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Research report

Acquisition of ischemic tolerance by repetitive transcranial magnetic stimulation in the rat hippocampus

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Abstract

We investigated the acquisition of ischemic tolerance in the rat hippocampus using repetitive transcranial magnetic stimulation (rTMS). Rats received 1000 pulses/day for 7 days, and the field excitatory postsynaptic potentials were measured in the hippocampal CA1. After slices were exposed to ischemic conditions, long-term potentiation (LTP) was induced. The LTP of the stimulated group was enhanced compared with the LTP of the sham control group in each ischemic condition, suggesting that rTMS has the potential to protect hippocampal function from ischemia.

Theme: Disorders of the nervous system
Topic: Ischemia

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

Transcranial magnetic stimulation (TMS) is a non-invasive technique that stimulates the brain using magnetically induced eddy currents through a coil positioned on the surface of the head [1,22]. Repetitive TMS (rTMS) has become an increasingly important therapeutic tool for the treatment of neurological and psychiatric disorders, such as depression and Parkinson's disease [13,15,21]. There is a possibility that rTMS might have therapeutic effects on ischemia; however, few studies have described the effects of rTMS on brain ischemia.

CA1 pyramidal neurons in the hippocampus are highly vulnerable to cerebral ischemia. Brief periods (minutes) of severe ischemia cause neuronal degeneration in the CA1 region 3 to 7 days after the ischemia by apoptosis (so-called "delayed neuronal death, DND") [12]. Notable increases in extracellular glutamate concentration during cerebral ischemia, excessive excitation of neurons by the accumulation of glutamate, and Ca^{2+}influx during ischemia are thought to cause neuronal hyperactivity and trigger neuronal injury (so-called "excitotoxicity") [5].

Long-term potentiation (LTP) is a long-lasting increase in synaptic efficacy resulting from the high-frequency stimulation of afferent fibers [3,4]. LTP in the hippocampus is thought to be representative of the type of synaptic plasticity that is related to learning and memory [3,16]. 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, changes in the number of synaptic-spine contacts and alterations in the shape of the spine heads [2,3,14].

Previously, we reported that the effect of rTMS depends on the stimulus intensity and that rTMS administered at the appropriate intensity (0.75 T) enhanced LTP in area CA1 in the rat hippocampus. These data suggest that 0.75 T rTMS

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might activate hippocampal neurons [19,20]. It was also reported that rTMS protected neurons in the hippocampal CA1 from delayed neuronal death induced by transient ischemia [8]. Therefore, it is possible that TMS might prove to be a useful therapeutic treatment for ischemia. In this study, we investigated the ability of 0.75 T rTMS to protect the hippocampal function of the rat from ischemic injury.

All experimental procedures performed in this study were approved by the Animal Ethics Committee of the University of Tokyo. Experiments were performed on male Wistar rats (4 weeks old, weight = 60 to 80 g; Saitama Experimental Animals Supply, Saitama, Japan). 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 using a round coil (inner diameter = 15 mm, outer diameter = 75 mm, thickness = 10 mm) positioned over the head of each rat. Conscious rats were held beneath the coil by the nape of the neck during the stimulation delivery. The stimulator (NIHON KOHDEN, Tokyo, Japan) delivered biphasic cosine current pulses for 238 μs. The peak magnetic field was set to 0.75 T at the center of the coil (80% of the motor threshold), which induces an eddy current of approximately 9 A/m^2 maximum in the brain [20]. 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. The coil was cooled during the intertrain intervals. The sham control rats were treated with a sham coil (that is, non-stimulated) and exposed to the same noise as that produced during the stimulation. After rTMS for 7 days in vivo, ischemia experiments were performed in vitro using electrophysiological methods.

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 dampened with artificial cerebrospinal fluid (ACSF) (composition (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 cut with a microslicer (D.S.K, Kyoto, Japan) into 400 μm transverse slice sections. The slices were incubated and then 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. After stimulation of Schaffer collaterals with a tungsten bipolar stimulating electrode, field excitatory postsynaptic potentials (fEPSP) were recorded from the dendrites of CA1 pyramidal cells using a tungsten recording electrode. A single stimulus was administered at 20-s intervals. The stimulus intensity was set to generate an fEPSP with a slope that was approximately 30% of the maximum, as determined from the input–output curve. After obtaining stable fEPSP recordings for 20 min, the ACSF was replaced by ischemic ACSF (composition (mM): NaCl 125, KCl 3, NaH_2PO_4 1.2, $NaHCO_3$ 26, $CaCl_2$ 2.0, $MgCl_2$ 1.0 and sucrose 10) bubbled with 95% N_2/5% CO_2 (pH 7.4). Ischemic periods were set to 5, 10, 30, 40 and 50 min. After 5, 10, 30, 40 and 50 min, ischemic ACSF was replaced by normal ACSF and fEPSP recordings were continued. fEPSP peaks were continuously measured before and after ischemia. When fEPSPs did not recover after ischemia, the recording was stopped. We defined the viability of the slices as the percentage of the slices with fEPSPs that recovered after ischemia (the number of recovered slices/the total number of slices). When the fEPSP recovered and became stable after ischemia, LTP was induced by tetanus stimulation (100 Hz for 1 s; 0.1 ms duration). fEPSP recordings continued for 60 min after tetanus stimulation. LTP is achieved if the potentiation is maintained for at least 1 h. Pre-tetanus and post-tetanus fEPSPs were compared using their maximum leftward slopes, and the fEPSP slope data for each experiment were expressed as percentages of the pre-tetanus EPSP baseline average. Each slice was used for only one experiment and then discarded. Data were analyzed using pCLAMP software (Axon Instrument). LTP without ischemia was also induced for each rat. All LTP data for each group were averaged and expressed as the mean ± standard error of the mean (S.E.M.). The average value of the maintenance phases of LTP (ranging from 10 min after tetanus stimulation to 60 min) were statistically analyzed by two-way ANOVA followed by post-hoc Dunnett's test. A probability (P) level of less than 0.05 was considered to be statistically significant.

Fig. 1 shows the viability of the slices after ischemia. The viabilities of the slices in the sham control groups after 5,

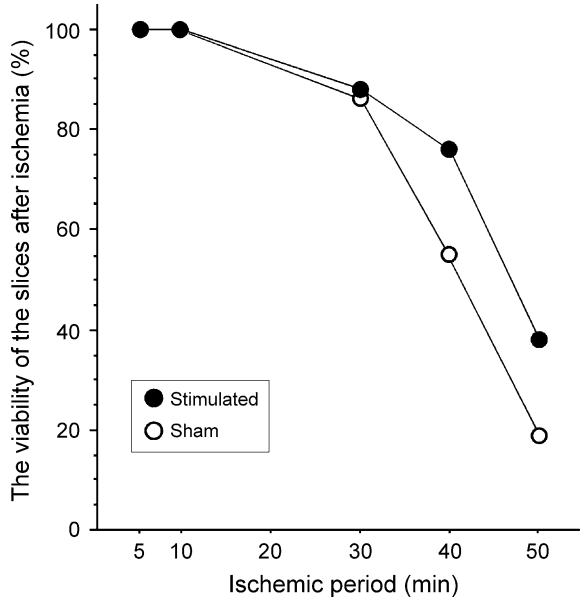

Fig. 1. The viability of the slices after ischemia (the number of recovered slices/the total number of slices). The viabilities in the sham control groups after 5, 10, 30, 40 and 50 min of ischemia were 100% (22/22), 100% (17/17), 86% (19/22), 55% (11/20) and 19% (3/16), respectively (open circle). The viabilities of the slices in the stimulated groups after 5, 10, 30, 40 and 50 min of ischemia were 100% (21/21), 100% (17/17), 88% (15/17), 76% (13/17) and 38% (5/13), respectively (closed circle).

10, 30, 40 and 50 min of ischemia were 100% (22/22), 100% (17/17), 86% (19/22), 55% (11/20) and 19% (3/16), respectively. The viabilities of the slices in the stimulated groups after 5, 10, 30, 40 and 50 min of ischemia were 100% (21/21), 100% (17/17), 88% (15/17), 76% (13/17) and 38% (5/13), respectively. With the extension of the period of ischemia, the viability of the slices decreased in both the stimulated and sham control groups. However, the viabilities of the slices of the stimulated groups were higher than those of the sham control groups under each ischemic condition, suggesting that 0.75 T rTMS has a protective effect from ischemic damage in the rat hippocampal CA1.

Fig. 2A shows examples of pre-tetanus and post-tetanus fEPSPs after each ischemic condition. Fig. 2B shows the LTPs of the stimulated and sham control groups after each ischemic condition, and Fig. 2C shows the average value of the maintenance phases of LTP (ranging from 10 min after tetanus stimulation to 60 min). The LTPs of the non-ischemia group, and the 5, 10 and 30 min ischemia groups, were 213 ± 7% (n = 42), 194 ± 9% (n = 21), 161 ± 13% (n = 14) and 142 ± 12% (n = 18) in the sham control groups, respectively. The LTPs of the non-ischemia group, and the 5, 10 and 30 min ischemia groups, were 265 ± 20% (n = 39), 217 ± 16% (n = 19), 201 ± 15% (n = 14), and 185 ± 16% (n = 14) in the stimulated groups, respectively. LTP levels in both the stimulated and sham control groups after 40 and 50 min of ischemia were also obtained. However, these data are not presented because the viabilities of the slices were low (Fig. 1), and sufficiently stable LTP data could not be obtained. The degree of LTP was suppressed depending upon the ischemic condition in both the stimulated and sham control groups ($F_{3,173}$ = 9.695, $P < 0.0001$), suggesting that a longer ischemic period leads to an increase in impairment in hippocampal function.

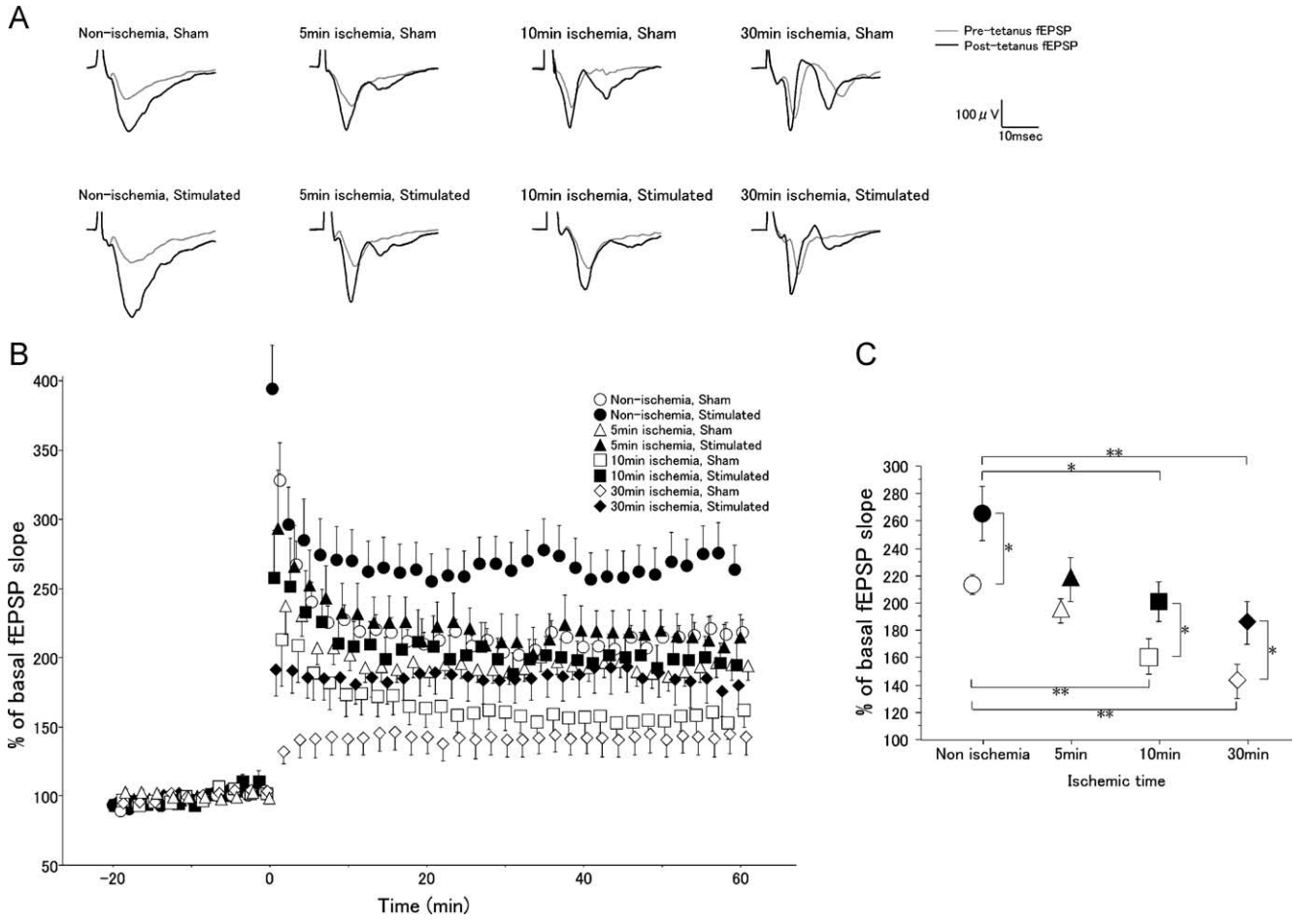

Fig. 2. (A) Examples of pre-tetanus and post-tetanus fEPSPs after each ischemic condition (gray line: pre-tetanus EPSP, black line: post-tetanus EPSP). (B) LTP in the stimulated and sham control groups in each ischemic condition. Tetanus stimulation was applied at time = 0 min (black arrow). Each symbol represents the average of six successive responses (for 2 min). Error bar = ±1 S.E.M. (C) The average value of the maintenance phases of LTP (ranging from 10 min after tetanus stimulation to 60 min). The LTPs in the non-ischemia group, and the 5, 10 and 30 min ischemia groups, were 213 ± 7% (n = 42, open circle), 194 ± 9% (n = 21, open triangle), 161 ± 13% (n = 14, open square) and 142 ± 12% (n = 18, open diamond) in the sham control groups, respectively. The LTPs in the non-ischemia group, and the 5, 10 and 30 min ischemia groups, were 265 ± 20% (n = 39, closed circle), 217 ± 16% (n = 19, closed triangle), 201 ± 15% (n = 14, closed square), and 185 ± 16% (n = 14, closed diamond) in the stimulated groups, respectively. Error bar = ±1 S.E.M. *$P < 0.05$, **$P < 0.01$.

The LTPs of the stimulated groups were enhanced compared with the sham control groups ($F_{1,173} = 10.857$, $P = 0.0012$), suggesting that 0.75 T rTMS has a protective effect from ischemic damage in the hippocampal function. The interaction between the group effect (the stimulated group and the sham control group) and the ischemic condition (non-ischemia, 5 min, 10 min, 30 min) was not statistically significant ($F_{3,173} = 0.349$, $P = 0.7901$).

Our results suggest that 0.75 T rTMS has the potential to protect hippocampal function from impairment due to ischemia-induced damage. Morphologically, Fujiki and colleagues [8] reported that rTMS protected neurons in the hippocampal CA1 from neuronal damage induced by transient ischemia. Our results support the findings of this previous study.

Previously, we reported that the effect of rTMS depends on the stimulus intensity, that rTMS administered at the appropriate stimulus intensity (0.75 T) enhanced LTP in the rat hippocampal CA1, and that 0.75 T rTMS might enhance hippocampal function [19,20]. Other studies reported that rTMS induces the gene expression of c-*fos*, glial fibrillary acidic protein (GFAP), which is the principal constituent of intermediate filaments in astrocytes, and brain-derived neurotrophic factor (BDNF) in the rat hippocampus [7,10,18]. Astrocytes are intimately associated with LTP [17], and BDNF induces long-lasting enhancement of synaptic transmission [11]. Therefore, it is possible that the 0.75 T rTMS enhanced hippocampal function by inducing the expression of genes that have important roles in maintaining neuronal function. This might be the mechanism by which the hippocampus was protected from ischemic damage.

The rTMS was used in this study as a "preconditioning" treatment; therefore, it is effective when delivered before ischemia occurs. Sub-threshold ischemic insult applied to the brain can help to reduce the damage caused by subsequent ischemic episodes—a phenomenon known as "ischemic preconditioning (IP)" [6]. The mechanism of IP is still unknown, but sub-threshold ischemia might induce the expression of neurotrophic factors and protect the brain from damage caused by subsequent ischemic episodes. Furthermore, any stimulus capable of causing injury to a tissue or organ might activate endogenous protective mechanisms when applied close to (but below) the threshold of damage [6]. Electrical stimulation is able to damage living tissues, but the eddy current induced by rTMS is below the threshold of damage. Therefore, we suggest that preconditioning rTMS has a similar role to IP, and has the potential to activate endogenous protective mechanisms.

Recent studies have focused on applying electrical stimulation to the brain via electrodes to potentially reduce ischemic symptoms [9], however, the invasiveness of electrical stimulation (for example, surgery and anesthetization) reduces its attractiveness as a therapeutic treatment. In contrast, rTMS is a painless and noninvasive method of brain stimulation. We suggest that rTMS administered at the appropriate stimulus intensity might become an important therapeutic tool for the treatment of ischemia. Further studies in vitro and in vivo as well as pathological studies are necessary to clarify the ability of rTMS to protect against ischemia-induced neural damage.

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References

[1] A.T. Barker, R. Jalinous, I.L. Freeston, Non-invasive magnetic stimulation of human motor cortex, Lancet 1 (1985) 1106–1107.
[2] J.M. Bekkers, C.F. Stevens, Presynaptic mechanism for long-term potentiation in the hippocampus, Nature 346 (1990) 724–972.
[3] T.V. Bliss, G.L. Collingridge, A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361 (1993) 31–39.
[4] 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) 331–356.
[5] D.W. Choi, S.M. Rothman, The role of glutamate neurotoxicity in hypoxic–ischemic neuronal death, Annu. Rev. Neurosci. 13 (1990) 171–182.
[6] U. Dirnagl, R.P. Simon, J.M. Hallenbeck, Ischemic tolerance and endogenous neuroprotection, Trends Neurosci. 26 (2003) 248–254.
[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] M. Fujiki, H. Kobayashi, T. Abe, T. Kamida, Repetitive transcranial magnetic stimulation for protection against delayed neuronal death induced by transient ischemia, J. Neurosurg. 99 (2003) 1063–1069.
[9] S.B. Glickstein, C.P. Ilch, D.J. Reis, E.V. Golanov, Stimulation of the subthalamic vasodilator area and fastigial nucleus independently protects the brain against focal ischemia, Brain Res. 912 (2001) 47–59.
[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] H. Kang, E.M. Schuman, Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus, Science 267 (1995) 1658–1662.
[12] T. Kirino, Delayed neuronal death in the gerbil hippocampus following ischemia, Brain Res. 239 (1982) 57–69.
[13] E. Klein, I. Kreinin, A. Chistyakov, D. Koren, L. Mecz, S. Marmur, D. Ben-Shachar, M. Feinsod, Therapeutic efficacy of right prefrontal slow repetitive transcranial magnetic stimulation in major depression: a double-blind controlled study, Arch. Gen. Psychiatry 56 (1999) 315–320.
[14] 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) 247–258.
[15] J. Mally, T.W. Stone, Improvement in Parkinsonian symptoms after repetitive transcranial magnetic stimulation, J. Neurol. Sci. 162 (1999) 179–184.

[16] 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.
[17] 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. 93 (1996) 6361–6366.
[18] 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.
[19] M. Ogiue-Ikeda, S. Kawato, S. Ueno, The effect of transcranial magnetic stimulation on long-term potentiation in rat hippocampus, IEEE Trans. Magn. 39 (2003) 3390–3392.
[20] M. Ogiue-Ikeda, S. Kawato, S. Ueno, The effect of repetitive transcranial magnetic stimulation on long-term potentiation in rat hippocampus depends on stimulus intensity, Brain Res. 993 (2003) 222–226.
[21] A. Pascual-Leone, B. Rubio, F. Pallardo, M.D. Catala, Rapid-rate transcranial magnetic stimulation of left dorsolateral prefrontal cortex in drug-resistant depression, Lancet 348 (1996) 233–237.
[22] 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.