Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 327 (2005) 218–224
BBRC
www.elsevier.com/locate/ybbrc

Modulation of monoamine transporter expression and function by repetitive transcranial magnetic stimulation

Tetsurou Ikeda[a], Masaru Kurosawa[a], Chiharu Uchikawa[a], Shigeo Kitayama[b], Nobuyuki Nukina[a,*]

[a] *Laboratory for Structural Neuropathology, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan*
[b] *Department of Dental Pharmacology, Okayama University, Graduate School of Medicine and Dentistry, Shikata 2-5-1, Okayama 700-8525, Japan*

Received 26 November 2004
Available online 10 December 2004

Abstract

Repetitive transcranial magnetic stimulation (rTMS) is a new tool for the treatment of neuropsychiatric disorders. However, the mechanisms underlying the effects of rTMS are still unclear. In this study, we analyzed mRNA expression changes of monoamine transporter (MAT) genes, which are targets for antidepressants and psychostimulants. Following a 20-day rTMS treatment, these genes were found to be differentially expressed in the mouse brain. Down-regulation of serotonin transporter (SERT) mRNA levels and the subsequent decrease in serotonin uptake and binding were observed after chronic rTMS. In contrast to the SERT changes, increased mRNA levels of dopamine transporter (DAT) and norepinephrine transporter (NET) were observed. For NET, but not DAT, there were accompanying changes in uptake and binding. Similar effect on NET was observed in PC12 cells stimulated by rTMS for 15 days. These results indicate that modulation of MATs by chronic rTMS may be one therapeutic mechanism for the treatment of neuropsychiatric disorders.

Keywords: Repetitive transcranial magnetic stimulation; Serotonin transporter; Dopamine transporter; Norepinephrine transporter; Monoamine transporter; Neuropsychiatric disorders; Uptake; Binding

Repetitive transcranial magnetic stimulation is a novel tool which is considered to induce electric current in the brain through a coil [1–4]. Initially, Merton and Morton [5] showed that it was possible to stimulate the motor areas of the human brain electrically through the intact scalp (transcranial electrical stimulation, TES). Barker and his group later showed that it was possible to stimulate both nerves and the brain using external magnetic stimulation [1], a technique known as transcranial magnetic stimulation (TMS). Recently, TMS has been used as a diagnostic tool in neurology for measuring the central motor conduction, because it is painless and noninvasive [6]. In contrast, electroconvulsive therapy (ECT) is known to provide highly reliable relief from depressive symptoms, however, intense electrical stimulation is required because the skull resists electric currents [7]. The intensity of stimulation usually used to treat patients induces a sustained after-discharge in cortical neurons, which causes convulsive seizures. Once TMS was introduced, TMS was found to have several therapeutic benefits for neuropsychiatric disorders such as depression, Parkinson's disease, and schizophrenia [8–10]. These neuropsychiatric disorders are considered to be associated with monoamine systems. Because of its safety and relative painlessness, TMS has many possible applications as a therapeutic device, and may be beneficial for treating some psychiatric disorders that have not yet been explored. If it ultimately proves useful for the treatment of neuropsychiatric disorders, TMS may well become a standard medical tool.

* Corresponding author. Fax: +81 48 462 4796.
E-mail address: nukina@brain.riken.jp (N. Nukina).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.12.009

The precise molecular mechanisms underlying the effects of TMS are unknown. Recent studies have reported monoamine release measurement using microdialysis and imaging study using raclopride after acute rTMS [11,12]. But there are few reports about monoamine transporters following either acute or chronic rTMS. This prompted us to investigate the molecular effects of TMS in rodents by evaluating the expression of monoamine transporters (MATs). We found that the expression levels of MATs, including the serotonin transporter, were modulated. Our study also showed that serotonin re-uptake was decreased after rTMS, suggesting that rTMS modulates MAT gene expression and function, and may be used in the treatment of several neuropsychiatric disorders.

Materials and methods

Mice and conditions of rTMS. Male C57Black R6 mice (8 weeks old, 20–25 g) were chronically treated for 20 days ($n = 42$) or acutely for 1 day ($n = 24$) with rTMS. Mice were housed in a light-controlled room (8:00 a.m. on, 8:00 p.m. off). Stimulation was performed using a round-coil (7.5 cm outer diameter) and a Nihon Kohden Rapid Rate Stimulator (Nihon Kohden, Japan). Stimulation conditions were as follows: 20 Hz, 2 s; 20 times/day; inter-stimulus interval 1 min (30% machine output, representing about 0.75 T). The coil was placed over the head without touching the skull. Sham control mice were 'stimulated' from a distance of more than 10 cm from the head. rTMS did not produce either notable seizures or changes in behavior, such as excessive struggling. Twenty-four hours after the last stimulation, the animals were sacrificed and their brains were processed for further analysis.

RNA extraction. Whole mouse brain was divided at the midbrain into cerebrum and cerebellum with brain stem (CBS). Total RNA was isolated from cerebrum and CBS by acid–phenol extraction [13]. Poly(A)$^+$ RNA was isolated from the samples using an mRNA purification kit (TaKaRa Bio, Japan).

Expression analysis by TaqMan real-time RT-PCR. We synthesized the TaqMan primer and probe sets with Primer Express Software (Applied Biosystems, Foster City, CA). The nucleotide sequence of the primers is shown below. Total RNA was extracted from the mice brains by acid–phenol extraction [13]. Contaminating genomic DNA was removed with RNase-free DNase I (TaKaRa Bio, Japan). Complementary DNAs were synthesized using MMLV Reverse Transcription Reagents (Invitrogen, Carlsbad, CA). We used 1 μg of mRNA for the 100 μl reaction. The TaqMan PCR was performed as follows. We used 15 μl of TaqMan Universal PCR Master Mix (Applied Biosystems) in a 30 μl reaction. Primers and probes in optimal concentrations were added. We used 1 μl of RT mix for each PCR. Each sample was amplified in duplicate and the experiment was repeated at least three times. PCR conditions were standard for the 7700 Sequence Detector System (Applied Biosystems): 2 min at 50 °C, 10 min at 95 °C followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min. The mRNA quantity for the gene of interest was normalized by the quantity of GAPDH in each sample. Primers and TaqMan probes are as follows: (forward primer, reverse primer, and TaqMan probe): SERT: 5′-AGACGTGTCCGAGGTGGC-3′, 5′-GCTATTGCCTC CGCATATGTG-3′, 5′-FAM-CGGGCCCCAGCCTCCTTTTCA-TAMURA-3′; DAT: 5′-GGCTCTCGGGCAGTTCAA-3′, 5′-CACCT TTCAGGACAGGGCA-3′, 5′-FAM-AGAGAAGGAGCTGCTGG TGTCTGGAAGAT-TAMURA-3′; NET: 5′-GCATCGCCCTCTCA TCCAT-3′, 5′-GCCGCGTATGCTGAGGAA-3′, 5′-FAM-TCCTGG TGCCTGCCTATGTCATCTACAA-TAMURA-3′; c-fos: 5′-CCCC TTCTCAACGACCCTG-3′, 5′-GCTCCACGTTGCTGATGCT-3′, 5′-FAM-CCAAGCCATCCTTGGAGCCAGTCAA-TAMURA-3′; and junB: 5′-GCGTCTATGCTGGTCCGG-3′, 5′-TGCAGAGGC TGGAGAGTAACTG-3′, 5′-FAM-CCGCCTCCCGTCTACACCAA CCT-TAMURA-3′.

Monoamine uptake assay. Synaptosomes were prepared as previously described [14] using oxygenated Krebs–Ringer Hepes-buffered solution (KRH: 125 mM NaCl, 5.2 mM KCl, 1.2 mM $CaCl_2$, 1.4 mM $MgSO_4$, 1.2 mM KH_2PO_4, 5 mM glucose, and 20 mM Hepes, pH 7.3) and incubated for 5 min at 37 °C with [^{3}H]NE (293.78 Gbq/mmol Perkin–Elmer, Japan), [^{3}H]SE (1002.7 Gbq/mmol Perkin–Elmer), and [^{3}H]DA (1968.4 Gbq/mmol Perkin–Elmer) in KRH containing 50 μM pargyline (Sigma, St. Louis, MO, USA), 50 μM OR486 (Sigma), and 100 μM ascorbate (Sigma). Ten micromolar nisoxetine and 10 μM citalopram were added for total DA uptake, 10 μM nisoxetine and 10 μM GBR12935 were added for total SE uptake, and 10 μM citalopram and 10 μM GBR12935 were added for total NE uptake. Synaptosomes were washed four times rapidly with ice-cold KRH using filters (Whatman GF/B, England) for removal of excess radioligands, and any radioactivity remaining in the filters was measured by liquid scintillation spectrometry (TRI-CARB2900TR, Packard, Japan). Nonspecific uptake was determined in the presence of 10 μM nisoxetine (Sigma), 10 μM citalopram (Sigma), and 10 μM GBR12935 (Sigma) or 100 μM cocaine (Sankyo, Japan).

Ligand binding assay for monoamine transporter. Synaptosomes were prepared by the above methods, using ice-cold buffer (50 mM Tris–HCl, 120 mM NaCl, and 5 mM KCl) and incubated for 4 h at 4 °C with [^{3}H]nisoxetine (3163.5 Gbq/mmol), [^{3}H]GBR1235 (1609.5 Gbq), and [^{3}H]citalopram (9.08 Gbq/mmol Amersham, USA), in binding buffer (50 mM Tris–HCl, 300 mM NaCl, and 5 mM KCl) containing 10 μM nisoxetine and 10 μM citalopram (for DAT binding), 10 μM nisoxetine and 10 μM GBR12935 (for SERT binding), and 10 μM citalopram and 10 μM GBR12935 (for NET binding). Synaptosomes were washed four times rapidly with ice-cold buffer using filters (Whatman GF/B) for removal of excess radioligands, and any radioactivity remaining in the filters was measured by liquid scintillation spectrometry. Nonspecific uptake was determined in the presence of 10 μM nisoxetine, 10 μM citalopram, and 10 μM GBR12935.

Cell culture. PC12 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS) at 37 °C under 5% CO_2/95% air. Cells at subconfluence were harvested, diluted in culture medium, and plated in a 24-well culture plate for the uptake assay or in a 25 cm^2 culture flask for total RNA extraction. The cells were then cultured with or without daily stimulation of chronic rTMS.

NE uptake for cells. Cells grown in culture were washed three times with oxygenated Krebs–Ringer Hepes-buffered solution (KRH:125 mM NaCl, 5.2 mM KCl, 1.2 mM $CaCl_2$,1.4 mM $MgSO_4$, 1.2 mM KH_2PO_4, 5 mM glucose, and 20 mM Hepes, pH 7.3) and incubated for 10 min at 37 °C with [^{3}H]NE (293.78 Gbq/mmol) in KRH containing 50 μM pargyline and 100 μM ascorbic acid [15]. After removal of excess radioligands, the cells were washed three times rapidly with ice-cold KRH and any radioactivity remaining in the cells was extracted with NaOH and measured by liquid scintillation spectrometry. Proteins were measured by Bradford methods. Nonspecific uptake was determined in the presence of 10 μM nisoxetine.

Reporter assay. PC12 cells stimulated by rTMS for 13 days were placed in 35 mm petri dishes, and cells were grown to about 40–60% confluency to perform transfection (rTMS stimulation was maintained for 2 days after this transfection). At 14 days treated with rTMS, transient transfection of PC12 cells with NET2.1 luciferase [16] and control luciferase was performed using Lipofectamine2000 (Invitrogen), according to the manufacturer's protocol. At 24 h after the final stimulation, luciferase activity in the transfected cells was measured using a luminometer (1420MULTI LABEL COUNTER, WALLAC, Finland).

^{3}H-labeled autoradiography. We prepared 10-μm frozen sections of mouse brain treated by rTMS for 20 days using a freezing microtome. Adjacent sections were labeled with 5 nM [^{125}I]RTI-55 (Perkin–Elmer) [17], [^{3}H]GBR12935, and [^{3}H]nisoxetine. Autoradiograms were obtained using an Imaging Plate (Fuji Film, Japan). Photomicrographs were measured using BAS2500 (Fuji Film, Japan). Autoradiograms were quantitatively analyzed using Image Gauge 3.4 (Fuji Film).

Data analysis. The data were represented as means ± SE of at least three independent experiments, each performed in triplicate or duplicate. Statistical analysis was performed using ANOVA (Figs. 2 and 5) and Student's *t* test as appropriate.

Results

Expression changes of MAT genes in mouse brain stimulated by rTMS

We used 8-week-old C57Black mice and stimulated their brains for 20 days by rTMS. We prepared mRNA from cerebrum and cerebellum with brain stem (CBS) from rTMS-treated mice and control mice. Then we analyzed MAT expression in mouse brain stimulated by rTMS using TaqMan RT-PCR analysis, and found that serotonin transporter (SERT) mRNA was decreased significantly in CBS, but not in the cerebrum (Figs. 1A and B). To evaluate the effects on the other monoamine transporters by rTMS, we further measured dopamine transporter (DAT) and norepinephrine transporter (NET) mRNA levels by RT-PCR. DAT mRNA expression increased in cerebrum (Fig. 1A). NET mRNA expression increased in CBS, but not in the cerebrum (Figs. 1A and B). To further investigate the effects of rTMS on monoamine transporter genes, we measured these mRNAs at different times with different amounts of stimuli. After 10 days of rest following 20 days of rTMS, MAT mRNA levels were measured to investigate the long-term effects of rTMS. DAT mRNA level still increased in cerebrum (Fig. 1C). Although SERT mRNA levels in cerebrum recovered to the control level, the SERT mRNA level still decreased in CBS (Fig. 1D). However, NET expression became rather decreased in CBS after 10 days of rest (Figs. 1C and D). Conversely, to check the immediate effect of rTMS, we examined MAT expression levels at 1, 4, 12, and 24 h after acute rTMS (single repetitive stimulation). SERT mRNA levels increased in CBS at 1 h (Fig. 2A). NET mRNA levels decreased in cerebrum at 1 h and did not change in CBS (Fig. 2C).

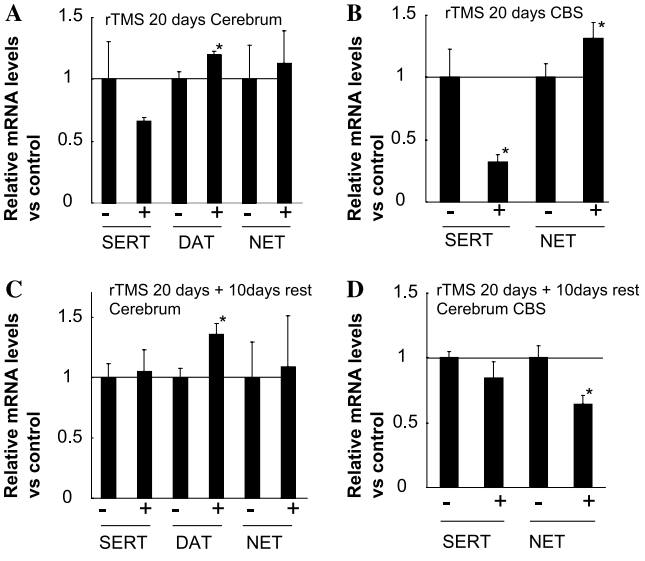

Fig. 1. Effects of chronic rTMS on SERT, DAT, and NET mRNA levels. (A,B) Effects of chronic rTMS on SERT, DAT, and NET mRNA levels on the cerebrum (A) and CBS (B) assessed by TaqMan RT-PCR. (C,D) Effects of rTMS treatment for 20 days followed by 10 days without stimulation on the cerebrum (C) and CBS (D). Values represent means ± SEM of three experiments each performed in duplicate. *Significantly different from control at $P < 0.05$.

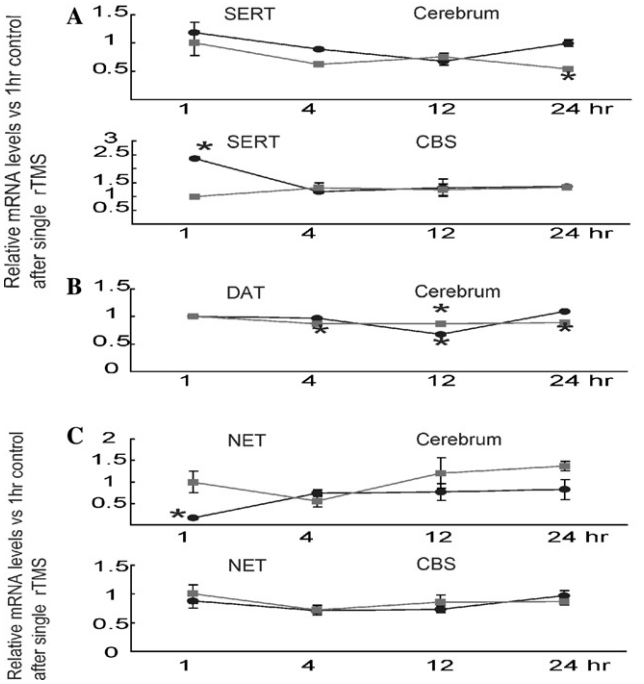

Fig. 2. Effects of acute rTMS on SERT, DAT, and NET mRNA levels. (A–C) Effects of acute rTMS on SERT, DAT, and NET mRNA levels. Mouse brains were treated by single rTMS, designated acute rTMS. The mRNA level was determined within 24 h after stimulation. Filled circle: rTMS-treated; filled square: control. Upper panels in (A,C) and (B) show the cerebrum and lower panels of (A,C) show the CBS. Values represent means ± SEM of three experiments each performed in duplicate. *Significantly different from control 1 h at $P < 0.05$ (ANOVA).

Effects of chronic rTMS on MAT function in vivo.

We next investigated the effect of chronic rTMS on MAT function in vivo. Effects of rTMS on monoamine uptake were examined using the synaptosomes of mouse whole brain at 24 h after a 20-day rTMS treatment. We found that [^{3}H]serotonin (SE) uptake was decreased, but

[^{3}H]dopamine (DA) and [^{3}H]norepinephrine (NE) were increased. Kinetic analysis revealed a decrease in both K_m and V_{max} (28.2% decrease of K_m and 21.0% decrease of V_{max} compared to control), reflecting an increase in apparent affinity for substrate and decrease in transport rate for SE uptake (Table 1). Kinetic analysis for DA uptake only revealed an increase in V_{max} (26.0% increase of V_{max} compared to control), reflecting an increase in transport rate. Kinetic analysis for NE uptake only revealed an increase in V_{max} (34.4% increase of V_{max} compared to control), reflecting an increase in transport rate. To confirm these effects, we used a [^{3}H] ligand binding assay with the synaptosomes of mouse whole brain 24 h after rTMS treatment for 20 days. We verified that [^{3}H]citalopram binding was decreased, and [^{3}H]GBR12935 and [^{3}H]nisoxetine binding to synaptosomes was increased. In the binding assay, a decrease in both K_D and B_{max} of [^{3}H]citalopram binding was observed (75% decrease of K_D and 67.6% decrease of B_{max} compared to control). There were no differences in the K_D or B_{max} of [^{3}H]GBR12935 binding, whereas we found a decrease in K_D and increase in B_{max} of [^{3}H]nisoxetine binding (33.5% decrease of K_D and 187.7% increase of B_{max} compared to control). The results confirmed the rTMS effects at the protein level using several ligands. In addition, we performed ^{3}H-, ^{125}I-labeled autoradiography using frozen sections to observe which brain regions are specifically affected by rTMS. [^{125}I]RTI-55 autoradiography showed a signal decrease in the raphe nucleus after 20 days of rTMS (Fig. 3A). There were no differences detectable by [^{3}H]GBR12935 autoradiography in the striatum, comparing 20-day rTMS treatment with the control (Fig. 3B). [^{3}H]Nisoxetine autoradiography showed an increased signal in the locus coeruleus (LC) after the 20-day rTMS compared to control (Fig. 3C).

Table 1
Kinetic analysis of effects of rTMS on monoamine uptake of [^{3}H]SE, [^{3}H]DA, [^{3}H]NE and on binding of [^{3}H]citaroplam, [^{3}H]GBR12935, [^{3}H]nisoxetine

	rTMS−	rTMS+
Uptake		
Serotonin		
K_m	3.9 ± 0.6	2.8 ± 0.9*
V_{max}	167608.3 ± 941	132275.1 ± 10583*
Dopamine		
K_m	2.9 ± 0.2	3.3 ± 1.2
V_{max}	320261.5 ± 13017.9	433333.3 ± 33333.3*
Norepinephrine		
K_m	2.9 ± 0.2	2.6 ± 0.6
V_{max}	333333.7 ± 0.4	508771.9 ± 8771.9*
Binding		
Citalopram		
K_D	3.3 ± 0.6	0.8 ± 0.07*
B_{max}	77.2 ± 14.7	35 ± 2.6*
GBR12935		
K_D	1.3 ± 0.4	2.1 ± 0.8
B_{max}	1726.2 ± 390.3	2222.2 ± 555.6
Nisoxetine		
K_D	13.1 ± 0.01	8.7 ± 2.3*
B_{max}	13.1 ± 2.8	37.7 ± 4.9*

Values represent means ± SEM of three experiments each performed in duplicate.
* Significantly different from control at $P < 0.05$.

Effects of chronic rTMS on the NET functions in PC12 cells

To confirm these effects in vitro, we treated PC12 cells for 15 days by rTMS under the same conditions used for the mice. We first investigated the activity of the NET promoter in PC12 cells treated by chronic rTMS. Chronic rTMS increased the luciferase activity driven by the rat NET2.1 promoter compared to the control in PC12 cells (Fig. 4A). Next, we measured mRNA levels of NET in rTMS-treated PC12 cells and found an increase in NET mRNA (Fig. 4B). We further investigated the function of NET in PC12 cells treated by chronic rTMS. A [^{3}H]NE uptake assay was performed. Kinetic analysis only revealed an increase in V_{max}, reflecting an increase in transport rate for NE uptake (K_m: rTMS treated, 3.3 ± 0.1 μM; control, 3.2 ± 0.001 μM; V_{max}: rTMS treated, 254167.0 ± 29581.9 fmol/μg of protein/min; control,

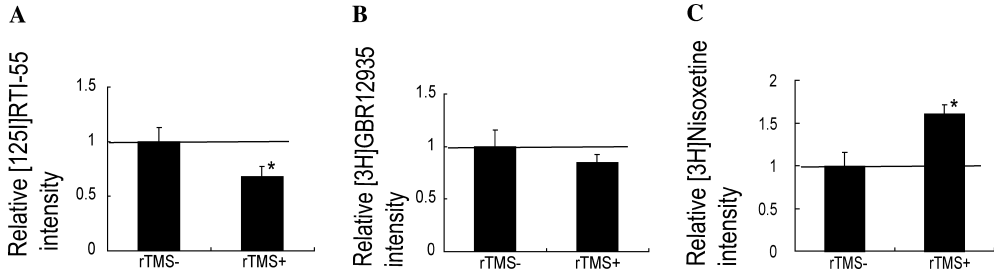

Fig. 3. [^{125}I]RTI-55, [^{3}H]GBR12935, and [^{3}H]nisoxetine binding in brain sections. Adjacent sections were labeled with [^{125}I]RTI-55, [^{3}H]GBR12935, and [^{3}H]nisoxetine, and the autoradiograms were obtained using an imaging plate (Fuji Film). Photomicrographs were obtained by directly photographing the film images. The autoradiograms of each area were quantitatively analyzed. Values represent means ± SEM of three experiments each performed in three adjacent sections. *Significantly different from control at $P < 0.05$.

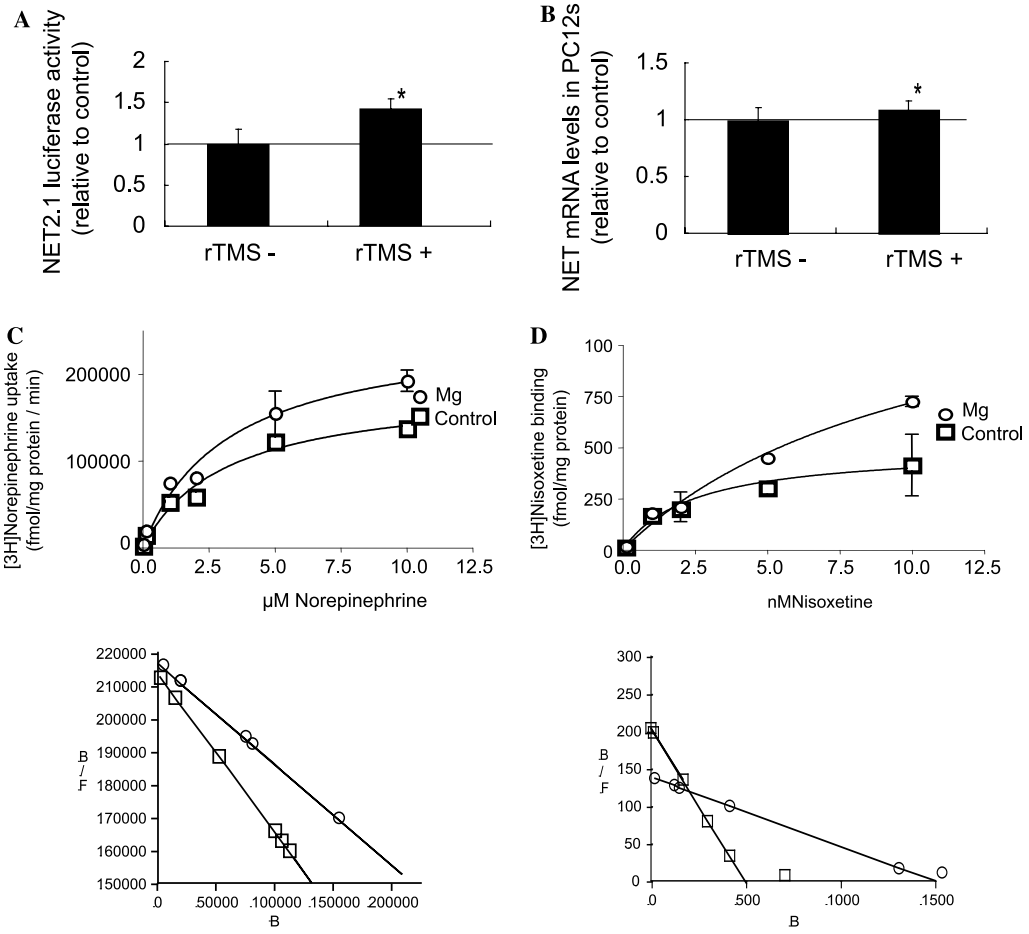

Fig. 4. Effects of chronic rTMS (15 days) on NET promoter activity, NET mRNA levels, [^{3}H]NE uptake, and the binding of [^{3}H]nisoxetine in PC12 cells. (A) Effects of chronic rTMS on the transcriptional activity of the rat NET promoter. Values represent means ± SEM, $N = 3$ (triplicate). *$P < 0.05$ vs control. (B) Effects of chronic rTMS on NET, mRNA levels in PC12 cells. (C) Kinetic analysis of the effects of chronic rTMS on [^{3}H]NE uptake in PC12 cells. Lower panel shows the Eadie–Hofstee plot analysis of [^{3}H]NE uptake. Each point represents the mean of three independent experiments obtained in (C, upper panel). (D) Nisoxetine binding assay-specific ligands for NET were evaluated using radiolabeled nisoxetine. The lower panel shows the saturation analysis of [^{3}H]nisoxetine. Points and bars represent means ± SEM, $N = 3$. Lower panel shows the Eadie–Hofstee plot of [^{3}H]nisoxetine binding. Each point represents the mean of three independent experiments obtained in (D, upper panel). Values represent means ± SEM of three experiments each performed in duplicate. *Significantly different from control at $P < 0.05$.

184958.6 ± 6710.7 fmol/μg of protein/min) (Fig. 4C). To confirm these effects at the protein level, we performed a [^{3}H]nisoxetine binding assay. In the binding assay, an increase in both K_D and B_{max} of [^{3}H]nisoxetine binding was observed (K_D: rTMS treated, 10.77 ± 4.031 nM; control, 2.436 ± 2.095 nM; B_{max}: rTMS treated, 1493 ± 329.9 fmol/μg of protein/min; control, 496.8 ± 142.0 fmol/μg of protein/min) (Fig. 4D).

Effects of chronic rTMS on the c-fos and junB mRNA levels in the mouse brain

Previous study showed the changes in the c-fos mRNA levels after acute rTMS. We therefore measured c-fos and junB mRNA by real-time RT-PCR. The expression of both genes increased at 1 h after acute rTMS, whereas a decrease in mRNA levels of both genes was observed after chronic rTMS (Fig. 5).

Discussion

rTMS is widely used to treat neuropsychiatric disorders, but the mechanism is not well known. To study the effects of chronic rTMS, we treated mouse brains with a chronic rTMS protocol for 20 days. Mice treated by only single rTMS (acute TMS) showed increased c-fos expression similar to a previous report [18], while mouse brains treated with chronic rTMS in this study showed a decrease in c-fos expression. There are no changes in jun-B expression. In addition, we found chronic rTMS changed MAT expression.

MATs, which include SERT [19], DAT [20–22], and NET [23,24], function to terminate monoaminergic synaptic transmission rapidly by taking up monoamines (SE, DA, and NE) released from nerve terminals. SERT, DAT, and NET are thought of as major targets for antidepressants, cocaine and amphetamines, since these

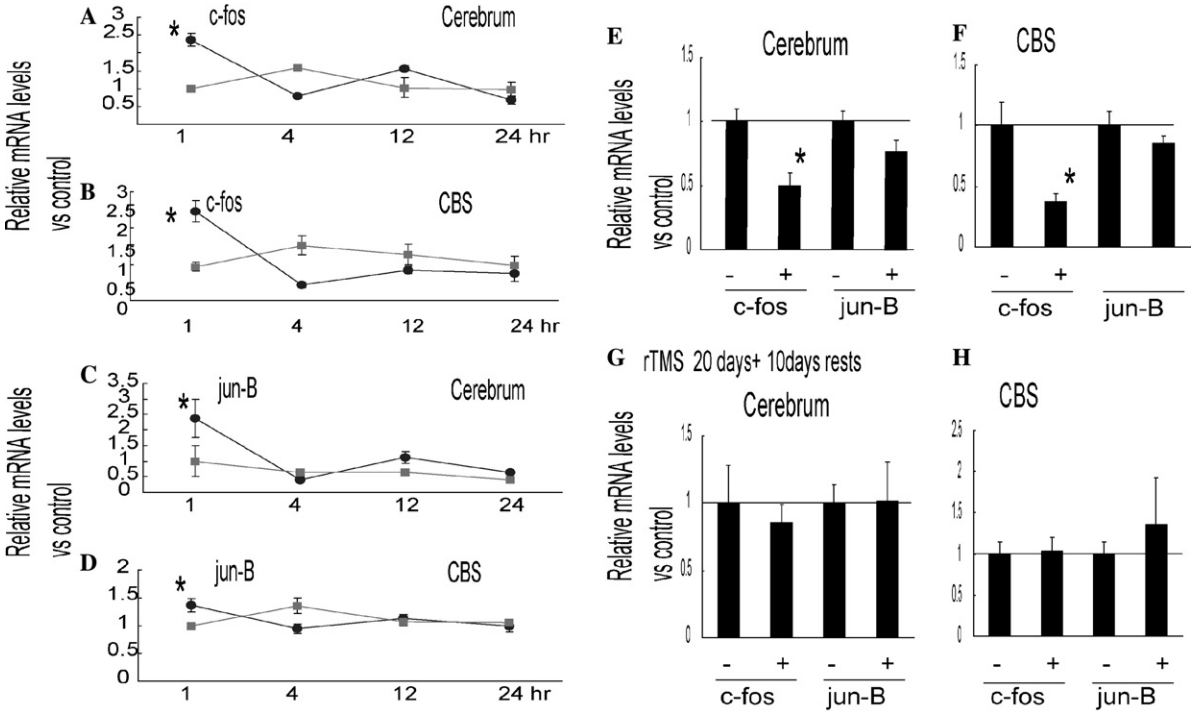

Fig. 5. Effects of acute rTMS on c-fos and junB mRNA levels. (A–D) Effects of acute rTMS on c-fos and junB mRNA levels. Mouse brain was stimulated by single rTMS. (A,B) The cerebrum (C,D) the CBS. Filled circle: rTMS-treated; filled square: control. (E–H) Effects of chronic rTMS on c-fos, junB mRNA levels. Mouse brain was treated by rTMS for 20 days (E,F) and kept for 10 days without stimulation (G,H). (E,G) The cerebrum (F,H) the CBS. Values represent means ± SEM of three experiments each performed in duplicate. *Significantly different from control at $P < 0.05$. We used ANOVA for acute effects. *Significantly different from 1 h control at $P < 0.05$.

drugs can block the re-uptake of monoamines effectively in vitro and in vivo [25–27]. By studying the effect of chronic rTMS on gene expression in mice, using an uptake assay, a ligand binding assay, and autoradiography, we confirmed that chronic rTMS down-regulated SERT mRNA expression, resulting in a functional change similar to SSRI treatment. On the other hand, chronic rTMS increased NET mRNA, NE uptake, and binding in mouse brains. Furthermore, even in PC12 cells treated with rTMS for 15 days, the same changes were observed for NET expression. Results from a luciferase assay of the NET promoter, RT-PCR analysis, and uptake and binding assays indicated that chronic rTMS increased the transcription of NET and the number of NET molecules on the membrane. Because K_m and K_D were changed, modifiers of NET may be involved in these changes. Neuronal activity, drugs, and MAT modifiers such as PKC and PICK1 may modulate the transporter expression at various stages such as transcription, translation or sorting, as well as the stability of the transporter protein in the plasma membrane [25–27]. The change in SERT expression was different from those of DAT and NET after chronic rTMS. The increase in NET mRNA expression and function after chronic rTMS is opposite to the effects of antidepressants on NET. Thus, the clinical significance of our results on mood disorders requires further investigations.

Hausmann et al. [28] reported that rTMS does not affect tyrosine hydroxylase and dopamine-β-hydroxylase (DBH) expression in rats in vivo. In contrast to Hausman's data, we confirmed the up-regulation of DBH and unchanged tyrosine hydroxylase and tryptophan hydroxylase mRNA levels by chronic rTMS (data not shown). This discrepancy may be due to the different coil size or different species, which activate different regions in the brain.

Ji et al. [18] reported that acute rTMS induced the transcription of c-fos mRNA in the paraventricular nucleus of the thalamus (PVT) in the rat brain. In our experiments, at 1 h after acute rTMS, c-fos mRNA was increased. However, after chronic rTMS, RT-PCR analyses revealed a decrease in c-fos mRNA levels. Ji et al. suggested that circadian rhythm genes change after c-fos induction by acute rTMS, preventing seasonal depression. We also found changes in mRNA levels of circadian rhythm genes. Chronic rTMS decreased Period2 mRNA levels and increased Period3, BMAL1, Casein Kinase 1 epsilon, and Clock mRNA levels (data not shown). So our data are partly consistent with previous data. PVT connects with the LC, and the LC projects to the hypothalamus and raphe nucleus [29–31]. Thus, chronic rTMS may change NET expression in LC and SERT expression in the raphe nucleus indirectly through its connection with PVT, although we cannot exclude the possibility of direct activation by rTMS as

suggested by the result of our in vitro study on NET expression changes in PC12.

Our results suggest the involvement of MAT expression changes in the therapeutic effects of chronic rTMS. Further research on the regulation of MAT expression by chronic rTMS will be needed to develop more effective rTMS therapies for neuropsychiatric disorders in which monoaminergic systems are involved. Moreover, it will be important to fine-tune the method or apparatus in order to stimulate certain brain areas more specifically, to reduce unnecessary side-effects.

Acknowledgment

This work was supported partly by a grant from the JSPS (Japan Society for the Promotion of Science).

References

[1] A.T. Barker, I.L. Freeston, R. Jalinous, J.A. Jarratt, Magnetic stimulation of the human brain and peripheral nervous system: an introduction and the results of an initial clinical evaluation, Neurosurgery 20 (1987) 100–109.
[2] A. Post, M.E. Keck, Transcranial magnetic stimulation as a therapeutic tool in psychiatry: what do we know about the neurobiological mechanisms? J. Psychiatr. Res. 35 (2001) 193–215.
[3] M. Hallett, Transcranial magnetic stimulation and the human brain, Nature 406 (2000) 147–150.
[4] B.J. Roth, J.M. Saypol, M. Hallett, L.G. Cohen, A theoretical calculation of the electric field induced in the cortex during magnetic stimulation, Electroencephalogr. Clin. Neurophysiol. 81 (1991) 47–56.
[5] P.A. Merton, H.B. Morton, Stimulation of the cerebral cortex in the intact human subject, Nature 285 (1980) 227.
[6] M.S. George, E.M. Wassermann, R.M. Post, Transcranial magnetic stimulation: a neuropsychiatric tool for the 21st century, J. Neuropsychiatry Clin. Neurosci. 8 (1996) 373–382.
[7] T. Zyss, Deep magnetic brain stimulation—the end of psychiatric electroshock therapy?, Med. Hypotheses 43 (1994) 69–74.
[8] T. Paus, J. Barrett, Transcranial magnetic stimulation (TMS) of the human frontal cortex: implications for repetitive TMS treatment of depression, J. Psychiatry Neurosci. 29 (2004) 268–279.
[9] N. Dragasevic, A. Potrebic, A. Damjanovic, E. Stefanova, V.S. Kostic, Therapeutic efficacy of bilateral prefrontal slow repetitive transcranial magnetic stimulation in depressed patients with Parkinson's disease: an open study, Mov. Disord. 17 (2002) 528–532.
[10] M.S. George, S.H. Lisanby, H.A. Sackeim, Transcranial magnetic stimulation: applications in neuropsychiatry, Arch. Gen. Psychiatry 56 (1999) 300–311.
[11] M. Kanno, M. Matsumoto, H. Togashi, M. Yoshioka, Y. Mano, Effects of acute repetitive transcranial magnetic stimulation on dopamine release in the rat dorsolateral striatum, J. Neurol. Sci. 217 (2004) 73–81.
[12] T. Ohnishi, T. Hayashi, S. Okabe, I. Nonaka, H. Matsuda, H. Iida, E. Imabayashi, H. Watabe, Y. Miyake, M. Ogawa, N. Teramoto, Y. Ohta, N. Ejima, T. Sawada, Y. Ugawa, Endogenous dopamine release induced by repetitive transcranial magnetic stimulation over the primary motor cortex: an [^{11}C]raclopride positron emission tomography study in anesthetized macaque monkeys, Biol. Psychiatry 55 (2004) 484–489.
[13] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[14] S.M. Tejani-Butt, D.J. Brunswick, A. Frazer, [3H]nisoxetine: a new radioligand for norepinephrine uptake sites in brain, Eur. J. Pharmacol. 191 (1990) 239–243.
[15] S. Kitayama, S. Shimada, H. Xu, L. Markham, D.M. Donovan, G.R. Uhl, Dopamine transporter site-directed mutations differentially alter substrate transport and cocaine binding, Proc. Natl. Acad. Sci. USA 89 (1992) 7782–7785.
[16] T. Ikeda, S. Kitayama, K. Morita, T. Dohi, Nerve growth factor down-regulates the expression of norepinephrine transporter in rat pheochromocytoma (PC12) cells, Brain Res. Mol. Brain Res. 86 (2001) 90–100.
[17] J.W. Boja, W.M. Mitchell, A. Patel, T.A. Kopajtic, F.I. Carroll, A.H. Lewin, P. Abraham, M.J. Kuhar, High-affinity binding of [125I]RTI-55 to dopamine and serotonin transporters in rat brain, Synapse 12 (1992) 27–36.
[18] 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. USA 95 (1998) 15635–15640.
[19] B.J. Hoffman, E. Mezey, M.J. Brownstein, Cloning of a serotonin transporter affected by antidepressants, Science 254 (1991) 579–580.
[20] S. Shimada, S. Kitayama, C.L. Lin, A. Patel, E. Nanthakumar, P. Gregor, M. Kuhar, G. Uhl, Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA, Science 254 (1991) 576–578.
[21] J.E. Kilty, D. Lorang, S.G. Amara, Cloning and expression of a cocaine-sensitive rat dopamine transporter, Science 254 (1991) 578–579.
[22] B. Giros, S. el Mestikawy, L. Bertrand, M.G. Caron, Cloning and functional characterization of a cocaine-sensitive dopamine transporter, FEBS Lett. 295 (1991) 149–154.
[23] T. Pacholczyk, R.D. Blakely, S.G. Amara, Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter, Nature 350 (1991) 350–354.
[24] B. Lingen, M. Bruss, H. Bonisch, Cloning and expression of the bovine sodium- and chloride-dependent noradrenaline transporter, FEBS Lett. 342 (1994) 235–238.
[25] T. Dohi, S. Kitayama, K. Kumagai, W. Hashimoto, K. Morita, Pharmacology of monoamine neurotransmitter transporters, Nippon Yakurigaku Zasshi 120 (2002) 315–326.
[26] S. Kitayama, T. Dohi, Cellular and molecular aspects of monoamine neurotransmitter transporters, Jpn. J. Pharmacol. 72 (1996) 195–208.
[27] G.E. Torres, R.R. Gainetdinov, M.G. Caron, Plasma membrane monoamine transporters: structure, regulation and function, Nat. Rev. Neurosci. 4 (2003) 13–25.
[28] A. Hausmann, C. Schermer, H. Hinterhuber, C. Humpel, Chronic repetitive transcranial magnetic stimulation (rTMS) does not affect tyrosine hydroxylase (TH) and dopamine-beta-hydroxylase (DBH) expression in rats in vivo, Synapse 46 (2002) 45–47.
[29] P.G. Luiten, G.J. ter Horst, H. Karst, A.B. Steffens, The course of paraventricular hypothalamic efferents to autonomic structures in medulla and spinal cord, Brain Res. 329 (1985) 374–378.
[30] P. Levitt, R.Y. Moore, Origin and organization of brainstem catecholamine innervation in the rat, J. Comp. Neurol. 186 (1979) 505–528.
[31] E.R. Marchand, J.N. Riley, R.Y. Moore, Interpeduncular nucleus afferents in the rat, Brain Res. 193 (1980) 339–352.