



Effects of repetitive transcranial magnetic stimulation on ER stress-related genes and glutamate, γ -aminobutyric acid and glycine transporter genes in mouse brain



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ABSTRACT

Repetitive transcranial magnetic stimulation (rTMS) is an emerging therapy for the treatment of psychiatric disorders. However, the mechanisms underlying the therapeutic effects of rTMS are still unclear, limiting its optimisation. Lasting effects suggest changes in disease-related genes, so we conducted gene chip and qRT-PCR analyses of genes associated with psychiatric diseases in the mouse brain at various times following 1, 20, 30 or 40 days of rTMS. Many genes were differentially expressed in the rTMS-treated mouse brain compared to sham controls, including genes encoding neurotransmitter transporters (upregulation of EAAT4, GLAST, GLT-1, GAT2, GAT4, GLYT1 and GLYT2), and endoplasmic reticulum (ER)-stress proteins (downregulation of IRE1 α , IRE1 β , and XBP1, upregulation of ATF6 and GRP78/Bip). Expression changes in many of these genes were also observed 10 days after the last rTMS treatment. In PC12 cells, rTMS upregulated GRP78/Bip mRNA and enhanced resistance against H₂O₂ stress. These results suggest that rTMS differentially modulates multiple genes associated with psychiatric and neurodegenerative disorders. Sustained changes in the expression of these genes may underlie the therapeutic efficacy of chronic rTMS.

1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a novel non-invasive therapy for neurological and psychiatric diseases [1–4]. Since Barker et al. first demonstrated that it is possible to activate both peripheral nerves and brain tissue using external magnetic stimulation [1], TMS has gained acceptance as a pain-free and non-invasive diagnostic tool in neurology, such as for evaluating peripheral neuropathies [5].

In addition, several studies have reported therapeutic benefits of TMS for patients with psychiatric disorders, such as depression, Parkinson's disease and schizophrenia [6–8]. These psychiatric disorders are associated with dysfunction in monoaminergic and glutamatergic neurotransmitter systems, suggesting that the benefits of rTMS arise from modulation of these neurotransmitter signalling pathways. For example, decreased expression of glutamate and GABA transporter has been reported in the post mortal brain of Schizophrenia patients

[9–11]. Based on the NMDAR (N-methyl-D-aspartate receptor) hypofunction hypothesis in schizophrenia, we speculated that rTMS might have effects on glutamatergic, GABAergic and glycinergic systems, including NMDAR, non-NMDAR, metabotropic GluR (glutamate receptor), glutamate transporter, GABA transporter, and glycine transporter. The glycine transporter is expressed in glia surrounding glutamatergic synapses and regulates synaptic glycine concentrations influencing NMDA receptor-mediated neurotransmission. Conversely, increased expression of GluR1 is found in the post mortal brain of Schizophrenia patients [12]. Because GluR1 is essential for the proliferation and growth of melanoma [13,14]; increased GluR1 might protect glutamatergic neurons. Because TMS is safe and relatively painless, it holds many possible applications as a therapeutic device for psychiatric disorders. However, the precise molecular mechanisms underlying the effects of TMS are unknown, which has impeded further optimisation for targeted regulation of processes involved in disease

Abbreviations: GLT-1, glial glutamate transporter-1; GLYT, glycine transporter; GLAST, glutamate/aspartate transporter; EAAT1, excitatory amino acid carrier 1; EAAT4, excitatory amino acid transporter 4; GABA, γ -aminobutyric acid; GAT, GABA transporter; ER, endoplasmic reticulum; GRP78/Bip, glucose-regulated protein 78/immunoglobulin heavy chain-binding protein; ATF6, activating transcription factor 6

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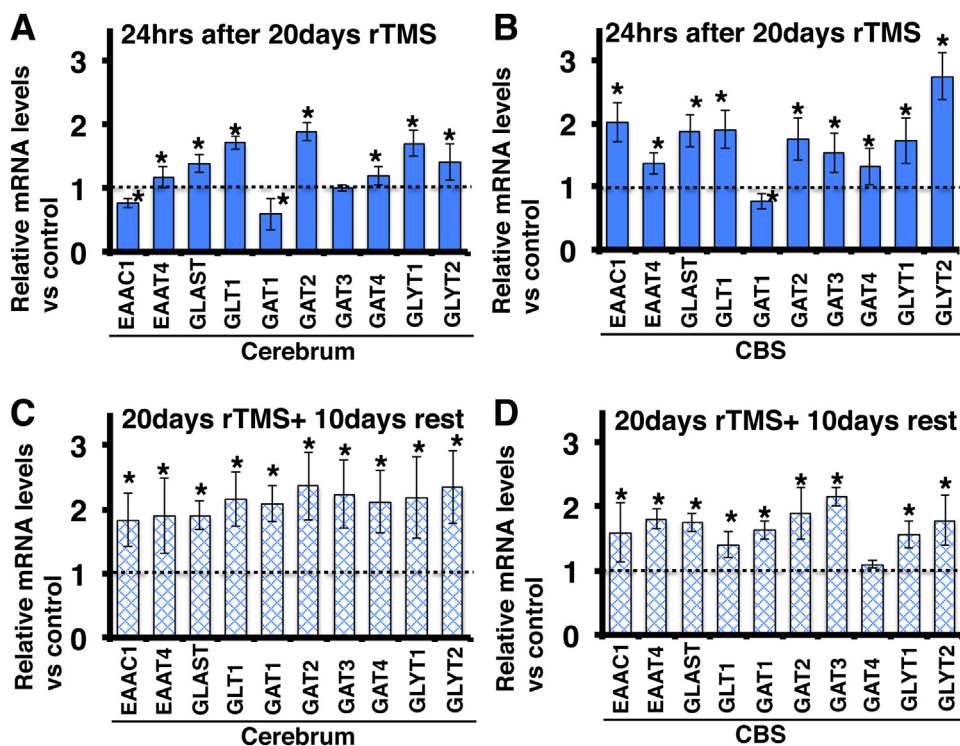


Fig. 1. Effects of chronic rTMS on glutamate, GABA and glycine transporter gene expression in mouse brain. The mRNA levels were determined at 24 h (A, B) and 10 days after 20 days of rTMS stimulation (C, D). (A, C) Effects of chronic rTMS on gene expression in the cerebrum. (B, D) Effects of chronic rTMS on gene expression in the cerebellum plus brain stem (CBS). All mRNA levels are normalised to GAPDH expression within the same sample. Values presented as mean \pm SEM of five independent experiments, each performed in triplicate. *Significantly different from control at $P < 0.05$.

aetiology. Recent studies have demonstrated altered monoamine release after acute rTMS [15,16]. In addition, we reported changes in the expression levels of monoamine transporters, dopamine receptor 2, HSP70 and circadian rhythm-related genes after acute and chronic rTMS [17,18].

However, there have been few reports on changes in gene expression profiles following acute or chronic rTMS. This prompted us to evaluate gene expression changes in mouse brain following rTMS using gene chip technology. We demonstrate that rTMS induces lasting changes in the expression levels of multiple neurotransmitter transporter genes as well as several ER stress-related genes. Furthermore, we demonstrate that upregulation of the ER-stress gene GRP78/Bip in PC12 cells by rTMS enhances resistance against oxidative stress.

2. Materials and methods

2.1. Mice and rTMS conditions

Male C57Black mice (8 weeks old, 20–25 g) were chronically treated with rTMS for 20, 30 or 40 days ($n = 50$) or acutely for 1 day ($n = 24$). During treatment, the mice were housed in a light-controlled room (8:00 a.m. on, 8:00 p.m. off). A round coil (7.5 cm outer diameter) and a Nihon Kohden Rapid Rate Stimulator (Nihon Kohden, Japan) were used to perform the stimulation. For chronic rTMS, stimulation conditions were as follows: 20 Hz for 2 s, 20 times/day, inter-stimulus interval of 1 min and 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 notable seizures or changes in behaviour, such as excessive struggling. Twenty-four hours after the last stimulation, the animals were sacrificed and their brains processed for further gene expression analysis. Mice subjected to acute rTMS (1 day using the same stimulus conditions) were sacrificed after 1, 4, 12 and 24 h for gene expression analysis. All the animal experiments were performed in compliance with institutional guidelines.

This study was approved by the Experimental Animal Committee of the RIKEN Institute and performed according to the guidelines for the

care and use of experimental animals of RIKEN Institute (approval # H15-2B046).

2.2. 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 [19]. Poly(A)⁺ RNA was isolated from the samples using an mRNA purification kit (TaKaRa Bio, Japan) for expression analysis by TaqMan real-time RT-PCR. Primer Express Software (Applied Biosystems, Foster City, CA) was used to design the TaqMan primer and probe sets. Supplementary data 1 shows the nucleotide sequences of the primers. Contaminating genomic DNA was removed with RNase-free DNase I (TaKaRa Bio, Japan). Complementary DNAs were synthesised from 1 μ g of mRNA per 100- μ l reaction using MMLV Reverse Transcription Reagents (Invitrogen, Carlsbad, CA). The TaqMan PCR reaction mixture contained 15 μ l of TaqMan Universal PCR Master Mix (Applied Biosystems) in a 30 μ l reaction. Primers and probes were added in optimal concentrations. We used 1 μ l 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 $^{\circ}$ C and 10 min at 95 $^{\circ}$ C, followed by 40 cycles of 95 $^{\circ}$ C for 15 s and 60 $^{\circ}$ C for 1 min. The mRNA quantity for each gene of interest was normalised to the quantity of GAPDH mRNA within each sample.

2.3. Cell culture

PC12 cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum (FCS) at 37 $^{\circ}$ C under 5% CO₂/95% air. Cells at subconfluence were harvested, diluted in culture medium and seeded in 24-well culture plates for cell viability assays or in 25 cm² culture flasks for total RNA extraction. The cells were cultured with or without daily rTMS for 15 days. Then, the cells in 24-well plates were treated with H₂O₂ for 2 h. Viability was evaluated by MTT assay [20].

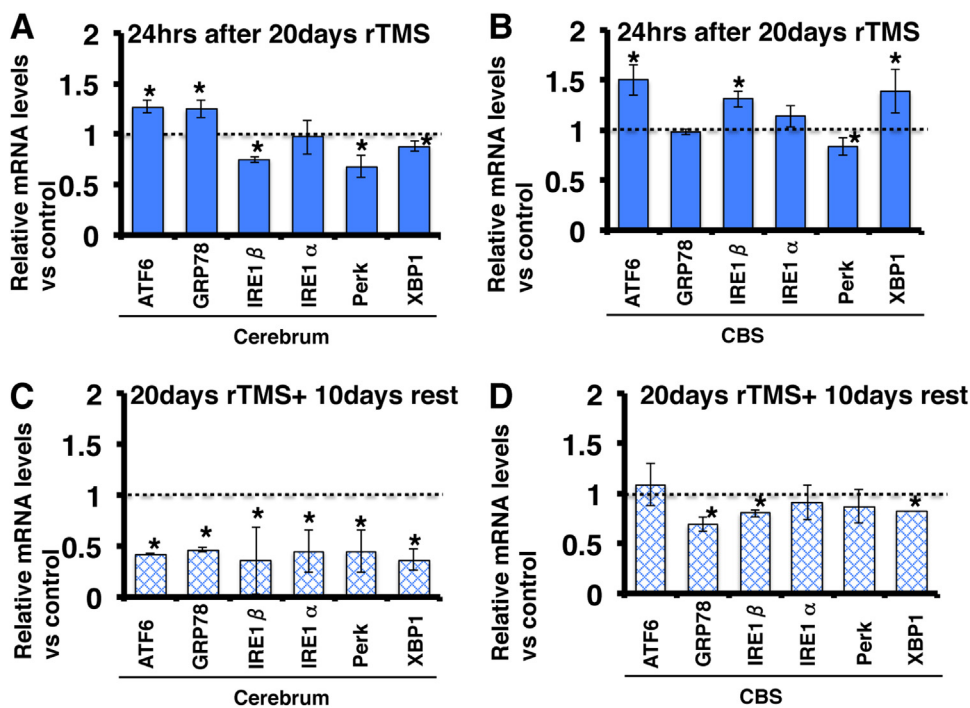


Fig. 2. Effects of rTMS on ER stress-related gene expression in mouse brain. The mRNA levels were determined at 24 h (A, B) and 10 days after 20 days of rTMS stimulation (C, D). (A, C) Effects of chronic rTMS on gene expression in the cerebrum. (B, D) Effects of chronic rTMS on gene expression in cerebellum plus brain stem (CBS). All mRNA levels are normalised to GAPDH expression in the same sample. Values presented as mean \pm SEM of five independent experiments, each performed in triplicate. *Significantly different from control at $P < 0.05$.

Table 1
Summary of glutamate transporter gene expression changes following acute rTMS.

			1 h	4 h	12 h	24 h
EAAC1	Cerebrum	Control	1 \pm 0.08	0.83 \pm 0.06	1.04 \pm 0.07	1.01 \pm 0.13
		Mg+	1.04 \pm 0.57	1.67 \pm 0.27*	0.91 \pm 0.06*	1.03 \pm 0.08
	CBS	Control	1 \pm 0.28	1.72 \pm 0.35	1.79 \pm 0.26	2.09 \pm 0.3
		Mg+	0.93 \pm 0.03	1.73 \pm 1.28	1.69 \pm 0.61	1.61 \pm 0.28
EAAT4	Cerebrum	Control	0.99 \pm 0.13	0.82 \pm 0.07	1.05 \pm 0.09	0.99 \pm 0.1
		Mg+	1.01 \pm 0.13	1.7 \pm 0.29*	0.88 \pm 0.07*	1.05 \pm 0.08
	CBS	Control	1 \pm 0.31	1.53 \pm 0.38	1.74 \pm 0.46	1.89 \pm 0.15
		Mg+	1.06 \pm 0.05	1.56 \pm 0.85	1.62 \pm 0.7*	1.57 \pm 0.3
GLAST	Cerebrum	Control	1 \pm 0.14	0.79 \pm 0.05	0.97 \pm 0.05	0.98 \pm 0.14
		Mg+	0.99 \pm 0.12	1.64 \pm 0.33 [†]	0.87 \pm 0.05*	0.99 \pm 0.07
	CBS	Control	1 \pm 0.02	1.6 \pm 0.07	1.49 \pm 0.29	1.98 \pm 0.31
		Mg+	0.81 \pm 0.04*	1.15 \pm 0.09*	1.38 \pm 0.14	1.45 \pm 0.05*
GLT1	Cerebrum	Control	1 \pm 0.17	0.73 \pm 0.04	0.86 \pm 0.05	0.93 \pm 0.11
		Mg+	0.96 \pm 0.11	1.45 \pm 0.31*	0.76 \pm 0.04*	0.88 \pm 0.09
	CBS	Control	1 \pm 0.34	1.51 \pm 0.32	1.38 \pm 0.33	2.39 \pm 0.54
		Mg+	0.76 \pm 0.05	1.34 \pm 0.9*	1.56 \pm 0.22*	1.36 \pm 0.26*
GAT1	Cerebrum	Control	1 \pm 0.06	0.8 \pm 0.05	0.97 \pm 0.05	0.91 \pm 0.11
		Mg+	0.99 \pm 0.13	1.64 \pm 0.32 [†]	0.83 \pm 0.04*	0.95 \pm 0.08
	CBS	Control	1 \pm 0.38	1.78 \pm 0.47	1.85 \pm 0.45	1.97 \pm 0.32
		Mg+	0.92 \pm 0.06	1.76 \pm 1.05	1.73 \pm 0.5	1.69 \pm 0.41
GAT2	Cerebrum	Control	0.99 \pm 0.1	0.79 \pm 0.07	0.95 \pm 0.07	0.99 \pm 0.19
		Mg+	0.87 \pm 0.04*	1.52 \pm 0.3*	0.84 \pm 0.04*	0.89 \pm 0.04
	CBS	Control	1 \pm 0.16	1.8 \pm 0.14	1.82 \pm 0.16	1.81 \pm 0.09
		Mg+	0.84 \pm 0.03 [†]	2.11 \pm 1.37*	1.55 \pm 0.06*	1.51 \pm 0.6*

Values are mean \pm SEM of three independent experiments, each performed in triplicate.

* Significantly different from control at $P < 0.05$.

2.4. Data analysis

The data are presented as mean \pm SE of at least three independent experiments, each performed in triplicate or duplicate. Means were compared by ANOVA (Figs. 1 and 2) or Student's *t*-test as appropriate.

3. Results

3.1. Gene expression changes in mouse brain following rTMS

We stimulated the brains of 8-week-old C57 Black mice for 20, 30 or

40 days by rTMS and analysed the changes in gene expression using the Affymetrix GeneChip microarray. GeneChip analysis revealed altered expression levels of multiple mRNAs, including those encoding glutamate and glycine transporters as well as ER stress-related genes, in both cerebrum and CBS [21,22]. In order to quantify these changes, we then measured mRNA levels by qRT-PCR (Figs. 1 and 2, Tables 1–3).

3.2. Effects of acute and chronic rTMS on transporter genes in mouse brain

The GeneChip data showed that glutamate and glycine transporter mRNAs were altered after chronic rTMS. Thus, we examined the mRNA

Table 2
Summary of GABA and glycine transporter gene expression changes following rTMS.

			1 h	4 h	12 h	24 h
GAT3	Cerebrum	Control	0.99 ± 0.15	0.8 ± 0.07	0.94 ± 0.07	0.95 ± 0.15
		Mg +	0.89 ± 0.07	1.6 ± 0.31*	0.88 ± 0.05	0.92 ± 0.08
	CBS	Control	0.99 ± 0.02	1.47 ± 0.22	1.47 ± 0.35	1.55 ± 0.05
		Mg +	1.12 ± 0.19	1.61 ± 0.98*	1.18 ± 0.29	1.33 ± 0.24*
GAT4	Cerebrum	Control	0.99 ± 0.06	0.8 ± 0.03	0.99 ± 0.04	0.96 ± 0.11
		Mg +	0.99 ± 0.1	1.69 ± 0.32*	0.85 ± 0.04*	0.97 ± 0.08
	CBS	Control	1 ± 0.04	2.12 ± 0.22	1.92 ± 0.29	2.42 ± 0.39
		Mg +	1.32 ± 0.07*	1.5 ± 0.46*	2.14 ± 0.62	2.16 ± 0.47
GLYT1	Cerebrum	Control	1 ± 0.12	0.81 ± 0.05	1.02 ± 0.06	1.04 ± 0.18
		Mg +	0.94 ± 0.12	1.6 ± 0.21*	0.85 ± 0.04*	1.03 ± 0.1
	CBS	Control	1 ± 0.32	1.65 ± 0.42	1.63 ± 0.36	1.77 ± 0.21
		Mg +	0.96 ± 0.03	1.55 ± 0.96	1.77 ± 0.44	1.74 ± 0.46
GLYT2	Cerebrum	Control	1 ± 0.09	0.72 ± 0.07	1.02 ± 0.02	0.97 ± 0.49
		Mg +	0.87 ± 0.11*	1.57 ± 0.17*	0.77 ± 0.02*	1.28 ± 0.4
	CBS	Control	0.52 ± 0.3	1 ± 0.14	1.02 ± 0.19	1.18 ± 0.16
		Mg +	0.37 ± 0.03	1.12 ± 0.79	1.07 ± 0.43	0.82 ± 0.16*

Values are mean ± SEM of three independent experiments, each performed in triplicate.

* Significantly different from control at P < 0.05.

Table 3
Summary of ER stress-related gene expression changes following acute rTMS.

			1 h	4 h	12 h	24 h
ATF6	Cerebrum	Control	1 ± 0.01	1.04 ± 0.05	0.86 ± 0.06	1.24 ± 0.15
		Mg +	0.89 ± 0.08*	0.87 ± 0.14*	0.88 ± 0.05	0.8 ± 0*
	CBS	Control	1 ± 0.05	0.87 ± 0.07	1.02 ± 0.09	1.06 ± 0.17
		Mg +	0.52 ± 0.11*	1.05 ± 0.25	0.8 ± 0.01*	0.41 ± 0.61*
GRP78/Bip	Cerebrum	Control	1 ± 0.15	1.42 ± 0.31	0.81 ± 0.02	1.19 ± 0.19
		Mg +	1.38 ± 0.19*	0.86 ± 0.08*	1.14 ± 0.13*	0.76 ± 0.05*
	CBS	Control	1 ± 0.02	0.95 ± 0.04	1.16 ± 0.13	2.19 ± 0.15
		Mg +	1.4 ± 0.29*	1.06 ± 0.05	0.74 ± 0.04*	1.49 ± 0.45*
IRE1β	Cerebrum	Control	1 ± 0.1	1.57 ± 0.18	1.27 ± 0.07	1.34 ± 0.05
		Mg +	1.03 ± 0.12	1.21 ± 0.16*	0.96 ± 0.1*	1.2 ± 0.08
	CBS	Control	1 ± 0.18	1.39 ± 0.25	0.97 ± 0.44	1 ± 0.1
		Mg +	1.15 ± 0.14*	1.63 ± 0.02*	0.99 ± 0.09*	1.16 ± 0.13*
IRE1α	Cerebrum	Control	1 ± 0.04	1.43 ± 0.07	1.05 ± 0.06	1.22 ± 0.09
		Mg +	0.86 ± 0.05*	1.04 ± 0.19*	1.02 ± 0.03	0.92 ± 0.04*
	CBS	Control	1 ± 0.11	1.04 ± 0.12	1 ± 0.12	1.34 ± 0.07
		Mg +	0.56 ± 0.12*	1.05 ± 0.25	1 ± 0.09	1.37 ± 0.04
PERK	Cerebrum	Control	1 ± 0.09	1.12 ± 0.16	0.91 ± 0.04	1.04 ± 0.1
		Mg +	0.82 ± 0*	1.07 ± 0.11	0.91 ± 0.06	1.03 ± 0.05
	CBS	Control	1 ± 0.11	0.92 ± 0.12	0.95 ± 0.12	1.05 ± 0.07
		Mg +	0.79 ± 0.12*	0.84 ± 0.25	0.73 ± 0.09*	1.46 ± 0.04*
XBP1	Cerebrum	Control	1 ± 0.1	1 ± 0.03	0.83 ± 0.04	1 ± 0.02
		Mg +	1.14 ± 0.03*	0.85 ± 0.09*	0.97 ± 0.06*	0.77 ± 0.05*
	CBS	Control	1.01 ± 0.01	1.08 ± 0.02	0.94 ± 0.01	1.94 ± 0.11
		Mg +	1.3 ± 0.11*	1.26 ± 0.01*	0.79 ± 0.09*	1.24 ± 0.46*

Values are mean ± SEM of three independent experiments, each performed in triplicate.

* Significantly different from control at P < 0.05.

levels of the glutamate transporters EAAC1, EAAT4, GLAST and GLT1, the GABA transporters GAT1–4, and the glycine transporters GLYT1 and 2 both 24 h and 10 days after 20 days of rTMS. Twenty-four hours after the last rTMS application, EAAT4, GLAST, GLT1, GAT2, GAT4, GLYT1 and GLYT2 mRNA levels were upregulated in the cerebrum (Fig. 1A), and EAAC1, EAAT4, GLAST, GLT1, GAT2, GAT3, GAT4, GLYT1 and GLYT2 mRNA levels were upregulated in the CBS (Fig. 1B). Conversely, EAAC1 and GAT1 mRNA levels were downregulated in the cerebrum (Fig. 1A), while GAT1 mRNA level was downregulated in the CBS (Fig. 1B). Ten days after the last rTMS treatment, EAAC1, EAAT4, GLAST, GLT1, GAT1, GAT2, GAT3, GAT4, GLYT1 and GLYT2 mRNA levels were upregulated in both cerebrum and CBS (Fig. 1C, D), while GAT4 mRNA level was upregulated only in the cerebrum (Fig. 1D).

We next examined the acute effects of a single days' rTMS administration after 1, 4, 12 and 24 h. These measurements revealed temporally complex and region-specific expression changes in transporter and ER stress-related genes. Expression changes in glutamate transporter

genes are summarised in Table 1, GABA and glycine transporter genes in Table 2, and ER-stress genes in Table 3. After acute rTMS, EAAC1, GAT1 and GLYT1 mRNA levels were upregulated at 4 h and downregulated at 12 h in the cerebrum (Tables 1 and 2). EAAT4 mRNA levels were upregulated at 4 h and downregulated at 12 h in the cerebrum, while expression levels were unchanged in CBS at 4 h before decreasing at 12 h (Table 1). GLAST mRNA levels were upregulated at 4 h and downregulated at 12 h in the cerebrum. In contrast, expression was downregulated after 1, 4 and 24 h in CBS (Table 1). GAT2 mRNA levels were upregulated at 4 h and downregulated at 1 and 12 h in the cerebrum, but upregulated at 4 h and downregulated after 1, 4 and 24 h in CBS (Table 1). GAT3 mRNA levels were upregulated at 4 h in the cerebrum and CBS, but then downregulated only in the CBS at 24 h (Table 2). GAT4 mRNA levels were upregulated at 4 h and downregulated at 12 h in the cerebrum, but upregulated at 1 h and downregulated at 12 h in CBS (Table 2). GLYT2 mRNA levels were upregulated at 4 h and downregulated at 1 and 12 h in the cerebrum, but

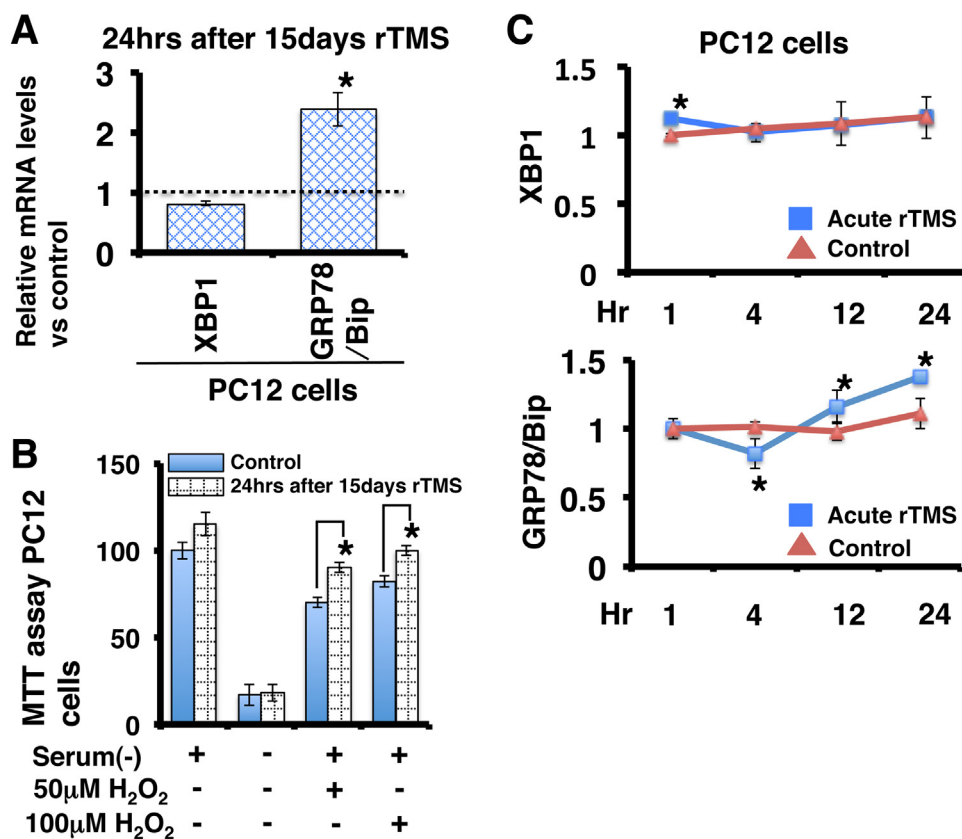


Fig. 3. Effects of chronic and acute rTMS on XBP1 and GRP78/Bip mRNA expression levels and H₂O₂ resistance in PC12 cells. (A) Effects of chronic rTMS on XBP1 and GRP78/Bip mRNA levels in PC12 cells. (B) Viability of rTMS-treated and control PC12 cells exposed to H₂O₂ for 2 h. (C) Effects of acute rTMS on XBP1 and GRP78/Bip mRNA levels in PC12 cells at 1, 4, 12 and 24 h after stimulation. Values presented as mean ± SEM of three independent experiments, each performed in triplicate. *Significantly different from control at P < 0.05.

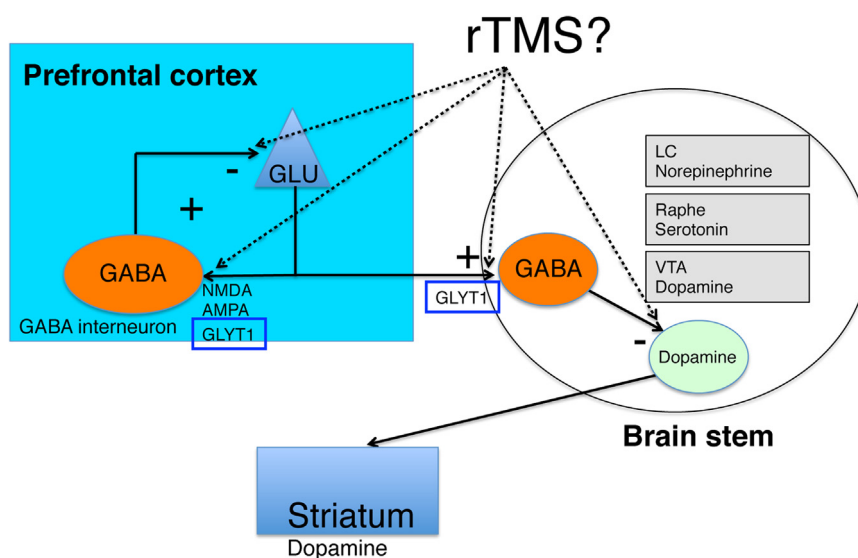


Fig. 4. Model for differential regulation of neurotransmitter transporters in mouse brain by rTMS. Chronic rTMS may alter glutamatergic, GABAergic and glycinergic neurotransmission by changing the rate of transmitter uptake from the synaptic cleft.

downregulated at 24 h in CBS (Table 2). In summary, rTMS appears to differentially regulate neurotransmitter transporter genes both dynamically following acute treatment and in a more sustained manner following chronic treatment.

3.3. Effects of acute and chronic rTMS on ER stress-related genes in mouse brain

GeneChip data revealed that multiple ER stress-related genes were also altered by rTMS [22], so examined the effects of acute and chronic rTMS on specific ER stress-related genes by qRT-PCR. Twenty-four

hours after 20 days of rTMS, ATF6 and GRP78/Bip mRNA levels were upregulated in the cerebrum (Fig. 2A), and ATF6, IRE1β and XBP1 mRNA levels were upregulated in CBS (Fig. 2B). However, IRE1β, PERK and XBP1 mRNA levels were downregulated in the cerebrum (Fig. 2A) and PERK mRNA level was downregulated in CBS (Fig. 2B). Ten days after the last rTMS treatment, ATF6, GRP78/Bip, IRE1α, IRE1β, PERK and XBP1 mRNA levels were downregulated in the cerebrum (Fig. 2C), and GRP78/Bip, IRE1 β, PERK and XBP1 mRNA levels were downregulated in CBS (Fig. 2D). There were no changes in ATF6, IRE1α and PERK mRNA levels in CBS (Fig. 2D).

We then examined the acute effects of rTMS at 1, 4, 12 and 24 h

(Table 3). ATF6 mRNA level was downregulated in the cerebrum at 1, 4 and 24 h, and in CBS at 1, 12 and 24 h after acute rTMS. GRP78/Bip mRNA level was upregulated at 1 and 12 h in the cerebrum and at 1 h in CBS, but was downregulated at 4 and 24 h in the cerebrum and at 12 and 24 h in CBS. IRE1 β mRNA level was upregulated at 1, 4 and 24 h and downregulated at 12 h in CBS, but was downregulated at 4 and 12 h in the cerebrum. IRE1 α mRNA level was downregulated at 1 h in the CBS, and downregulated at 1, 4 and 12 h in the cerebrum. PERK mRNA level was downregulated at 1 and 12 h in CBS and at 1 h in the cerebrum, but upregulated at 24 h in the CBS. XBP1 mRNA level was downregulated at 12 and 24 h in CBS and at 4 h in the cerebrum, but upregulated after 1 and 12 h in the cerebrum and at 1 and 4 h in CBS. Thus, similar to neurotransmitter transporter genes, rTMS induced temporally complex and region-specific expression changes in ER stress-related genes.

3.4. Effects of acute and chronic rTMS on ER stress-related genes in PC12 cells

The effects of rTMS on ER stress-related genes were also examined in PC12 cells to investigate the functional significance. GRP78/Bip mRNA expression was upregulated after 15 days of rTMS in PC12 cells (Fig. 3A). After acute rTMS, XBP1 mRNA level was upregulated at 1 h, while GRP78/Bip mRNA level was downregulated at 4 h and upregulated at 12 and 24 h (Fig. 3C). ER stress-related genes are known to be induced by H₂O₂ via glutamate and glycine stimulation as a compensatory response to prevent cytotoxic protein misfolding, so we investigated whether rTMS increases PC12 cell resistance against H₂O₂. Indeed, 15 days of rTMS increased cell viability following 2 h of H₂O₂ exposure compared to controls (Fig. 3B).

4. Discussion

Repetitive TMS is a promising treatment for psychiatric disorders. Persistent therapeutic effects suggest that rTMS induces lasting changes in the expression of genes involved in these disorders, such as genes associated with stress-responses and neurotransmission. To study such effects, we measured gene expression changes by gene chip arrays and qRT-PCR in mouse brain following an acute (1-day) or chronic (20-day) rTMS protocol. We have previously shown that acute and chronic rTMS alters monoamine transporter (MAT) mRNA expression, protein levels and function [17], dopamine receptor 2 mRNA expression, protein levels and function, circadian rhythm-related gene expression and both mRNA and protein expression levels of the stress response gene HSP70 [18]. Here we confirmed differential modulation of transporter and ER stress-related genes reported in previous studies [21,22] and provide evidence for enhanced neuroprotection following rTMS.

In addition to changes in mouse cerebrum, we found that GRP78/Bip mRNA level was upregulated in PC12 cells by acute and chronic rTMS. Furthermore, 15 days of rTMS increased the resistance of PC12 cells against H₂O₂ toxicity. These results indicate that rTMS may also slow the progression of neurodegenerative disorder, such as Huntington's disease, through GRP78/Bip induction. Further investigations are needed on the effects of chronic rTMS because acute rTMS increased GRP78/Bip mRNA expression in the cerebrum 24 h after 20 days of rTMS, whereas other stress-associated genes such as ATF6, IRE1 β , PERK and XBP1 were downregulated. Thus, the ER-stress response induced by rTMS appears highly specific.

Expression levels of EAAT4, GLAST, GLT1, GAT2, GAT4, GLYT1 and GLYT2 were upregulated in the cerebrum after chronic rTMS (Fig. 1A), while these same genes plus EAAC1 and GAT3 were upregulated in CBS (Fig. 1B). Furthermore, EAAC1, EAAT4, GLAST, GLT1, GAT1, GAT2, GAT3, GAT4, GLYT1 and GLYT2 mRNA levels were still upregulated in the cerebrum and CBS 10 days after the last rTMS administration (Fig. 1C, D). Thus, rTMS appears to induce widespread and sustained increases in glutamate, GABA and glycine transporters, possibly

resulting in altered transmitter signalling. Indeed, the efficacy of NMDA receptor antagonists such as memantine for the treatment of dementia, obsessive-compulsive disorder (OCD), and certain schizophrenia symptoms suggests that chronic rTMS may result in symptom improvement by upregulating transporter-mediated glutamate uptake. Our results also suggest that ER stress-related genes may be involved in glutamate and glycine transporter expression changes induced by chronic rTMS.

In conclusion, the therapeutic effects of chronic rTMS may depend on modulation of ER stress-related genes and glutamate, glycine and GABA transporter genes (Fig. 4). Further research is needed to identify the region-specific functional changes resulting from up- and down-regulation of these genes following rTMS. Such information could facilitate the development of more effective rTMS protocols with fewer side effects.

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Appendix A. Transparency document

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.bbrep.2018.10.015](https://doi.org/10.1016/j.bbrep.2018.10.015).

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