] A. Pascual-Leone, B. Rubio, F. Pallardo, and M. D. Catala, "Rapid-rate transcranial magnetic stimulation of left dorsolateral prefrontal cortex in drug-resistant depression," *Lancet*, vol. 348, pp. 233–237, Jul 1996.
[6] J. Mally and T. W. Stone, "Improvement in Parkinsonian symptoms after repetitive transcranial magnetic stimulation," *J. Neurol. Sci.*, vol. 162, pp. 179–184, Jan 1999.
[7] T. V. P. Bliss and T. Lomo, "Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path," *J. Physiol.*, vol. 232, pp. 357–374, Jul 1973.
[8] S. Maren and M. Baudry, "Properties and mechanisms of long-term synaptic plasticity in the mammalian brain: relationships to learning and memory," *Neurobiol. Learn. Mem.*, vol. 63, pp. 1–18, Jan 1995.
[9] A. Hausmann, C. Weis, J. Marksteiner, H. Hinterhuber, and C. Humpel, "Chronic repetitive transcranial magnetic stimulation enhances c-fos in the parietal cortex and hippocampus," *Brain Res. Mol. Brain Res.*, vol. 76, pp. 355–362, Mar 2000.
[10] M. Fujiki and 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.*, vol. 44, pp. 301–308, Mar 1997.
[11] M. Sekino and S. Ueno, "Comparison of current distributions in electroconvulsive therapy and transcranial magnetic stimulation," *J. Appl. Phys.*, vol. 91, pp. 8730–8732, May 2002.
[12] M. Ogiue-Ikeda, M. Sekino, and S. Ueno, "Effects of electrical stimulation on PC12 cells," in *Proc. Bioelectromagn. Soc. 24th Annu. Meeting*, June 2002, pp. 208–209.
[13] T. V. P. Bliss and G. L. Collingridge, "A synaptic model of memory: long-term potentiation in the hippocampus," *Nature*, vol. 361, pp. 31–39, 1993.
[14] K. S. Lee, F. Schottler, M. Oliver, and G. L. Lynch, "Brief bursts of high-frequency stimulation produce two types of structural changes in rat hippocampus," *J. Neurophys.*, vol. 44, pp. 247–257, 1980.
[15] H. Uno, R. Tarara, J. G. Else, M. A. Suleman, and R. M. Sapolsky, "Hippocampal damage associated with prolonged and fatal stress in primates," *J. Neurosci.*, vol. 9, pp. 1705–1711, May 1989.
[16] M. P. Armanini, C. Hutchins, B. A. Stein, and R. M. Sapolsky, "Glucocorticoid endangerment of hippocampal neurons is NMDA-receptor dependent," *Brain Res.*, vol. 532, pp. 7–12, Nov 1990.