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Effects of high-frequency repetitive transcranial magnetic stimulation (rTMS) on spontaneously hypertensive rats, an animal model of attention-deficit/hyperactivity disorder

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ABSTRACT

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 is 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 behavioural 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. Behavioural 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 analysed using high performance liquid chromatography and Western blotting. 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.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most prevalent developmental disorders among children (Biederman, 2011). Patients with ADHD exhibit varying levels of hyperactivity/impulsivity and inattention that result in impaired functioning and academic performance (American Pshchiatric Association, 2013). Research has indicated that the prefrontal cortex, caudate nucleus, and cerebellum—which constitute a neuronal network involved in the regulation of attention, emotion, and behaviour—are important structures in the pathophysiology of ADHD (Zang et al., 2007). Indeed, delayed development and decreased size of the prefrontal cortex, caudate nucleus, and

Abbreviations: rTMS, repetitive transcranial magnetic stimulation; ADHD, attention deficit/hyperactivity disorder; SHR, spontaneously hypertensive rat; BDNF, brain-derived neurotrophic factor; WKY, Wistar-Kyoto; MPH, methylphenidate; PND, postnatal day; ANOVA, analysis of variance; SEM, standard error of the mean; PFC, prefrontal cortex.

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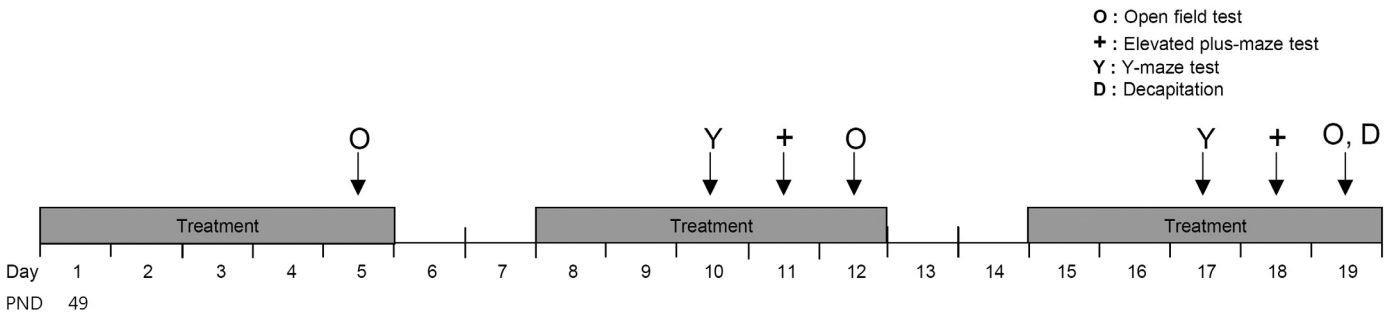

Fig. 1. Experimental design.

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 further contributes to the pathophysiology of ADHD (Sharma and Couture, 2014). As the aetiology 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 (Storebø et al., 2015). 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 (Charach et al., 2004; Stiefel and Besag, 2010). 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 (George et al., 2007) that exerts its effects by modulating cortical excitability, either increasing or decreasing neuronal excitability (or inhibition), depending on the administered stimulation (Rossi et al., 2000). The therapeutic effect 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 (Croarkin et al., 2010; Fukudome et al., 2002). 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 (Weaver et al., 2012; Bloch et al., 2010). 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 (Pascual-Leone et al., 1994; Chen et al., 1997). 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 aetiology of ADHD, we aimed to activate the depressed brain area with high-frequency stimulation and evaluate alterations in the behavioural ADHD symptoms of spontaneously hypertensive rats (SHRs) (Lefaucheur et al., 2014).

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 cortex, and that the application of psychostimulants (methylphenidate, amphetamine) increases BDNF expression and improves behavioural symptoms (Saadat et al., 2015). In addition to those of dopamine and noradrenaline, we analyzed serotonin levels due to their association with emotional behaviour.

2. Materials and methods

2.1. 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 (Bizot et al., 2007). 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 °C) and humidity (50 ± 10 °C) 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.

2.2. 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.