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Effects of high-frequency repetitive transcranial magnetic stimulation on spontaneously hypertensive rats, an animal model of Attention Deficit Hyperactivity Disorder

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Effects of high-frequency repetitive transcranial magnetic stimulation on spontaneously hypertensive rats, an animal model of Attention Deficit Hyperactivity Disorder

Directed by Professor Dong Ho Song

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Woo-Young Im

December 2017

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This certifies that the Doctoral Dissertation of Woo-Young Im is approved.

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ABSTRACT

Effects of high-frequency repetitive transcranial magnetic stimulation on spontaneously hypertensive rats, an animal model of Attention Deficit Hyperactivity Disorder

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(Directed by Professor Dong Ho Song)

The current treatment of choice for attention deficit hyperactivity disorder (ADHD) is pharmacotherapy. A search for new treatment options is underway, however, as the wide application of drugs to the general population of patients with ADHD are limited by side effects and the variance of pharmacokinetic effects of the drugs in each patient. In the present study, we applied repetitive transcranial magnetic stimulation (rTMS), a non-invasive treatment used in a number of other psychiatric disorders, to spontaneously hypertensive rats (SHRs), an animal model of ADHD, in order to assess the efficacy of the treatment in modifying behavior symptoms as well as levels of dopamine, noradrenaline, serotonin, and brain-derived neurotrophic factor (BDNF).

A total of fifteen sessions of high-frequency rTMS treatment were administered. Behavior symptoms were observed using open field, Y-maze, and elevated plus-maze tests. Upon completion of the experiments, rats were sacrificed, and the neurochemical changes in brain tissue were analyzed using high performance liquid chromatography and Western blotting.

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The SHRs treated with rTMS tended to exhibit less locomotor activity in the open field test over the course of treatment, but there was no improvement in inattention as measured by the Y-maze test. Furthermore, BDNF concentration increased and noradrenaline concentration decreased in the prefrontal cortex of SHRs treated with rTMS.

The results of the present preclinical study indicate that rTMS may constitute a new modality of treatment for patients with ADHD, through further evaluation of specific treatment parameters as well as safety and efficacy in humans are required.

Key words : Attention Deficit Hyperactivity Disorder, Repetitive transcranial magnetic stimulation, Spontaneously hypertensive rat, Catecholamines, Brain derived neurotrophic factor , Behavior analysis

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I. INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is one of the most prevalent developmental disorders among children.[1] Patients with ADHD exhibit varying levels of hyperactivity/impulsivity and inattention that result in impaired functioning and academic performance.[2] Research has indicated that the prefrontal cortex, caudate nucleus, and cerebellum—which constitute a neuronal network involved in the regulation of attention, emotion, and behavior—are important structures in the pathophysiology of ADHD.[3] Indeed, delayed development and decreased size of the prefrontal cortex, caudate nucleus, and cerebellum have been observed in patients with ADHD. Alterations in the activity of monoamine neurotransmitters such as dopamine or norepinephrine due to the dysfunction of neurons associated with this network

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further contributes to the pathophysiology of ADHD.[4] As the etiology of ADHD remains unknown, the search for an effective treatment using medication or non-invasive psychosocial therapies is ongoing.

Currently, treatment with methylphenidate, amphetamine, or atomoxetine is the first-line therapy for patients with ADHD. The most commonly prescribed medication for the treatment of ADHD symptoms is methylphenidate, which is known to block dopamine and noradrenaline reuptake, though the exact mechanism underlying the resultant improvements in ADHD symptoms remains uncertain.[5] In addition, not only do the therapeutic effects of medication vary among individual patients, but methylphenidate or atomoxetine may also occasionally cause cardiovascular side effects, anxiety, headaches, and anorexia.[6,7] Many parents of children with ADHD are hesitant to pursue treatment with psychotropic medications. Unfortunately, there are no evidence-based alternative treatments currently available. The present work was undertaken to provide preclinical rationale for the application of non-invasive brain stimulation in the treatment of ADHD.

Repetitive transcranial magnetic stimulation (rTMS) is a promising non-invasive treatment in psychiatry[8] that exerts its effects by modulating cortical excitability, either increasing or decreasing neuronal excitability (or inhibition), depending on the administered stimulation.[9] The therapeutic effect

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of rTMS in patients with major depression has been well documented, and its use has extended to the treatment of anxiety disorders, childhood autism, and Parkinson's disease.[10,11] Recently, a few studies have applied rTMS to patients with ADHD, though an appropriate protocol and the precise mechanisms by which the effects occur remain undocumented.[12,13] The frequency of stimulation is the most important determinant in formulating an rTMS protocol. The number of frequency cycles in a given time produces different effects, and high-frequency stimulation is noted to enhance working memory and attention.[14,15] In the present study, we utilized a high-frequency rTMS protocol that has been successful in treating patients with major depression in order to examine the efficacy of such non-pharmacological treatment in an animal model of ADHD. As research has suggested that the functional abnormality of the right prefrontal cortex is involved in the etiology of ADHD, we aimed to activate the depressed brain area with high-frequency stimulation and evaluate alterations in the behavioral ADHD symptoms of spontaneously hypertensive rats (SHRs) .[16]

Additionally, we assessed the neurochemical mechanisms underlying the pharmacological action of methylphenidate and rTMS by evaluating changes in the concentration of extracellular dopamine/noradrenaline and the expression of brain-derived neurotrophic factor (BDNF)—a key protein that regulates synaptic plasticity and dendrite growth. Research has indicated that patients with ADHD exhibit decreased expression of BDNF in the prefrontal

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cortex, and that the application of psychostimulants (methylphenidate, amphetamine) increases BDNF expression and improves behavioral symptoms.[17] In addition to those of dopamine and noradrenaline, we analyzed serotonin levels due to their association with emotional behavior.

II. MATERIALS AND METHODS

1. Animals

Male SHR/Izm (n = 26) and Wistar-Kyoto (WKY)/Izm (n = 8) rats of postnatal day (PND) 39 weighing 150–180 g (Japan SLC Inc., Central Lab Animal Inc., Korea) were used as subjects and kept in groups of two to three animals per cage. According to research by Bizot et al., psychostimulants improve impulsivity in juvenile SHRs but not adult SHRs.[18] Furthermore, as ADHD manifests during childhood, we considered the use of juvenile rats to be more relevant in examining the effects of our experimental procedures. Animals were maintained in a room under controlled temperature (22 ± 3 ℃) and humidity (50 ± 10 ℃) under a 12-h light/dark cycle (lights on at 08:30) with free access to food and water. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Chungnam National University Hospital in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

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2. Study design

Animals were acclimated to their home cages in a maintenance room for 10 days, and treatment was initiated on PND 49. The experiment was carried out for 3 weeks (19 days), alternating 5 days of treatment with 2 days of rest. The WKY group consisted of four WKY rats treated with saline and four WKY rats treated with sham rTMS. The Sham group consisted of four SHRs treated with saline and four SHRs treated with sham rTMS. The methylphenidate (MPH) group consisted of nine SHRs treated with methylphenidate, and the TMS group consisted of nine SHRs treated with rTMS. The open field test was conducted on days 5, 12, and 19; while the Y-maze test was conducted on days 10 and 17, and the elevated plus-maze test was conducted on days 11 and 18 (Fig. 1). Animals were anaesthetized and decapitated on day 19, 7 h after the last open field test. The front 3 mm of the prefrontal cortex was extracted for catecholamine analysis, while the following 2 mm of the right motor cortex was extracted for Western blot analysis.

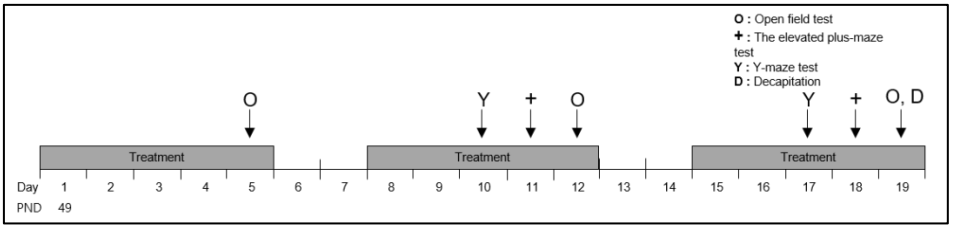

Figure 1. Experimental design.

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3. Drug and rTMS application

Methylphenidate (Penid, Hwanin Pharmaceutical Co. Korea) was administered orally to the MPH group at 1 mg/kg, twice a day (9:00 and 13:00). Pure saline was administered to control groups of four SHRs and four WKY rats.

The TMS group received rTMS with a commercially available stimulator MagPro R30 and X100-type magnetic device (MagVenture, Denmark), and a figure-8-shaped Cool-B35 Butterfly Coil (10-mm inner diameter and 46-mm outer diameter). The daily stimulation protocol included twenty 5-s trains of 10 Hz, at 40% of the machine's intensity (100% of motor threshold), with a 15-s intertrain interval. Motor threshold was measured as proposed by Sohn et al.[19] The intensity that coincides with the motor threshold was the mean value attained from five rats. One thousand pulses were applied in each of 15 daily sessions, resulting in 15,000 pulses in total. The center of the coil was accurately aimed as close to the lateral angle of the right eye as possible by a skilled experimenter in order to stimulate the right prefrontal cortex. Animals were awake during the treatment and thus free from the potential effects of repetitive anesthetic use and were restrained during treatment by a skilled experimenter holding them down on a table firmly by hand. The same operation was performed on animals of the sham rTMS treatment group (4 SHRs and 4 WKY rats) except that the coil was turned upside down and held 10 cm above the animal's head. This kept the animals

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away from direct stimulation while allowing them to hear the sound from the coil.

4. Behavioral analyses

A. Open field test

An open field test was used to evaluate hyperactivity and locomotor activity of the animals. Forty minutes after each rTMS treatment, each rat was placed in the center of a black acrylic box (48 cm × 48 cm × 42 cm), and the body's center-point was captured to record the total distance moved. The center of the box was lit at 160 lx. The movement of the animals was recorded for 10 min and divided into two 5-min sections to observe any shift in total distance moved that might be caused by habituation. A Sony camera (HDR-PJ670, Sony, Japan) was used in recording, and the video tracking data were analyzed using the animal behavior research software tool EthoVision XT (Noldus Information Technology, Wageningen, Netherlands). The behavior test was carried out in a soundproof room, undisturbed by any external (environmental) conditions. The apparatus was cleaned with 70% ethanol between trials.

B. Y-maze test

In the Y-maze test, the spontaneously alternating choice of which arm to enter provides information on a subject's attentiveness. The Y-maze test apparatus was black, with each 12-cm-wide arm extending 45 cm, and shielded

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with 30 cm high walls. The center of the maze was lit at 92 lx, and the animals were all initially placed at the end of the same arm. Each rat's movement was recorded for 8 min, and the spontaneous alternation was calculated by dividing successful alternations by (total arm entries – 2). Entrances to an arm were determined to be valid when all four of the animal's paws had entered the arm.

C. Elevated plus-maze test

This behavior analysis was used to observe anxiety-related impulsive behavior associated with open-arm exploration. The maze was elevated 50 cm above the ground and consisted of two open arms, 50 cm long and 10 cm wide, with a 1-cm transparent raised sill around the edge, and two closed arms of the same dimensions with 40 cm high walls. The elevated plus-maze was also black, and the center (the intersection of the closed and open arms) was lit at 160 lx, the open arms at 124 lx, and the closed arms at 69 lx. Every rat was placed in the center at the beginning of the experiment, with its head pointing toward a corner between an open and closed arm. An 8-min recording was used to calculate the number of entrances and duration spent in the open arms for each rat.

5. Western blotting

The expression of BDNF was assessed using Western blotting. The Western blot protocol used in this study was the same as that used in our

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previous study.[20] The extracted 70 mg of the right prefrontal cortex was first homogenized in 500μl RIPA buffer containing protease and phosphatase inhibitor. It was then incubated in ice for 30 min and centrifuged at 13,000g for 15 min at 4 ℃ to pellet the debris. The supernatant was then collected and maintained in a −70 °C freezer until the time of analysis. Blots were probed overnight with diluted rabbit anti-BDNF (1:1000, Millipore, USA). Mouse anti-β-actin (1:1000, Santa Cruz Biotechnology, USA) was used as an internal control to analyze the amount of protein expressed.

6. High performance liquid chromatography (HPLC)

Dopamine, noradrenaline, and serotonin (5-hydroxytryptamine) levels were measured using high performance liquid chromatography (HPLC). Dopamine hydrochloride, l-noradrenaline bitartrate monohydrate, and serotonin were acquired from Sigma-Aldrich (St. Louis, Missouri, United States) to plot a standard curve. The prefrontal cortex was homogenized and separated by Prominence UFLC XR with RF-20A fluorescence detector (Shimadzu, Kyoto, Japan), and ACE 5 C18 (250 × 4.6 mm, 5μm) from ACE (Scotland, UK). The tissue preparation and preconditioning processes for HPLC followed the protocol described by De Benedetto et al.[21] Fluorescence detection was measured at 320 nm with excitation at 279 nm.

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7. Statistics

For parametric data, comparison between groups was performed using analysis of variance (ANOVA). Differences between two groups calculated to be statistically significant by ANOVA were compared using Student's t-tests.

III. RESULTS

1. Open field test

Throughout the experiment, the MPH group travelled shorter distance ($p = 0.057$) than the Sham group in the open field test (Fig. 2A–C). Especially in the first week, when the 10-min distance was divided into two 5-min tracks, the Sham group displayed more hyperactive tendencies than the WKY group ($p < 0.05$, Fig. 2D). Moreover, in the latter 5 min, the TMS group covered less distance than the Sham group ($p < 0.05$, Fig. 2D). In the second and third weeks, the TMS group maintained the same tendency to move less in the latter 5 min, though this result was not statistically significant (Fig. 2E,F).

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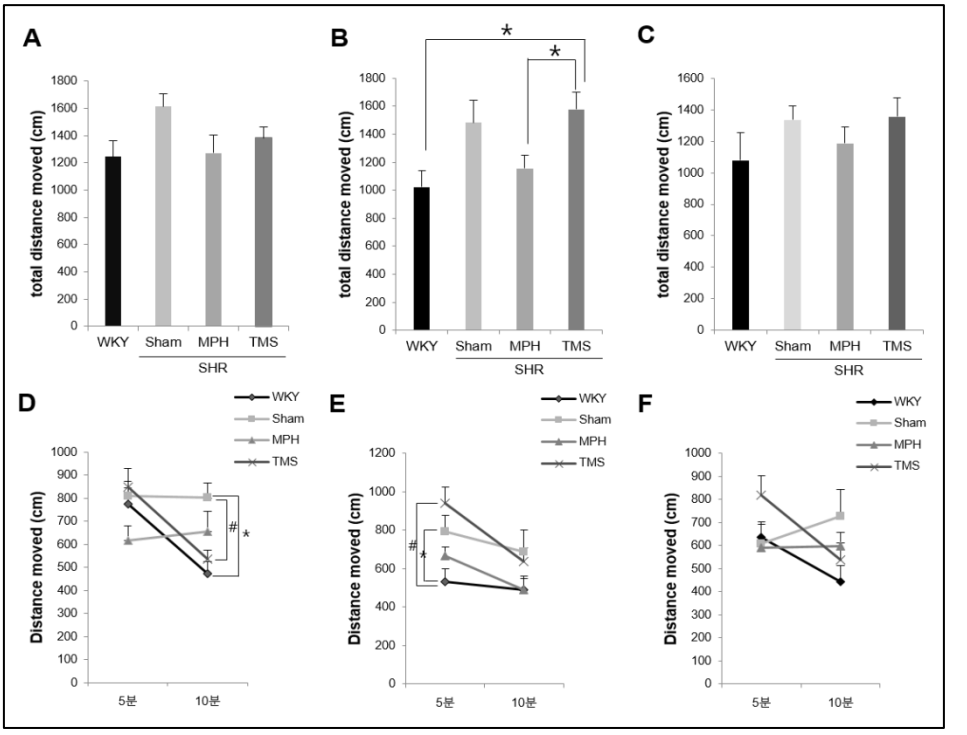

Figure 2. Measured locomotor activity (s) in the open field test in the first (A, D), second (B, E), and third (C, F) weeks. The total distance moved during 10 min in the open field (A, B, C), and in two 5-min parts: exploration (0–5 min) and habituation (5–10 min) (D, E, F). The mean ± SEM distance moved for each group is shown. $^{\#}$ or $*p < 0.05$. SEM: standard error of the mean. WKY: Wistar-Kyoto; MPH: methylphenidate; TMS: transcranial magnetic stimulation.

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2. Y-maze test

In the second week, no significant differences in spontaneous alternation were observed among the groups (Fig. 3A). All four groups made approximately 60–70% successful spontaneous alternations. In the third week, the MPH group exhibited a significant improvement in spontaneous alternation when compared to the Sham group ($p < 0.05$, Fig. 3B). The TMS group, however, exhibited no statistically significant change throughout the experiment (Fig. 3).

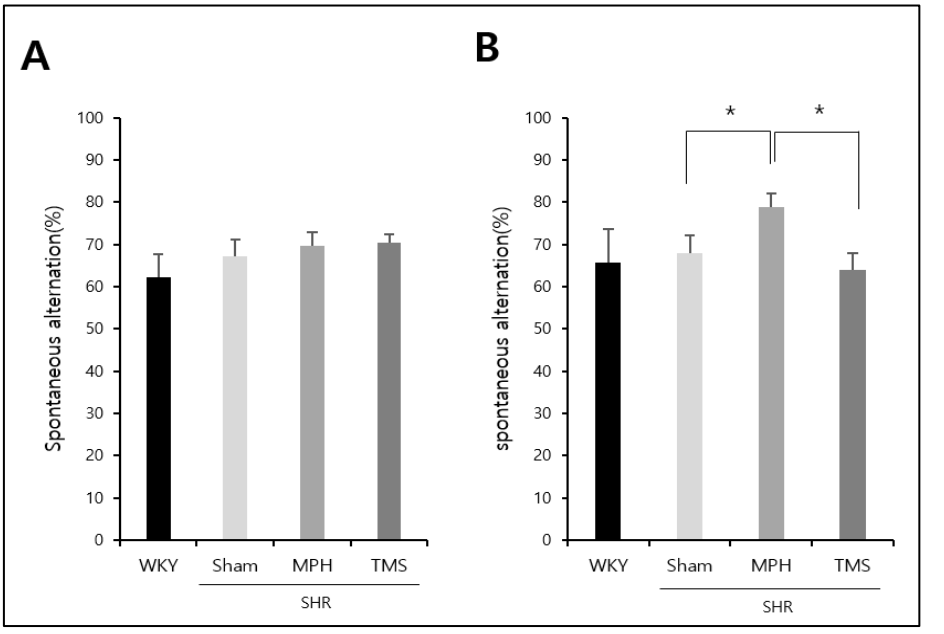

Figure 3. Spontaneous alternation (%) for the Y-maze test in the second (A) and third (B) weeks. Shown are the mean ± SEM spontaneous alternations of each group. *$p < 0.05$. SEM: standard error of the mean. WKY: Wistar-Kyoto group; MPH: methylphenidate group; TMS: transcranial magnetic stimulation group.

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3. Western blotting

BDNF expression in the prefrontal cortex of the MPH group was similar to that in the Sham group. BDNF concentration was higher in the TMS group than the Sham group, though the difference was not statistically significant (Fig. 4), indicating that rTMS may have exerted a slight positive effect in increasing BDNF concentration.

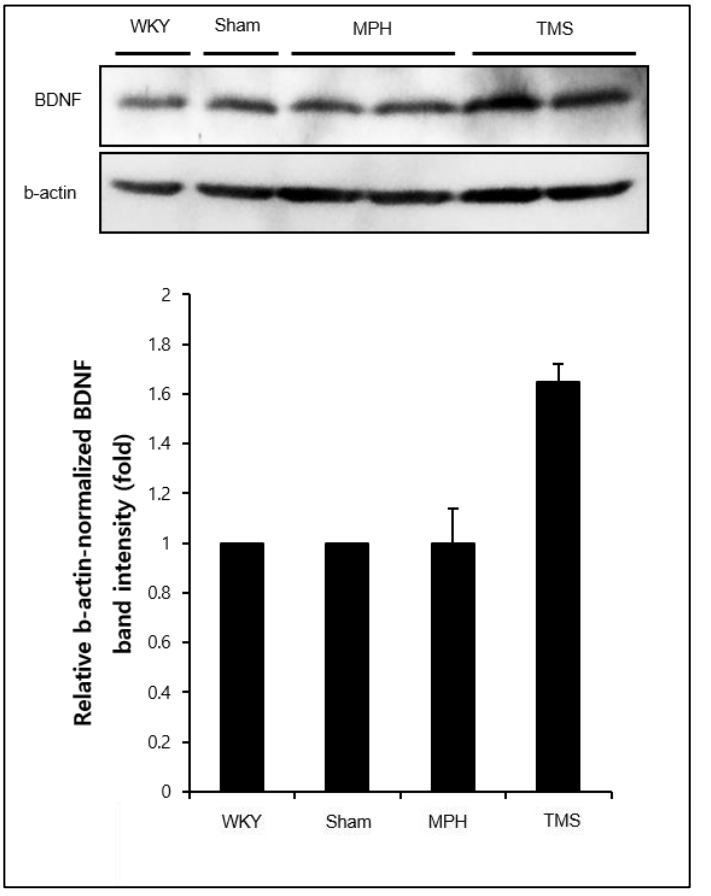

Figure 4. Western blot analysis of BDNF in the prefrontal cortex. BDNF: brain-derived neurotrophic factor; WKY: Wistar-Kyoto; MPH: methylphenidate; TMS: transcranial magnetic stimulation group.

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4. High performance liquid chromatography (HPLC)

The average concentration of noradrenaline in the TMS group was significantly lower than in the Sham and MPH groups ($p < 0.05$, Table 1). The average concentration of serotonin was significantly lower in the WKY group than in the Sham, MPH, and TMS groups ($p < 0.05$), though no significant difference was observed among the three groups of SHR. The average dopamine level was significantly higher in the MPH group than in the WKY group ($p < 0.05$).

Table 1. Concentration of catecholamines in the prefrontal cortex

Groups	Noradrenaline	Serotonin	Dopamine
WKY	1.36 ± 0.08	0.08 ± 0.005	0.01 ± 0.01
Sham	1.61 ± 0.04$^{\#}$	0.11 ± 0.01$^{\#}$	0.06 ± 0.03
MPH	1.60 ± 0.06$^{\#}$	0.12 ± 0.01$^{\#}$	0.19 ± 0.07$^{\#}$
TMS	1.42 ± 0.05*	0.11 ± 0.005$^{\#}$	0.01 ± 0.01

WKY: Wistar-Kyoto group; MPH: methylphenidate group; TMS: transcranial magnetic stimulation group. The table values represent the mean (ng/mg in tissue) ± SEM. $^{\#}p < 0.05$ vs. WKY; $^{*}p < 0.05$ vs. Sham

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IV. DISCUSSION

In the present study, we examined whether rTMS can improve ADHD symptoms in spontaneously hypertensive rats. Either low (1 Hz) or high (5–20 Hz) frequency rTMS is administered to patients with psychiatric disorders.[22] At low frequencies, rTMS reduces neuronal excitability. Conversely, high frequency stimulation not only induces increased neuronal excitability, but also improves working memory and attention.[14,15] Whether high- or low-frequency stimulation is effective in patients with ADHD remains unclear. Bloch et al. demonstrated that high frequency rTMS at the right dorsolateral prefrontal cortex improved inattention in patients with ADHD.[13] Conversely, Niederhofer documented that low-frequency rTMS ameliorated ADHD symptoms and helped reduce the necessary dosage of methylphenidate.[23] Nevertheless, clinically most counselled psychiatric disorders are treated with high-frequency stimulation. The present study thus employed stimulation parameters that have been successful in patients with depression while targeting the right prefrontal cortex.

The application of rTMS to SHR did not reduce the total distance moved in the open field test, as methylphenidate did. Interestingly, however, the pattern of behavior in the TMS group over time (10 min in the open field apparatus) was similar to that of the control WKY group. The control group

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explored quite actively in the open field apparatus during the first 5 min, though movement decreased over time as the animals adapted to the location. Nonetheless, the SHR group moved restlessly throughout the box for the entire 10 min, resulting in relatively high total distance travelled. The application of rTMS to SHR resulted in decreased movement as time progressed. The reduced distance moved over time in a novel environment implies that the subject has adapted to it.[24] Control subjects adapted sufficiently and remained in a comfortable location; however, hyperactive SHRs consistently explored the open field box, regardless of the number of times they were exposed to the environment. Accordingly, the distance moved by each subject corresponds to the intensity of hyperactivity.[25] As we conducted only a 10-min open field test, it is possible that a statistically significant decrease in total distance may be observed if the total duration of the experiment were extended.

The extracellular noradrenaline concentration was lower in the TMS group than in the Sham group. Another characteristic of SHRs is dopaminergic hypofunction and noradrenergic hyperactivity.[26] Noradrenaline release is normally inhibited when the α2-adrenoceptor is stimulated, but in SHRs, the sensitivity of α2-adrenoceptors is decreased, which results in over-release of noradrenaline. Hence, α2-adrenoceptor agonists ameliorate ADHD symptoms.[27] An α2-adrenoceptor antagonist would, conversely, induce increased noradrenaline release and thus promote increased locomotor activity.[28] Our

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results may indicate that decreased concentrations of noradrenaline in brain regions where rTMS was applied may ameliorate hyperactivity. Since the prefrontal cortex (PFC) responds to the surrounding neurochemicals very sensitively, the functions of the PFC are decreased when the release of catecholamine is either insufficient or excessive.[29] Excessive release of noradrenaline causes problems in attention and hyperactivity, functions associated with altered PFC activity. Hence, balanced and appropriate release of dopamine and noradrenalin is necessary. Therefore, rTMS may have attenuated impaired attention and hyperactivity in SHRs due to reduction of excessive noradrenalin release.

Interestingly, the TMS group exhibited an increased tendency to enter the open arms in the elevated plus-maze test relative to the remaining groups, although this result was not statistically significant (13.4 s ± 6.8 vs. 9.9 s ± 6.5 (Sham group), 4.0 s ± 3.0 (MPH group), 12.9 s ± 4.6 (WKY group)). Increased noradrenaline release induces anxiety-like behavior.[30] In the present study, reduction of noradrenaline may have decreased anxiety in SHRs, which may have in turn affected the number of entrances into open arms. Stimulant drugs such as methylphenidate, which are the most widely used therapeutic agents in the treatment of ADHD, often cause anxiety, sleep disturbances, and anorexia due to excessive release of dopamine. In particular, since about 30% of children with ADHD are diagnosed with comorbid anxiety disorders,[1] they may be more

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susceptible to the adverse anxiogenic effects of methylphenidate. However, rTMS—which acts on noradrenaline rather than directly on dopamine—may have reduced not only hyperactivity but also anxiety by attenuating and controlling the release of noradrenaline in SHRs of the present study. In addition, BDNF expression, which modifies synaptic plasticity and neural development of the prefrontal lobe, was also increased following rTMS. These results align with those of previous reports, which have revealed that increases in BDNF expression result in improvements in ADHD symptoms.[31,32]

The present study possesses certain limitations. First, no standard protocol is available with respect to the application of rTMS to SHRs. However, rTMS studies of human patients with ADHD as well as previous studies on SHR depression were appropriately utilized to prepare our own standards. Therefore, the present study may be a pioneering work in the preparation of a standard protocol for treating animal models of ADHD with rTMS. Second, no significant differences in dopamine were observed among the three groups of SHRs in the present study. However, this result indicates that rTMS therapy may be particularly useful in patients experiencing adverse effects related to dopamine over-stimulation, which often occurs during as a side-effect of methylphenidate treatment. The finding that rTMS is not correlated with levels of dopamine also suggests a new mechanism by which ADHD may be identified. Thirdly, the response of BDNF to rTMS in humans depends upon

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individual genetic variations. Therefore, care should be taken when applying the methods/results of this study to humans due to the increased complexity of the associated systems in humans relative to animals.[33]

V. CONCLUSION

High-frequency rTMS treatment of SHRs resulted in decreased noradrenaline concentration and elevated BDNF expression in the prefrontal cortex, as well as a tendency to decrease locomotor activity. The results of this preclinical study indicate that rTMS may present a new modality of treatment for children with ADHD. However, further animal and clinical studies are required in order to examine the efficacy, safety, and optimal stimulation parameters prior to offering rTMS in routine clinical practice.

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REFERENCES

1. Biederman J. Attention-deficit/hyperactivity disorder: a selective overview. Biol. Psychiatry 2011;57: 1215–20.
2. Amer Psychiatric Pub Incorporated. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington: Amer Psychiatric Pub Incorporated; 2013.
3. Zang YF1, He Y, Zhu CZ, Cao QJ, Sui MQ, Liang M, et al. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. Brain Dev 2007;29(2):83-91.
4. Sharma A, Couture J. A review of the pathophysiology, etiology, and treatment of attention-deficit hyperactivity disorder (ADHD). Ann Pharmacother 2014;48(2) 209–25.
5. Storebø OJ, Ramstad E, Krogh HB, Nilausen TD, Skoog M, Holmskov M, et al. Methylphenidate for children and adolescents with attention deficit hyperactivity disorder (ADHD). Cochrane Database Syst Rev 2015;11: CD009885.
6. Charach A, Ickowicz A, Schachar R. Stimulant treatment over five years: adherence, effectiveness, and adverse effects. J Am Acad Child Adolesc Psychiatry 2004;43(5):559-67.
7. Stiefel G, Besag FM. Cardiovascular effects of methylphenidate, amphetamines and atomoxetine in the treatment of attention-deficit hyperactivity disorder. Drug Saf 2010;33:821–42.
8. George MS, Nahas Z, Borckardt JJ, Anderson B, Foust MJ, Burns C, et al. Brain stimulation for the treatment of psychiatric disorders. Curr Opin Psychiatry 2007;20:247–54.
9. Rossi S, Pasqualetti P, Rossini PM, Feige B, Ulivelli M, Glocker FX, et al. Effects of repetitive transcranial magnetic stimulation on movement-related cortical activity in humans. Cereb Cortex 2000;10(8):802-8.
10. Croarkin PE, Wall CA, McClintock SM, Kozel FA, Husain MM, Sampson SM. The emerging role for repetitive transcranial magnetic stimulation in optimizing the treatment of adolescent depression. J ECT 2010 Dec;26(4):323-9.
11. Fukudome T, Goto H, Izumoto H, Matsuo H, Shibuya N. The effects of repetitive transcranial magnetic stimulation (rTMS) in the patients with Parkinson's disease. Rinsho Shinkeigaku. 2002;42(1):35-7.
12. Weaver L, Rostain AL, Mace W, Akhtar U, Moss E, O'Reardon JP. Transcranial magnetic stimulation (TMS) in the treatment of attention-deficit/hyperactivity disorder in adolescents and young adults: a pilot study. J ECT 2012;28:98–103.
13. Bloch Y, Harel EV, Aviram S, Govezensky J, Ratzoni G, Levkovitz Y. Positive effects of repetitive transcranial magnetic stimulation on attention in ADHD subjects: a randomized controlled pilot study. World J. Biol. Psychiatry 2010;11(5): 755–8.

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14. Pascual-Leone A, Valls-Sole J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain 1994;117:847–58.
15. Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, et al. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 1997;48(5):1398-403.
16. Lefaucheur JP, André-Obadia N, Antal A, Ayache SS, Baeken C, Benninger DH, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). Clin Neurophysiol 2014;125: 2150-206.
17. Saadat F, Kosha M, Amiry A, Torabi G. Brain-derived neurotrophic factor as a biomarker in children with attention deficit-hyperactivity disorder. J KIMSU 2015;4:10-7.
18. Bizot JC, Chenault N, Houzé B, Herpin A, David S, Pothion S, et al. Methylphenidate reduces impulsive behavior in juvenile Wistar rats, but not in adult Wistar, SHR and WKY rats. Psychopharmacology (Berl) 2007;193(2):215-23
19. Sohn MK, Song H, Jee S. Plasticity associated changes in neurophysiological tests following non-invasive brain stimulation in stroke rat model. Korean J Clin Neurophysiol 2014;16:62–9.
20. Yu SL, Lee DC, Sohn HA, Lee SY, Jeon HS, Lee JH, et al. Homeobox A9 directly targeted by miR-196b regulates aggressiveness through nuclear Factor-kappa B activity in non-small cell lung cancer cells. Mol Carcinog 2015; 55(12):1915-26.
21. De Benedetto GE, Fico D, Pennetta A, Malitesta , Nicolardi G, Lofrumento DD, et al. A rapid and simple method for the determination of 3,4-dihydroxyphenylacetic acid, norepinephrine, dopamine, and serotonin in mouse brain homogenate by HPLC with fluorimetric detection. J Pharm Biomed Anal 2014;98:266-70.
22. Theiner P, Ustohal L, Skřont T, Bareš M, Kašpárek T. Repetitive transcranial magnetic stimulation in ADHD. In: Norvilitis JM, editors. ADHD–New Directions in Diagnosis and Treatment, InTech; 2015.
23. Niederhofer H. Additional biological therapies for attention-deficit hyperactivity disorder: repetitive transcranial magnetic stimulation of 1 Hz helps to reduce methylphenidate. Clin Pract 2012;2(1):e8.
24. Dai H, Krost M, Carey RJ. A new methodological approach to the study of habituation: the use of positive and negative behavioral indices of habituation. J Neurosci Methods 1995;62(1-2):169-74.
25. Knardahl S, Sagvolden T. Open-field behavior of spontaneously hypertensive rats. Behav Neural Biol 1979;27:187-200.
26. Tayebati SK, Tomassoni D, Amenta F. Spontaneously hypertensive rat as a model of vascular brain disorder: microanatomy, neurochemistry and behavior. J Neurol Sci 2012;322:241–9.

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27. Russell VA. Hypodopaminergic and hypernoradrenergic activity in prefrontal cortex slices of an animal model for attention-deficit hyperactivity disorder-the spontaneously hypertensive rat. Behav Brain Res 2002;130:191-6.
28. Ma CL, Arnsten AF, Li BM. Locomotor hyperactivity induced by blockade of prefrontal cortical alpha2-adrenoceptors in monkeys. Biol Psychiatry 2005;57(2):192-5.
29. Arnsten AF. Dopaminergic and noradrenergic influences on cognitive functions mediated by prefrontal cortex. In: Solanto MV, Arnsten AF, Castellanos FX, editors. Stimulant Drugs and ADHD: Basic and Clinical Neuroscience. Oxford: Oxford University Press; 2001. p. 185–208.
30. Dazzi L, Vignone V, Seu E, Ladu S, Vacca G, Biggio G. Inhibition by venlafaxine of the increase in norepinephrine output in rat prefrontal cortex elicited by acute stress or by the anxiogenic drug FG 7142. J Psychopharmacol 2002 Jun;16(2):125-31.
31. Amiri A, Torabi Parizi G, Kousha M, Saadat F, Modabbernia MJ, Najafi K, et al. Changes in plasma brain-derived neurotrophic factor (BDNF) levels induced by methylphenidate in children with attention deficit-hyperactivity disorder (ADHD). Prog Neuropsychopharmacol Biol Psychiatry 2013;47:20-4.
32. Kim H, Heo HI, Kim DH, Ko IG, Lee SS, Kim SE, et al. Treadmill exercise and methylphenidate ameliorate symptoms of attention deficit/hyperactivity disorder through enhancing dopamine synthesis and brain-derived neurotrophic factor expression in spontaneous hypertensive rats. Neurosci Lett 2011;504:35–9.
33. Tang A, Thickbroom G, Rodger J. Repetitive transcranial magnetic stimulation of the brain: mechanisms from animal and experimental models. Neuroscientist 2015:1–13.

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ABSTRACT (IN KOREAN)

주의력결핍 과잉행동장애 동물 모델인 자연발생 고혈압 쥐에 대한 고주파 반복적 경두개자기자극술의 효과

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주의력결핍 과잉행동장애에 대한 현재의 치료법은 약물요법이다. 그러나 주의력결핍 과잉행동장애를 지닌 환자의 일반적인 대상군에 대한 약물 사용은 부작용 및 각 환자의 약물에 대한 약동학적 차이로 인해 제한되기 때문에, 새로운 치료법에 대한 연구가 진행 중이다. 본 연구에서 우리는 주의력결핍 과잉행동장애의 동물 모델인 자연발생 고혈압쥐에게 기타 다른 정신 질환에서 사용하고 있는 비침습적 치료법인 반복적 경두개자기자극술을 적용하여, 도파민, 노르아드레날린, 세로토닌, 뇌-유래 신경영양인자에서의 농도 및 행동 증상에서도 변화 유무를 통해 치료 효과를 평가하려 한다.

총 15회의 고주파 경두개자기자극술 치료가 시행되었다. 오픈필드, Y- 미로 및 상승된 십자형-미로 검사를 사용하여 행동 증상을 관찰 하였다. 실험이 완료되면 쥐를 희생시키고, 뇌 조직에서의 신경화학적 변화를 고성능 액체 크로마토그래피 및 웨스턴 블롯팅을 사용하여 분석 하였다

반복적 경두개자기자극술로 치료한 자연발생 고혈압쥐는 치료기간 동안 오픈 필드 검사에서 운동 활동성이 덜 나타나는 경향이 있

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었으나, Y- 미로 검사에서는 부주의성에서의 호전은 없었다. 또한, 반복적 경두개자기자극술로 치료한 자연발생 고혈압쥐의 전두엽 피질에서 뇌-유래 신경영양 인자의 농도가 증가하였으며, 노르아드레날린의 농도는 감소하였다.

본 임상 전 연구의 결과는 반복적 경두개자기자극술은 주의력결핍 과잉행동장애 환자에게 새로운 치료법을 제공할 수 있다는 것과 사람에게 있어 안전성 및 효과뿐 만 아니라 특정한 치료 매개 요인에 대한 심화된 연구가 필요함을 보여주었다.

핵심되는 말: 주의력결핍 과다활동장애, 반복적 경두개자기자극술, 자연발생 고혈압, 카테콜아민, 뇌유래 신경영양 인자, 행동분석