Modulation of frequency and duration of repetitive magnetic stimulation affects catecholamine levels and tyrosine hydroxylase activity in human neuroblastoma cells: implication for the antidepressant effect of rTMS

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Abstract
Transcranial magnetic stimulation (TMS), which is produced by strong non-static magnetic fields, is a non-invasive means to stimulate the cerebral cortex. Studies from recent years show that TMS affects mood in healthy subjects and improves depressive symptoms in patients with major depression. However, the relationship between the clinical efficacy of TMS and stimulation parameters is still obscure. In the present study we have investigated the effects of different stimulation frequencies and number of treatments on catecholamine turnover in SH-SY5Y cell cultures. A single session of magnetic stimulation (1.7 T) caused a significant decrease in intracellular dopamine and L-DOPA and in noradrenaline (NE) release at a rate of 3 Hz for 10 s but increased NE release at a rate of 9 Hz. These alterations were associated with a reduction (47.8%) or an increase (48%) in tyrosine hydroxylase (TH) activity after 3 and 9 Hz magnetic stimulation, respectively. The latter may be related to the known sensitivity of TH to neuronal firing rates and NE concentrations. Higher stimulation frequencies (15, 20, 45 Hz) had no effect on catecholamine metabolism. Unlike 3 Hz acute treatment, chronic treatment (3 Hz, 11 sessions, for 4 d) had no effect on monoamines and TH activity was increased by 54.5% with no change in its protein level. The results of the present study demonstrate that in tissue culture system frequency and treatment duration of the magnetic stimulation are important factors in affecting catecholamine turnover. Considering the major role of catecholamine in the pathophysiology of depression, these findings may be of relevance to the application of rTMS in humans with major depression.

Received 11 August 2002; Reviewed 15 October 2002; Revised 17 December 2002; Accepted 18 December 2002

Key words: Frequency and duration, human neuroblastoma SH-SY5Y cells, magnetic stimulation, noradrenaline (NE), rTMS, tyrosine hydroxylase (TH).

Introduction
Alternating magnetic fields, primarily those of weak strength (<1 mT), display a broad-spectrum of biological effects. Low-field magnetic stimulation when applied either in vivo or in vitro, stimulates growth and differentiation of various types of cells (Basset et al., 1974; Ito and Basset, 1983; Macias et al., 2000) and accelerates regeneration and recovery of function following peripheral nerve crush or transection (Ito and Basset, 1983; Sisken et al., 1993; Walker et al., 1994). It has been suggested that weak magnetic fields can interact with different intracellular biochemical components, including ions such as calcium and phosphate groups, organic acids, coenzymes and proteins (Liboff, 1994; Uzdensky, 1999). In addition, they may interfere with electrostatic processes such as phosphate group transfer, ligand–receptor, association–dissociation, protein–protein and protein–nucleic acid interaction (Uzdensky, 1999). This wide range of effects
on different biological systems has been attributed to a resonance mechanism induced by the magnetic fields (Lednev, 1991).

In contrast to the extensive interest in the biological effects of alternating weak magnetic fields only few studies have looked at the biological effects of strong non-static magnetic fields, despite their wide practice in medicine. Strong non-static magnetic fields (1–2 T) have been used to stimulate the brain since the introduction of a novel technology in 1985 (Baker et al., 1985), which allowed for the storage and rapid discharge and recharge of powerful electrical currents transmitted in a spiral of wire inducing a magnetic field. The magnetic field produces an electric field which initiates ion flow and consequent membrane depolarization directly in brain tissues. Magnetic stimulation of the human brain (transcranial magnetic stimulation; TMS) is used for functional cortical mapping of the primary motor cortex and speech areas, and for the investigation of cortical functions related to cognition in both healthy and diseased states (Baker, 1991; Gates, 1995). Recently it has been shown that TMS in combination with different imaging techniques, including positron emission tomography (PET), single photon emission computerized tomography (SPECT) as well as functional magnetic resonance imaging (fMRI) enables assessment of the state of functional connectivity in the human brain without the need for the subject to be engaged in a specific behavioural task (Krings et al., 2001; Paus et al., 1997; Shajahan et al., 2002).

A growing number of reports have demonstrated that repetitive transcranial magnetic stimulation (rTMS) of prefrontal regions affects mood in both normal and depressed subjects (Feinsod et al., 1998; George et al., 1995; Grisaru et al., 1994; Grunhaus et al., 2000; Hallott, 2000; Klein et al., 1999; Pascual-Leone et al., 1996a). The effect on mood has been shown to depend on the site of stimulation. Thus, in normal volunteers, focal stimulation over the left prefrontal cortex induced a transient feeling of sadness, while right prefrontal stimulation induced a feeling of happiness (George et al., 1996; Pascual-Leone et al., 1996b).

Evidence suggests that stimulation frequency may also differentially affect the physiological outcome of rTMS (Kimble et al., 1999; Speer et al., 2000). It is believed that slow rTMS (≤1 Hz) has inhibitory effects, whereas fast stimulation facilitates neuronal activity in the primary motor cortex (Pascual-Leone et al., 1994; Sackeim, 2000). Moreover, it was shown that in depressed subjects the antidepressant effect of slow rTMS (3 and 5 Hz) tended to be more pronounced (although not statistically significant) than that of fast rTMS (10 and 20 Hz) (George et al., 2000; Klein et al., 2002). The importance of stimulation frequency for the behavioural effects of rTMS was also suggested in animal models of depression, where an increase in frequency of impulses from 20 to 30 Hz augmented the reduced immobility in the forced swimming test (Zyss et al., 1999).

Further studies in animal models of depression have shown that rTMS induces behavioural effects similar to those induced by antidepressant drugs and electroconvulsive shock (ECS) (Lisanby and Belmaker, 2000), and, likewise, at the cellular and molecular level. Thus, it was reported that a single session of rTMS (25 Hz for 2 s) caused significant and specific alterations in monoamines in the rat brain (Ben-Shachar et al., 1997a), while chronic rTMS treatment (15 Hz for 3.5 s daily, for 10 d) had no effect on monoamine levels but caused up-regulation of β-adrenergic and down-regulation of 5-HT2 receptors in the frontal cortex (Ben-Shachar et al., 1999). Others however, have reported a minimal down-regulation of β-adrenergic receptors in the cortex (Fleischman et al., 1996). Further studies have shown that similar to other antidepressant treatments, chronic administration of rTMS to rats caused subsensitivity of both the 5-HT1A and 5-HT1B autoreceptors (Gur et al., 2000). In restrained rats a single session of rTMS (20 Hz) resulted in increased binding to 5-HT1A and NMDA receptors (Kole et al., 1999). Moreover, a frequency-dependent stimulation with rTMS significantly increased mRNA levels of glial fibrillary acidic protein (GFAP) in the dentate gyrus of the hippocampus and less so in the cerebral cortex, as well as increasing the levels of c-fos in the paraventricular nucleus of the thalamus and the frontal and medial cortex (Fujiki and Steward, 1997; Ji et al., 1998).

Taken together, these studies suggest that rTMS exhibits antidepressant properties, which share considerable clinical and biochemical resemblance with other antidepressant treatments. However, given the state of evidence, it is clear that in order to become a routine clinical tool, optimization of stimulation parameters is required. In studies with weak alternating magnetic fields, frequencies of stimulation were reported to be of crucial importance for the biological outcome of the stimulation (Uzdensky, 1999). Similarly, there are indications for the importance of stimulation frequency for the antidepressant effect of rTMS (Kimble et al., 1999; Sackeim, 2000; Speer et al., 2000). In the present study we investigated the influence of both frequency and duration of treatment of a strong non-static magnetic field (1.72 T) on...
monoamine metabolism and synthesis using human neuroblastoma SH-SY5Y cell cultures. Neuronal cell cultures were used in order to investigate the latter without the interference of other factors such as stress, tissue characteristics, lateralization and connectivity.

Materials and methods

**Culture treatment by magnetic stimulation**

Human neuroblastoma SH-SY5Y cells, were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin at 37 °C in a 5% CO₂ humidified incubator. Twenty-four hours before stimulation the medium was replaced by serum-free medium. Magnetic stimulation was applied by a direct contact with cell culture dishes using a Magstim Rapid Stimulator with pulse duration of 250 μs and field intensity of 1.72 T (75% of maximum stimulator output). A 9 cm circular coil was used with stimulation frequencies ranging from 3 to 45 Hz for 10 s. Although 1 Hz was typically used as the low frequency in clinical studies with rTMS (Feinsod et al., 1998; Grisaru et al., 1994; Klein et al., 1999), we could not demonstrate in preliminary experiments any change in monoamine transmission in SH-SY5Y tissue cultures using the above-mentioned experimental protocol. Cell cultures received either a single (acute treatment) or repeated sessions (chronic treatment) by placing the 35-mm diameter dishes directly on the surface of the coil. Following acute treatment cells were immediately harvested. For chronic treatment cells were stimulated 3 times daily for 3 d and twice on the fourth day, and harvested 4 h after the last session. The control group received sham treatment, by placing the culture dishes on the coil directly on the surface of the coil. Following acute treatment the entire culture was used for further analysis.

**HPLC analysis**

SH-SY5Y cells and media were separated on ice and HClO₄ was added to a final concentration of 0.1 N. Both medium and cell homogenates were centrifuged (12,000 g, 4 °C), lyophilized, re-suspended and stored at −70 °C until use. Concentrations of catecholamines and their metabolite, i.e. L-dihydroxyphenylalanine (L-DOPA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and noradrenaline (NE), were determined by HPLC with an electrochemical detector, equipped with a column of 5 μm spherical C₁₈ particles as described previously (Ben-Shachar et al., 1997b). The dual electrode analytical cell operated in a redox mode with 0.3 V oxidation potential and −0.35 V reduction potential. The mobile phase consisted of 0.1 M phosphate buffer (pH 2.75) containing 0.2 mM octane sulfonic acid, 2.5% methanol and 4.5% acetonitrile.

**Tyrosine hydroxylase (TH) activity**

TH activity was determined by measuring l-DOPA accumulation after inhibition of DOPA decarboxylase with 100 μM 3-hydroxybenzylhydrazine (NSD-1015) for 60 min according to Hunter et al. (1993). Cells were treated with 3 Hz magnetic stimulation 15 min before terminating the reaction with 0.1 N HClO₄. In order to determine the effect of chronic magnetic stimulation on TH activity, NSD-1015 was added 3 h after the last magnetic stimulation for an additional 60 min.

**Western blot analysis**

Cells were washed with PBS, lysed for 1 h in 1 ml ice-cold lysis buffer (pH 7.4) containing 10 mM Tris, 250 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40 and TM protease inhibitors cocktail. Following centrifugation at 12,000 g for 5 min the supernatant was diluted 1:1 in electrophoresis loading buffer containing 20% (v/v) glycerol, 4% (w/v) SDS, 250 mM Tris–HCl (pH 6.8), 10% (v/v) 2-mercaptoethanol and 0.5 mg/ml Bromophenol Blue. The protein sample was separated on SDS acrylamide gel (7.5%) and transferred to a nitrocellulose membrane. Quality of transfer was assayed by Ponceau staining. Following blocking of non-specific binding sites, membranes were incubated at 4 °C overnight with primary monoclonal human TH antibody diluted 1:1600 in T-TBS containing 1% BSA. After washing, blots were incubated for 1 h at room temperature with anti-mouse IgG diluted 1:10,000 in T-TBS. The blots were then developed with Amersham’s ECL and exposed to XLS Kodak film for 20–30 s. Antibodies were purchased from Santa Cruz Biotechnology Inc., CA, USA.

**Cell survival and protein concentration**

Cell survival was assessed by Trypan Blue, which was added to the cell suspension at a final concentration of 0.1%. Protein concentration was measured using Bradford reagent (Bio-Rad Laboratories Inc., CA, USA).

**Statistical analysis**

All results were analysed separately for each monoamine and its metabolite with the SPSS software version 9.0. Inter-group differences of the various
dependent variables were assessed by one-way ANOVA followed by Dunnett post-hoc multiple comparisons test. \( p \) values lower than 0.05 were considered significant.

**Results**

**The effect of a single session magnetic stimulation on catecholamines and TH activity in SH-SY5Y cells**

Human neuroblastoma SH-SY5Y cells synthesize and accumulate in their vesicles releasable NE, and take up both NE and DA (Willets et al., 1993). A single session of magnetic stimulation (acute treatment) at a frequency of 3 Hz for 10 s resulted in a significant reduction in intracellular DOPA \( (p = 0.01) \) and DA \( (p = 0.02) \) with no significant change in DOPAC and NE concentrations (Figure 1). In the medium, a significant \( (p = 0.001) \) decrease in NE concentrations was observed, suggesting a reduction in the release of NE following 3 Hz stimulation (Figure 2). However, simulating the cells at a frequency of 9 Hz for 10 s resulted in a significant \( (p = 0.014) \) increase in NE in the medium (Figure 2) without any significant effect on catecholamine intracellular levels (Figure 3). Higher frequencies, i.e. 15, 20 and 45 Hz had no effect on the intracellular and extracellular levels of these catecholamines (data not shown).

It has been documented that in response to enhanced or reduced neuronal activity, endogenous levels of NE are not affected whereas its synthesis is altered due to a regulatory mechanism on the activity of TH, the rate-limiting enzyme in the biosynthesis of catecholamines. Thus, we determined changes in TH activity in response to a single session of 3 and 9 Hz magnetic stimulation by measuring the accumulation of \( \alpha \)-DOPA following NSD-1015 treatment. Magnetic stimulation at 3 Hz induced a reduction to 47.8\% \( (p = 0.001) \), while 9 Hz induced a 48\% \( (p = 0.005) \) increase in TH activity compared to untreated cells (Table 1).

**Figure 1.** Intracellular concentrations of NE, its precursors \( \alpha \)-DOPA and DA, and the DA metabolite DOPAC following a single 10-s session of 3 Hz magnetic stimulation. Tissue culture dishes were placed on the stimulating coil either stimulated (MS) or sham treated (control) and processed by HPLC for their catecholamine content as described in the Materials and methods section. Data are means \( \pm \) S.D. of a representative experiment out of three experiments each performed in five different culture dishes. The significant difference between MS and sham-treated cultures was analysed by ANOVA followed by Dunnett post-hoc multiple comparison \( (* p = 0.01, ** p = 0.02) \).

**Figure 2.** Concentrations of NE released from cells following a single 10-s session of 3, 9 and 15 Hz magnetic stimulation. Cell culture dishes were processed as described in Figure 1 and NE content in the medium was analysed by HPLC as described in the Materials and methods section. Data are means \( \pm \) S.D. of representative experiments for each stimulation frequency out of 3–5 experiments for every frequency each performed in five different culture dishes. The significant difference between MS and sham-treated cultures was analysed by ANOVA followed by Dunnett post-hoc multiple comparison \( (* p = 0.001, ** p = 0.014) \).

**Figure 3.** Intracellular concentrations of NE, its precursors \( \alpha \)-DOPA and DA, and the DA metabolite DOPAC following a single 10-s session of 9 Hz magnetic stimulation. Tissue culture dishes were treated and processed as described in Figure 1. Data are means \( \pm \) S.D. of a representative experiment out of four experiments each performed in five different culture dishes. No significant difference was observed between MS and sham-treated cultures.
The effect of chronic magnetic stimulation on catecholamines and TH activity in SH-SY5Y cells

It has been shown that alterations in monoamine metabolism in rat brains following acute stress or antidepressant treatments differ from those induced by chronic treatments (Ben-Shachar et al., 1997a, 1999; Glue et al., 1990; Plaznik et al., 1994; Yoshida et al., 1997; Zis et al., 1992). Since findings from clinical studies suggest a greater effect of low-frequency rTMS in major depression and 3 Hz stimulation demonstrated the most pronounced effects on both synthesis and release of NE in the acute treatment, we chose this frequency to further study the effect of chronic treatment. Cells received 11 sessions of magnetic stimulation at 3 Hz during 4 d. Unlike a single session (acute treatment) no change was observed in intracellular levels of DOPA, DA, DOPAC and NE following chronic treatment (data not shown). The accumulation of extracellular levels could not be measured since the medium had to be replaced during the treatment period in order to avoid cell starvation.

TH activity was increased by 54.5% ($p = 0.023$) following chronic treatment with 3 Hz stimulation (Table 1). No change was observed in TH protein levels measured by Western blot analysis after 11 sessions of a 3 Hz magnetic stimulation (Figure 4).

**Cell viability**

Cell viability was determined in treated and control cultures using Trypan Blue. Magnetic stimulation of both acute and chronic at 3, 9, 15, 20 and 45 Hz had no effect on cell viability (Figure 5).

**Discussion**

The present study demonstrates that the frequency of the magnetic stimulation, and the duration of treatment induced by a strong non-static magnetic field (1.5 T), are important factors in the biochemical effects of this treatment in SH-SY5Y human neuroblastoma cells. The use of an in-vitro system and its relevance to the in-vivo mode of action of rTMS might be questioned. However, it has been previously shown that in-vitro effects of magnetic stimulation were later confirmed in vivo. Thus, Massot et al. (2000) demonstrated that magnetic stimulation induced desensitization of 5-HT$_{1B}$ receptors in rat brain membranes, an effect that has also been shown in rats chronically treated with rTMS (Gur et al., 2000).

In the present study the relative contribution of two parameters namely, stimulation frequency and number of treatments (duration), which have been suggested to play an important role in the clinical effects of rTMS, was investigated. Thus, five different frequencies in the range of 3–45 Hz were applied in a single-session experiment, and acute (single treatment) vs. chronic (multiple) treatment at 3 Hz were
administered. For the latter, the low-frequency stimulus (3 Hz) was selected given the findings from clinical studies which suggested a greater efficacy of low-frequency rTMS in major depression (George et al., 2000; Klein et al., 2002).

A single session (acute) of low-frequency (3 Hz) magnetic stimulation inhibited NE release and synthesis as expressed by a reduction in its precursors, L-DOPA and DA levels as well as TH activity. At higher frequencies (9 Hz) acute stimulation caused an increase in NE release, which was accompanied by an increase in TH activity, but was not manifested in both L-DOPA and DA levels. The latter is probably due to an increase in the turnover rate, which can mask any change in the already low L-DOPA and DA intracellular concentrations. Further elevation of the stimulation frequency (15–45 Hz) had no effect on NE release or synthesis, suggesting that such high frequencies could not affect cellular components associated with NE release and synthesis.

Nonlinear modulation of neuronal output and transmission by electrical stimulation frequency has been described both in vivo and in vitro. For example, it was demonstrated that long-term potentiation (LTP) and long-term depression (LTD) result from high- and low-frequency conditioning stimulation of the middle layers of cortex, respectively (Kirkwood and Bear, 1995). In addition, the release of neurotransmitter at the synaptic release site was shown to be nonlinearly dependent on stimulation frequency in the presynaptic Lamprey reticulospinal axons (Pieribone et al., 1995). In the medial entorhinal cortex, cells of layers II and III innervating the hippocampus are increasingly activated during stimulation at low frequencies, but project only a few action potentials during higher frequency (>10 Hz) synaptic stimulation (Heinemann et al., 2000). In cultured cortical neurons it has been shown that input–output relations are nonlinearly tuned by stimulation (input) frequency (Tal et al., 2001). Thus, as the input frequency was increased the neuron started to fail responding linearly to stimuli and the ordered response of 1:1 mode which was defined at 1 Hz disappeared and became randomly distributed at higher frequencies (30 Hz). Moreover, Rice et al. (1997) reported that in substantia nigra pars compacta slices electrical stimulation over a range of 1–50 Hz for a constant duration of 10 s, resulted in a frequency-dependent increase in DA release over the range of 1–10 Hz, with significant differences between stimulation at 1 or 2 Hz vs. 10 Hz.

Weak magnetic fields also demonstrate frequency-dependent effects on biological systems. It has been suggested that the biphasic response (stimulation/inhibition) of biological systems to extremely low-frequency magnetic fields is a result of interference with enzyme–reaction cycles, which involve feedback control mechanisms (Eichwald and Walencewicz, 1996). The present findings clearly demonstrate that in SH-SY5Y cells, acute strong non-static magnetic fields alter NE metabolism in a frequency non-monotonous dependent manner. Indeed, strong non-static magnetic fields such as those produced by rTMS are believed to have inhibitory effects at low frequencies (<1 Hz) whereas higher frequencies facilitate neuronal activity of the primary motor cortex (Chen et al., 1997; Kimbrell et al., 1999; Pascual-Leone et al., 1994; Speer et al., 2000). A frequency-dependent relationship has been also reported in clinical trials using rTMS in depressed patients (George et al., 2000; Klein et al., 2002), further supporting the importance of stimulation frequency for the antidepressant efficacy of rTMS.

Chronic treatment with 3 Hz magnetic stimulation for 4 d had no effect on L-DOPA, DA and NE intracellular concentrations. A similar phenomenon regarding a different effect of acute vs. chronic treatments on monoamine levels has been observed following treatments of rats with antidepressants including ECS and rTMS. For example Yoshida et al. (1997) also found a marked increase in DA, DOPAC, HVA and 5-HIAA following a single ECS, which was significantly attenuated after the eighth ECS. Glue et al. (1990) showed that an acute ECS treatment had no effect on brain monoamines, while eight ECS treatments caused an increase in basal release of DA in the striatum. In the frontal cortex however there was no change in the basal levels and the effect of acute ECS to increase DA was the same after a single or eight ECS treatments. Sacchetti et al. (1999) reported that acute administration of reboxetine significantly increased, whereas chronic treatment did not change, extracellular levels of NE both in the frontal cortex and in the dorsal hippocampus. In all, these studies indicate that a single treatment with rTMS, or antidepressants, induces significant alterations in monoamine levels, which are either abolished or markedly attenuated following chronic treatment (Ben-Shachar et al., 1997a, 1999; Glue et al., 1990; Plaznik et al., 1994; Sacchetti et al., 1999; Yoshida et al., 1997; Zis et al., 1992). Apparently chronic treatment, unlike acute treatments, induces compensatory mechanisms, which result in a normalization of intracellular catecholamine levels. In line with this hypothesis TH activity, in our cellular experimental model, was significantly increased following chronic treatment with 3 Hz magnetic stimulation probably compensating for the reduction in norepinephrine release observed following a single treatment.
Further confirmation for the latter is still required; however, parallel findings have been reported for ECS. Koubi et al. (2001) found a decrease in TH activity in the locus coeruleus after a single 3 s ECS, while some other studies have reported an increase in TH activity following chronic ECS (Brady et al., 1994; Leviel et al., 1990).

Being the rate-limiting enzyme in monoamine synthesis, TH is the preferred target for both biochemical and physical compensatory mechanisms. Namely, increases or decreases in neuronal activity, electrical stimulations, stress, lesions, drug effects, endocrinological manipulations and experimental models of hypertension are all associated with alterations in TH (for review see Masserano and Weiner, 1983). It is well established that TH activity is increased following an increase in synaptic release of NE, due to increased impulse flow, while reduced during periods of synaptic quiescence associated with decreased NE release (Costa et al., 1974). In our SH-SY5Y cells, following a single 3 Hz stimulation, a decrease in NE release was associated with a decrease in TH activity, whereas following 9 Hz stimulation a parallel increase in both NE release and TH activity was observed.

Acute regulation of TH involves activation or suppression of its pre-existing molecules via phosphorylation and possibly through alternative splicing events, while long-term regulation results from an increased synthesis of the enzyme (Haycock and Wakade, 1992; Icard-Liepkalns et al., 1993). In line with this, in our SH-SY5Y cells, TH activity was decreased by a single 3 Hz stimulation, while being significantly increased following chronic 3 Hz stimulation. However this elevated activity was not associated with a detectable change in its protein levels as assessed by Western blot. The lack in measurable changes in the protein levels of TH could be due to the duration of treatment and heterogeneity in the number of stimulations administered to each cell in a constantly dividing cell population.

Along with an end product feedback regulation of TH activity, it has been suggested that TH might be influenced directly by neuronal depolarization (Salzman and Roth, 1980). Thus, a direct effect of the magnetic field on TH, which requires Fe$^{2+}$ as a cofactor, may also be of importance.

In summary, the results of the present study demonstrate that magnetic stimulation of SH-SY5Y cell cultures affects NE release and synthesis both at acute and chronic treatment. These results are in line with the findings in animal studies with rTMS, ECS and antidepressant drugs and may suggest that the putative antidepressant effect of rTMS is associated with alterations in monoaminergic neurotransmission. The differential effects of frequency and treatment duration, as shown in this study, might support the importance of optimizing stimuli parameters to maximize the therapeutic potency of rTMS in depression and other mood disorders. Nevertheless, the inference from tissue cultures to humans is a quantum leap and should thus be taken with due reservation. Neuronal cell cultures can thus provide a convenient and reliable tool for the study of the multitude of combination possibilities of stimuli parameters. A further advantage of this experimental model is that it diminishes the confounding effects of other factors such as stress lateralization and connectivity, which are inherent within animal and human studies. By the same token, this is also a disadvantage of cellular models when inferring these results to the human brain. Still, bearing in mind this limitation, similar studies could extend our understanding of the mechanism underlying the antidepressant effect of rTMS.

Acknowledgement

This study was supported by a grant from the Theodore & Vada Stanley Foundation.

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