

Functional Dopaminergic Neurons in Substantia Nigra are Required for Transcranial Magnetic Stimulation-Induced Motor Plasticity

Tsung-Hsun Hsieh^{1,2,4,†}, Ying-Zu Huang^{6,†}, Alexander Rotenberg⁷, Alvaro Pascual-Leone⁸, Yung-Hsiao Chiang^{1,2,9}, Jia-Yi Wang³ and Jia-Jin J. Chen^{4,5}

¹Graduate Institute of Neural Regenerative Medicine, ²Center for Neurotrauma and Neuroregeneration and, ³Graduate Institute of Medical Sciences, Taipei Medical University, Taipei, Taiwan, ⁴Department of Biomedical Engineering and, ⁵Medical Device Innovation Center, National Cheng Kung University, Tainan, Taiwan, ⁶Department of Neurology, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taipei, Taiwan, ⁷Department of Neurology, Boston Children's Hospital, ⁸Berenson-Allen Center for Noninvasive Brain Stimulation, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA, and ⁹Department of Neurosurgery, Taipei Medical University Hospital, Taipei, Taiwan

Address correspondence to Prof. Jia-Jin J. Chen, Department of Biomedical Engineering, National Cheng Kung University, 1 University Rd., Tainan 70101, Taiwan. Email: jasonbiolab@gmail.com

[†]Tsung-Hsun Hsieh and Ying-Zu Huang contributed equally to this work.

Repetitive magnetic stimulation (rTMS), including theta burst stimulation (TBS), is capable of modulating motor cortical excitability through plasticity-like mechanisms and might have therapeutic potential for Parkinson's disease (PD). An animal model would be helpful for elucidating the mechanism of rTMS that remain unclear and controversial. Here, we have established a TMS model in rat and applied this model to study the impact of substantia nigra dopamine neuron on TBS-induced motor plasticity in PD rats. In parallel with human results, continuous TBS (cTBS) successfully suppressed motor evoked potentials (MEPs), while MEPs increased after intermittent TBS (iTBS) in healthy rats. We then tested the effect of iTBS in early and advanced 6-hydroxydopamine (6-OHDA)-lesioned PD. Moreover, dopaminergic neurons in substantia nigra and rotation behavior were assessed to correlate with the amount of iTBS-induced plasticity. In results, iTBS-induced potentiation was reduced in early PD rats and was absent in advanced PD rats. Such reduction in plasticity strongly correlated with the dopaminergic cell loss and the count of rotation in PD rats. In conclusion, we have established a TMS PD rat model. With the help of this model, we confirmed the loss of dopaminergic neurons in substantia nigra resulting in reduced rTMS-induced motor plasticity in PD.

Keywords: motor evoked potential, Parkinson's disease, rat, theta burst stimulation, 6-OHDA

Introduction

Repetitive transcranial magnetic stimulation (rTMS) is capable of producing long-lasting changes of cortical excitability beyond the short period of stimulation. Although the exact neurobiologic substrate for the mechanisms of rTMS remains unclear, several lines of evidence support that rTMS induces changes similar to long-term potentiation (LTP) or long-term depression (LTD) of synaptic strength (Huang et al. 2007). Hence, rTMS has been applied to study plasticity phenomena in healthy humans and patients. Moreover, the modulation effect of rTMS is recently considered having therapeutic potentials in neurological or psychiatric disorders, for example, Parkinson's disease (PD), dystonia and depression (Dragasevic et al. 2002; Koch et al. 2005; Huang et al. 2010, 2012; Benninger et al. 2011; Berlin et al. 2013).

rTMS has recently been used to investigate plasticity in PD patients with or without complications (Huang et al. 2011). Impaired motor plasticity was reported using rTMS in chronic

and early PD patients with off medications and was restored by dopaminergic medications (Morgante et al. 2006; Ueki et al. 2006; Eggers et al. 2010; Huang et al. 2011; Suppa et al. 2011; Kishore et al. 2012). In contrast, others showed enhanced or normal motor plasticity in PD patients off medication (Bagnato et al. 2006; Zamir et al. 2012). Similar inconsistency was also found in the therapeutic effect of rTMS on PD. Although quite a few studies have shown clinical benefits of rTMS on PD (Lefaucheur et al. 2004; Elahi and Chen 2009; Degardin et al. 2012), the results so far have been inconclusive (Benninger et al. 2011; Shirota et al. 2013). Such discrepancy between studies could be due to the clinical heterogeneity in patients, long-term pharmacological effects, different severity of the disease, and the variability of protocols (Muller-Dahlhaus et al. 2008; Bologna et al. 2012; Hamada et al. 2013).

Disease animal models may provide a more stable and controllable condition than patients to reduce the discrepancy (Picconi et al. 2003; Morgante et al. 2006; Calabresi et al. 2007). However, the studies in animals were mostly done with invasive electrical stimulation, which is not suitable for humans. For the translational purpose, between animals and humans, it would be valuable to study the effect of rTMS on animals. Recently, rTMS protocols have been translated to use in animal models for understanding their mechanistic insights (Aydin-Abidin et al. 2008; Benali et al. 2011; Funke and Benali 2011; Ghiglieri et al. 2012; Hoppenrath and Funke 2013; Volz et al. 2013). However, most of these studies focused on the mechanism at the cellular level. The physiological response to rTMS as measured in humans, for example, changes in motor evoked potentials (MEPs), has rarely been tested in the animal models. This lacking piece of information in animal models may restrict the usage of the knowledge obtained from the animal model. On the other hand, the literature is scant in the use of disease animal models for studying the plasticity phenomena of rTMS in disease conditions, which may provide insights into the underlying pathophysiology of the disease for future diagnostic purposes and therapeutic applications targeting synaptic plasticity.

We therefore first set up a plasticity rat model, which is comparable with that established in humans, to test the effects of theta burst form of rTMS (theta burst stimulation, TBS), which is adapted from theta burst paradigm commonly used in animal preparations (Larson and Lynch 1986; Hess et al. 1996), on MEPs in anesthetized normal rats. Then, the rat model served as

a platform to examine TBS-elicited plasticity responses in a 6-OHDA-lesioned rat model of PD. Two stages of PD rats reflecting different severity of dopamine depletion quantified by counting dopaminergic cell loss and the behavioral presentation tested by drug-induced rotation were utilized to understand the correlation between the disease severity and plasticity change.

Materials and Methods

Animal Preparation

Experiments were carried out on 45 male Wistar rats (350–400 g) obtained from the Animal Center of National Cheng Kung University Medical College. All experiments were approved beforehand by National Cheng Kung University Medical College Animal Use Committee. Among them, 27 rats were equally assigned to sham control, iTBS and cTBS groups ($n=9$ for each group) for testing immediate effect of TBS. The other 18 rats were chosen for PD lesions and were separated into 2 equal groups to undergo iTBS at 1 week or 4 weeks postlesion (expressed as 1-wk PD and 4-wk PD, respectively) to identify motor plasticity during PD progression. The animals were housed in standard cages at a temperature of $25 \pm 1^\circ\text{C}$ with a 12/12-h light/dark cycle and ad libitum water and food access before the experimental procedures.

Parkinsonian Rat Model

For the 6-OHDA lesion, the rat was deeply anaesthetized by intraperitoneal injection of Tiletamine-Zolazepam (50 mg/kg, i.p.; Zoletil, Vibac, France) and xylazine (10 mg/kg, Rompun, Bayer, Germany), then placed in a stereotaxic apparatus (Stoelting, USA). A solution of 6-OHDA (8 μg , 4 μL , dissolved in 0.02% ascorbic saline, Sigma, USA) was injected intracranially at a rate of 0.5 $\mu\text{L}/\text{min}$ into the left medial forebrain bundle (AP: -4.3 mm; lateral: 1.6 mm; DV: 8.2 mm) according to the stereotaxic brain atlas of Paxinos and Watson using a 10- μL Hamilton microsyringe (Paxinos and Watson 2005). The needle was left in the brain for 5 min before being slowly retracted (Truong et al. 2006; Hsieh et al. 2011).

Electrophysiological Recordings

The test rats were deeply anesthetized for ~ 4 h with Tiletamine-Zolazepam (65 mg/kg, i.p.; Zoletil, Vibac, France) plus xylazine (10 mg/kg, i.p.; Rompun, Bayer, Germany) and mounted in a stereotaxic apparatus (Stoelting, USA). Anesthesia depth was adjusted to the absence of abdominal contractions during tail pinch. Recording of electromyographic (EMG) activity was obtained with monopolar uninsulated 27G stainless steel needle electrodes (Axon Systems, Inc., USA) inserted into the belly of each brachioradialis muscle. A reference electrode was positioned distally in the paw and a ground electrode was attached to the base of the rat's tail (Vahabzadeh-Hagh et al. 2011). The EMG signal was amplified (1000 \times) (Cyberamp 380, Axon Instruments, USA) and filtered using 60-Hz notch and 10-Hz to 1-kHz bandpass filters prior to digitization at 4 kHz (DAQPad-6015, National Instruments, USA).

All TMS and rTMS sessions were performed using a Rapid² magnetic stimulator and a figure-of-eight 25 mm coil (Magstim Co., Wales, UK). The coil was held in the stereotaxic frame and one of the rings (as a circular coil) was placed over the rat dorsal scalp, and was systematically adjusted to the best coordination for eliciting MEP in the right forelimb (see Supplementary Fig. 1). At the coil position, MEPs were reliably elicited from both forelimbs as previously reported (Magnuson et al. 1999; Nielsen et al. 2007; Gersner et al. 2011; Iglesias-Bregna et al. 2013). MEPs evoked by single-pulse TMS were recorded to evaluate the effect of TBS on cortical excitability. The resting motor threshold (RMT) was defined as the minimal intensity of stimulation required for eliciting MEPs from the right brachioradialis muscle with peak-to-peak amplitude of at least 20 μV in 5 of 10 consecutive trials performed during muscle relaxation due to anesthesia (Rotenberg et al. 2010; Vahabzadeh-Hagh et al. 2011). The coil was maintained at this location throughout the whole session. TMS intensity for RMT was documented

as percent machine output (% MO) with 100% corresponding to the maximal strength of the generated magnetic field.

TBS Protocols

Two TBS protocols, including iTBS and cTBS, consisting of 3-pulse bursts at 50 Hz repeated at 5 Hz were used in the present study. For iTBS, a 2-s train of TBS was repeated every 10 s for 20 repetitions (total 600 pulses). In the cTBS paradigm, a 40-s train of uninterrupted TBS was given (600 pulses) (Huang et al. 2005). Because active motor threshold (AMT) could not be obtained in animals under anesthesia, 80% RMT, instead of AMT, was used for TBS. For sham intervention, the coil was placed 8 cm laterally and above the rat's head (Aydin-Abidin et al. 2008) and the iTBS protocol was delivered.

MEP Assessment

For the assessment of MEP amplitudes, 15 single pulses were applied at 10- to 15-s random intervals at 120% RMT. The stimulus intensity remained unchanged throughout the whole session of experiment. Peak-to-peak amplitudes of MEP were analyzed offline.

Behavioral Tests

In addition, we measured the apomorphine-induced rotational behavior to correlate the severity of parkinsonian symptom with iTBS-induced changes in the MEP amplitude. Apomorphine (0.5 mg/kg in 0.1% ascorbic acid, s.c.; Sigma) was given to the PD rat (Yoon et al. 2007; Hsieh et al. 2011). Then the rat was placed in a 40-cm-diameter round bowl to assess the rotational behavior, which was recorded using a digital video camera to calculate the number of rotation at 10-min intervals offline, for 60 min. The net number of rotations was calculated by subtracting the number of ipsilateral rotations from the number of contralateral turns with respect to the 6-OHDA injection side.

Histology Investigation

After electrophysiological and behavioral tests, the PD rat was sacrificed for tyrosine hydroxylase (TH) staining to quantify dopaminergic neuron loss in the brain. The animals received sodium pentobarbitone (60 mg/kg i.p., Apoteksbolaget, Sweden) and transcardial perfusion of phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA). Brains were post-fixed 3 days and cryoprotected in 30% sucrose. The brains were frozen and sectioned at 30 μm "coronally" in the region spanning from -5.20 to -5.80 mm in the substantia nigra and from $+1.70$ to $+2.30$ mm in the striatum with respect to the bregma (Paxinos and Watson 2005). Free-floating sections were treated with 0.3% H_2O_2 in PBS for 10 min to remove endogenous peroxidase. After being washed, the sections were preincubated in PBS containing 0.3% Triton-X 100 (Merck, Germany), 3% horse serum (Vector Labs, USA), and 3% bovine serum albumin (BSA; Sigma, USA) for 30 min to block nonspecific antibody binding. Sections were then incubated in a 1:1000 dilution of rabbit primary anti-TH (AB152, Millipore, USA) containing 5% horse serum (Vector Labs) and 5% BSA for 15–18 h. Thereafter, consecutive sections were washed and then incubated for 1 h with an anti-rabbit horseradish peroxidase (1:200; ImmPRESS Anti-Rabbit Ig Kit # MP-7401, Vector Labs) containing 3% "normal horse serum" in PBS. Following rinsing with PBS, immunostaining was visualized with 3,3-diaminobenzidine (SK-4105, Vector Labs) for 3 min. The sections were finally mounted on slides, dehydrated in a series of alcohols, cleared in xylene, and cover-slipped in DPX. After TH staining, the stained images were digitalized with a camera, connected to a microscope and a computer. The TH-positive cell bodies in the substantia nigra from both hemispheres were counted manually in each section (Truong et al. 2006; Hsieh et al. 2011). The percentage loss of TH-positive cells in the lesioned hemisphere was calculated and normalized with respect to the nonlesioned side. In addition, we measured the optical density of TH-positive fibers in the striatal sections using Image-Pro Plus 6.0 software (Media Cybernetics, USA) to calculate the dopaminergic fibers in the striatum. The optical density measurements were corrected for nonspecific background density measured at the corpus callosum. The percentage loss of dopaminergic

fiber in the lesioned side was presented and normalized with respect to the nonlesioned side.

Experimental Design

The experimental design is illustrated in Figure 1. We first investigated the effect of different TBS protocols (i.e., iTBS, cTBS, and sham) on the MEP size in normal rats. To reach a stable condition of anesthesia, we waited for 60 min after anesthetics were injected to begin the investigation. MEPs were recorded every 5 min for 3 times before TBS as the baseline condition. Then TBS was given and MEPs were recorded again immediately and at 5, 10, 15, 20, 25, and 30 min after the end of TBS (Fig. 1A). For evaluating the effect of TBS in PD rats, we focused on the effect of iTBS. The same MEP assessment as above followed by apomorphine-induced rotational behavior test was performed. To prevent the possible interaction between MEP assessment and behavioral recording and to avoid any residual anesthesia, the behavioral test was done at 12 h after the MEP assessment. On the day after electrophysiological and behavioral tests, we sacrificed the rat for histological examination to correlate the TBS effect with dopamine depletion (Fig. 1B).

Data Analysis

Data were analyzed using SPSS for Windows version 17.0 with the significance level set at $P < 0.05$. All data are presented as the average \pm standard error of the mean (SEM). Statistics for the comparisons of TBS effects in healthy rats were performed on MEPs, which were normalized to the last 5-min pre-TBS baseline, using two-way repeated-measures analysis of variance (ANOVA) with PROTOCOL (iTBS, cTBS, sham) as between-subjects factor and TIME (0, 5, 10, 15, 20, 25, and 30 min after TBS) as within-subject factor. When it was needed, a separate one-way ANOVA with the time points of last 5-min pre-TBS

baseline and 0, 5, 10, 15, 20, 25, and 30 min after TBS was performed on absolute values to characterize the time-dependent change of each protocol.

To analyze the effect of iTBS in normal and PD rats, we used a two-way repeated-measure ANOVA on normalized MEPs with GROUP (normal rats, 1-wk PD rats, and 4-wk PD rats) as between-subjects factor and TIME (0, 5, 10, 15, 20, 25, and 30 min after TBS) as within-subjects factor. A separate one-way ANOVA on absolute values followed to characterize the time-dependent change of each protocol. Post hoc Fisher's LSD tests were used to compare between time points if needed.

Furthermore, to test whether the iTBS-induced plasticity was affected with the evolution of PD, the averaged iTBS-induced percentage change in the MEP size after iTBS were compared between 1-wk PD and 4-wk PD rats using *t*-tests. The percentage change of MEP size was calculated as the percentage of changes in averaged MEP size of all testing time points (i.e., 0, 5, 10, 15, 20, 25, and 30 min) after iTBS with respect to last 5-min pre-TBS MEP baseline.

We also used Pearson's correlation test for evaluating the correlation between the iTBS-induced percentage changes of MEP size and dopaminergic cell loss and the count of apomorphine-induced rotation in the PD rats.

Results

Effects of TBS on MEPs in Healthy Rats

RMT was $53.64 \pm 1.93\%$ (mean \pm SEM) maximum machine output (MMO) in normal rats, $52.83 \pm 2.04\%$ MMO in 1-wk PD rats and $51.23 \pm 1.33\%$ MMO in 4-wk PD rats. No significant difference was found between groups. Two-way repeated-

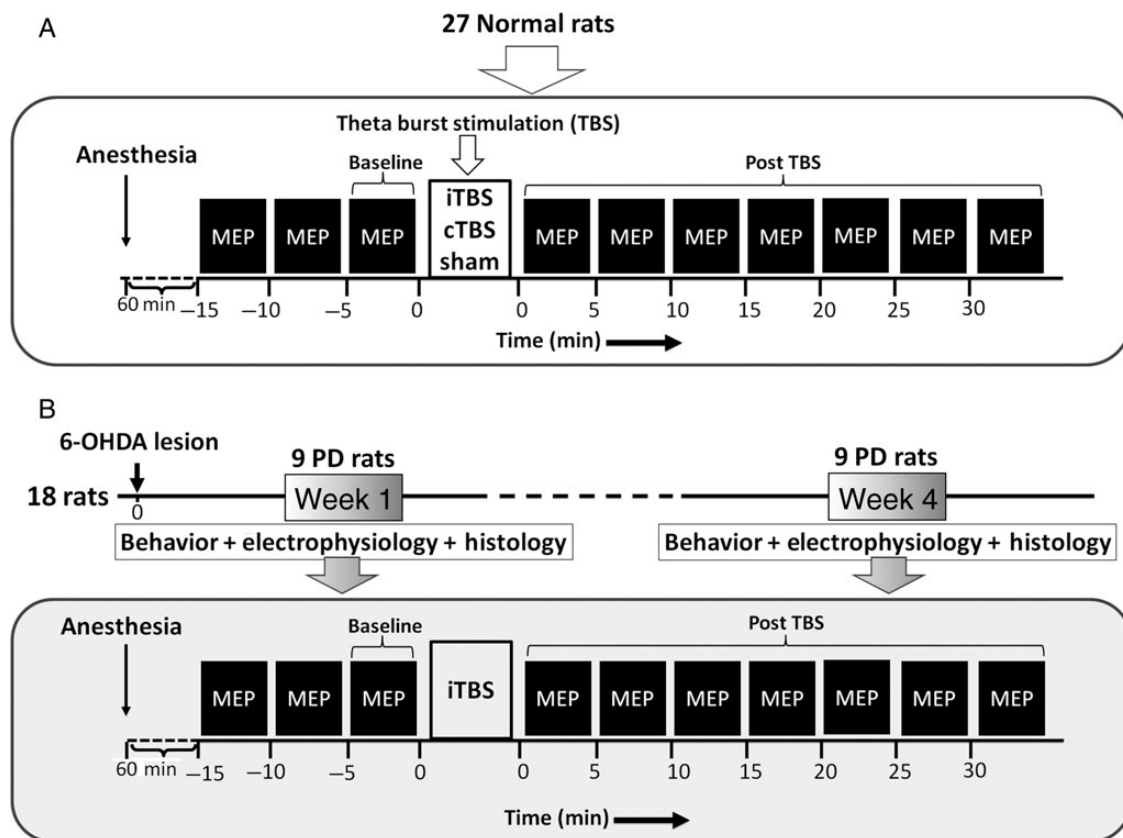


Figure 1. Experimental design. In the experiments for testing the effect of TBS on normal rats (A), 15 MEPs assessed every 10–15 s were recorded every 5 min for 3 times before TBS and every 5 min until 30 min after the end of TBS, including iTBS, cTBS, and sham stimulation. For the PD groups (B), the MEP assessment, behavioral, and histological investigations were performed on 1-wk and 4-wk PD rats.

measures ANOVA showed significant main effects of PROTOCOL ($F_{2,24} = 46.35$, $P < 0.001$ in left limb; $F_{2,24} = 26.78$, $P < 0.001$ in right limb) and TIME ($F_{6,144} = 2.64$, $P = 0.02$ in left limb; $F_{6,144} = 3.12$, $P = 0.007$ in right limb). Following one-way ANOVAs showed that MEPs were enhanced by iTBS ($F_{7,64} = 3.619$, $P = 0.002$ in left limb and $F_{7,64} = 2.60$, $P = 0.02$ in right limb), whereas they were suppressed by cTBS ($F_{7,64} = 2.51$, $P = 0.02$ in left limb and $F_{7,64} = 2.64$, $P = 0.02$ in right limb). Post hoc Fisher's LSD analysis revealed that MEPs were significantly enhanced in 5 min ($P = 0.024$ in left limb; $P = 0.041$ in right limb) and remained enhanced up to 30 min ($P < 0.001$ in both limbs) after iTBS when compared with the baseline MEP. MEPs were suppressed immediately ($P = 0.002$ in left limb; $P = 0.015$ in right limb) and lasted for 30 min or more ($P = 0.049$ in left limb; $P = 0.009$ in right limb) after cTBS. No effect was noted on the MEP size in rats that had received sham stimulation ($F_{7,64} = 0.32$, $P = 0.94$ in left limb and $F_{7,64} = 0.47$, $P = 0.85$ in right limb) (Fig. 2B–D).

Effects of iTBS on MEPs in PD Rats

Two-way repeated-measures ANOVA showed significant main effects of GROUP ($F_{2,24} = 5.783$, $P = 0.009$ in left limb; $F_{2,24} = 5.069$, $P = 0.015$ in right limb) and TIME ($F_{6,144} = 3.698$, $P = 0.002$ in left limb; $F_{6,144} = 4.789$, $P < 0.001$ in right limb). Further

two-way ANOVA with factor GROUP confirmed significant differences between the 3 groups (normal vs. 1-wk PD: $F_{1,112} = 7.444$, $P = 0.007$ in left limb; $F_{1,112} = 4.821$, $P = 0.03$ in right limb; normal vs. 4-wk PD: $F_{1,112} = 88.422$, $P < 0.001$ in left limb; $F_{1,112} = 62.521$, $P < 0.001$ in right limb; 1-wk PD vs. 4-wk PD: $F_{1,112} = 15.313$, $P < 0.001$ in left limb; $F_{1,112} = 18.711$, $P < 0.001$ in right limb). Following one-way ANOVAs revealed that MEPs were enhanced by iTBS ($F_{7,64} = 3.619$, $P = 0.002$ in left limb and $F_{7,64} = 2.60$, $P = 0.02$ in right limb) in the normal group but not in either 1-wk PD group ($F_{7,64} = 0.699$, $P = 0.673$ in left limb and $F_{7,64} = 1.425$, $P = 0.211$ in right limb) or 4-wk PD group ($F_{7,64} = 0.338$, $P = 0.934$ in left limb and $F_{7,64} = 0.695$, $P = 0.676$ in right limb) (Fig. 3B–D). We further averaged the MEPs at all time points after iTBS to compare with the baseline MEP, and revealed that MEPs were enhanced by iTBS in 1-wk PD ($t = 3.071$, $P = 0.015$), but not in 4-wk PD ($t = 0.413$, $P = 0.691$). This result suggests that there is still some facilitatory effect of iTBS in 1-wk PD, but not in 4-wk PD.

Dopaminergic Cell Depletion and Behavioral Deficits in PD Rats

The results of TH-immunohistochemistry in the substantia nigra and striatum in 1-wk and 4-wk PD are shown in Figure 4A,B. A moderate reduction of TH-immunoreactive density was observed in the substantia nigra and striatum of the infusion side in rat at

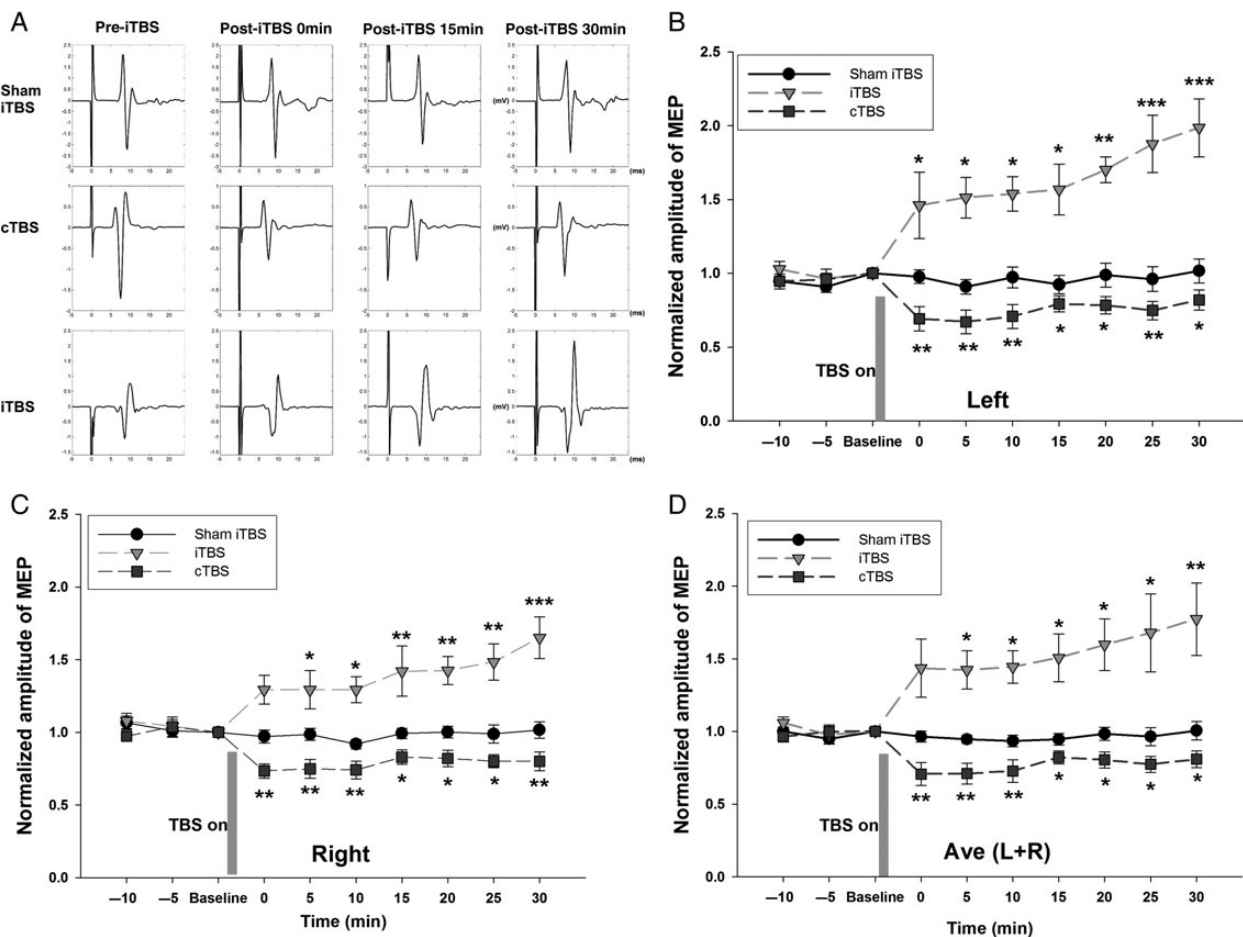


Figure 2. The effect of TBS on MEPs in healthy rats. Representative MEPs of a rat before and after sham stimulation, cTBS, and iTBS are shown at each measured time epoch (A). Averaged changes in the MEP amplitude after sham, cTBS, and iTBS interventions are presented for left (B), right (C), and average of both sides (D). Each point corresponds to the mean and standard error of MEP amplitude expressed as a ratio to the last block of baseline responses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significant post hoc Fisher's LSD differences on the MEP size when compared with each time point after intervention to the last block of pre-TBS baseline.

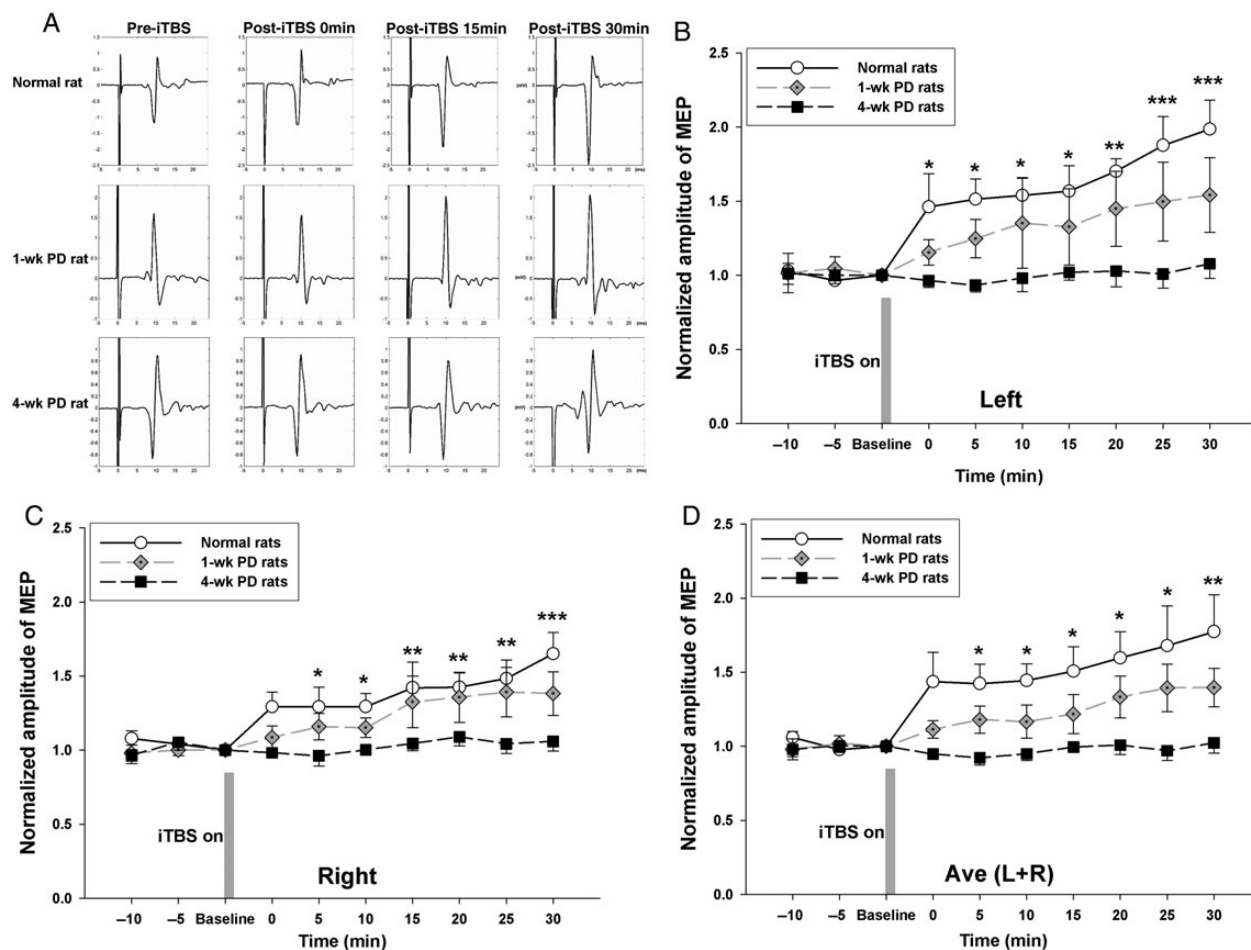


Figure 3. The effect of iTBS on MEPs in PD rats. Representative MEPs before and after iTBS in normal, 1-wk and 4-wk PD rats are shown at each measured time epoch (A). The MEP amplitude increased in the normal rat but was less facilitated in PD rats after iTBS. Time course of averaged changes in the MEP amplitude in normal, 1-wk PD and 4-wk PD rats are presented for left (B: ipsilateral side), right (C: contralateral side), and average of both sides (D). These effects of iTBS in the 3 groups were significantly different from each other. $*P < 0.05$, $**P < 0.01$, significant post hoc Fisher's LSD differences on MEP size compared between baseline and at each time point after iTBS.

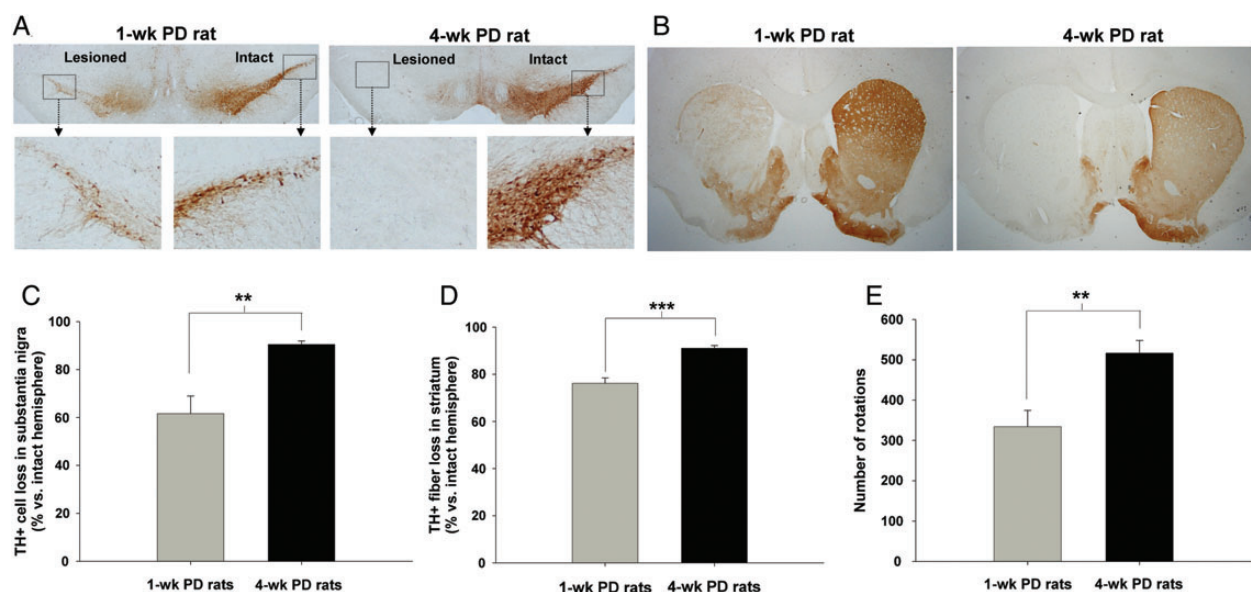


Figure 4. The histological and behavioral changes correlate with the disease stage in PD rats. Representative TH-immunoreactive fibers at the substantia nigra (A) and striatum (B) from animals sacrificed at 1 week and 4 weeks post-6-OHDA lesion are shown in upper row, while the quantification of TH-positive neuron loss (C), TH-positive fiber loss (D), and the count of rotational behavior (E) are shown in the lower row. $**P < 0.01$, $***P < 0.001$ (unpaired *t*-test).

one week after 6-OHDA injection. Four weeks after the injection, TH immunoreactivity in the substantia nigra and striatum was much less detectable on the side of the infusion when compared with that at 1-week postlesion. Quantification of TH-positive cell loss in the substantia nigra and TH-positive fiber loss in striatum at 1 week and 4 weeks postlesion are presented in Figure 4C,D, respectively. The average TH-positive cell loss in the substantia nigra region when compared with the intact hemisphere was 61.56 ± 7.35 and $90.47 \pm 1.43\%$ at 1 week and 4 weeks postlesion, respectively ($t = 3.93$; $P = 0.004$). The average TH-positive fiber loss in the striatum was 76.16 ± 2.26 and $91.05 \pm 1.10\%$ at 1 week and 4 weeks postlesion, respectively ($t = 5.90$; $P < 0.001$). With regard to apomorphine-induced rotational behavior, apomorphine induced 516.4 ± 31.5 turns, which was significantly higher than 366.7 ± 40.1 turns that was recorded in 1-wk PD rats ($t = 3.57$; $P = 0.003$), in 60 min in 4-wk PD rats (Fig. 4E).

The Correlation Between the Severity of PD and iTBS-Induced Plasticity

We then calculated the iTBS-induced percentage change in MEP size, which was significantly larger in 1-wk PD than in 4-wk PD rats (Fig. 5A: 1-wk PD: $22.83 \pm 5.74\%$; 4-wk PD: $-1.07 \pm 3.60\%$, $t = 3.524$, $P < 0.003$), to correlate with the level of dopamine denervation and the number of apomorphine-induced rotation in PD rats. The Pearson's correlation analysis showed a significant negative correlation between the percentage change of MEP size after iTBS and the loss level of dopaminergic neuron in substantia nigra ($r = -0.87$, $P < 0.001$) (Fig. 5B) and fibers in the striatum ($r = -0.87$, $P < 0.001$) (Fig. 5C). A similar significant negative correlation was also found between the rotational behavior and the percentage change of MEP size ($r = -0.81$, $P < 0.001$) (Fig. 5C). These results suggest that advanced PD rats have more evident deficit in plasticity in response to iTBS than early PD rats.

Discussion

In the present study, we successfully translated the human TMS protocols into an in vivo rat model. The study of motor plasticity in 6-OHDA-lesioned PD rats with this model showed that the amount of rTMS-induced plasticity decreased in PD rats and deteriorated as the disease progressed. Such impaired plasticity was strongly associated with the loss of dopaminergic nigral cells and striatal fibers proved histologically and the impairment of dopaminergic function tested by behavioral investigations.

rTMS-Induced Motor Plasticity in Vivo Rat Model

In the TMS rat model, we recorded consistent MEPs from anesthetized rats' limbs as previous reports (Rotenberg et al. 2010; Vahabzadeh-Hagh et al. 2011). Analogous to those observed in humans (Huang et al. 2005), iTBS increased the size of MEPs for 30 min or more, while cTBS decreased the size of MEPs for at least 30 min. To avoid major stress effects, we had to anesthetize the rat. In contrast, most TMS-MEP measurements were studied in conscious humans. To our knowledge, the effect of rTMS on MEPs has never been reported in anesthetized humans. It is more difficult to induce plasticity in freely moving animals than in brain slices or anesthetized animals (Trepel and Racine 1998; Froc et al. 2000). This may explain why the plasticity-like response in our rats seems to be larger and less variable than those reported in humans.

MEPs were recorded at several time points and sham controlled group was used to confirm no confounding effects of anesthesia. Eighty percent RMT instead of AMT was used for TBS because it is impossible to record AMT in anesthetized rats. Hence, TBS was given at a higher intensity when compared with that usually applied to human. In humans, it has been reported that TBS is sensitive to the given intensity. A subtle change in the intensity of TBS may selectively activate different circuit within the cortex and produce different effect on the cortical excitability (McAllister et al. 2009; Doeltgen and Ridding 2011). It has been demonstrated that cTBS at 90% AMT, which is close to 80% RMT, has stronger inhibition than cTBS at 80% AMT (Bruckner et al. 2013). This could be another reason why the TBS effects in the present study were larger than those in humans. However, the influence of the stimulus intensity on the effect of TBS deserves further careful studies. The current TMS rat model may be an ideal platform for this purpose.

MEPs were evoked by 120% RMT of the anesthetized rat. Thinking of the large size of the coil relative to the size of the rat brain and the relatively strong stimulus intensity, we do not propose that we are stimulating only the motor cortex. Nevertheless, the results were compatible with those in humans and showed pattern-dependent lasting effects on the size of MEP (Huang et al. 2005). Cellular studies of the similar protocols in rats showed that iTBS altered cortical neuronal activity along with significant changes in the expression of the synaptic plasticity-related proteins (Hoppenrath and Funke 2013). These lines of evidence suggest that plasticity-like effects were successfully activated by our magnetic stimulation protocol in the corticospinal pathway of rats in this model. Hence, this TMS model may serve as a bridge between animal and human plasticity studies, at least, in the motor pathway. On the other hand, the human protocols could be tested or manipulated in the rat model, for example, to clarify the underlying mechanisms, to improve protocols, and to test variability (Hamada et al. 2013) and the effect of stimulus intensity. If the disease models are available, it may also be used to match the findings between animal model and patients and clarify the pathophysiology or to test the therapeutic potential of rTMS protocols.

iTBS-Induced Plasticity in PD Rats

With the help of this model, we revealed that motor plasticity was reduced in PD rats. This result is in line with recent results showing reduced rTMS-induced plasticity in the motor cortex of PD patients and suggest that dopamine might play a key role for expression of plasticity (Morgante et al. 2006; Ueki et al. 2006; Huang et al. 2011; Suppa et al. 2011). Similar results have been shown in animal studies, in which plasticity was tested in vitro with brain slices and the corticostriatal pathway was tested (Centonze et al. 1999; Picconi et al. 2003; Ghiglieri et al. 2012). However, the corticostriatal pathway is difficult to test in humans, unless a deep brain stimulation electrode is implanted (Prescott et al. 2009). Hence, motor plasticity has commonly been studied instead.

The facilitatory effect of iTBS was absent in the later stage PD rats. In contrast, iTBS was still able to increase the MEPs amplitude in early-stage PD rats, although the amount of increase was less than that in normal rats. The result of TH staining suggests a lower level of functional dopaminergic cells in 4-wk than 1-wk PD. Previous studies have demonstrated that 6-OHDA induces an initial loss in dopaminergic phenotype

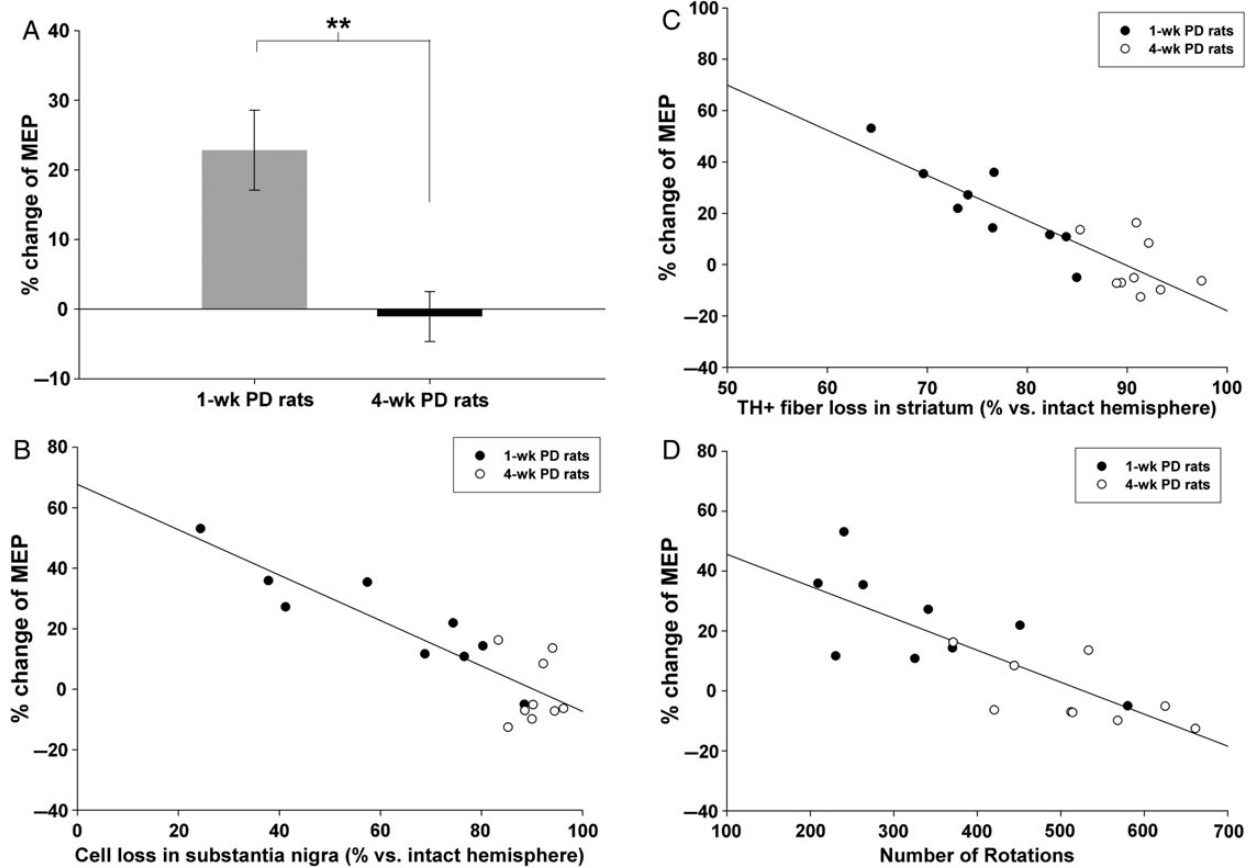


Figure 5. The correlation between the percentage change of MEP and the disease severity. iTBS was able to enhance MEPs in 1-wk PD, but not in 4-wk PD (A). There was a significant negative correlation between the change of MEP amplitude versus the level of dopaminergic neuron loss in substantia nigra (B), versus the level of dopaminergic fiber loss in striatum (C), and versus the count of apomorphine-induced rotation (D). Asterisks indicate a significant difference (unpaired *t*-test, $P < 0.01$).

and then induces more neuronal loss in late stage (Jeon et al. 1995; Bowenkamp et al. 1996; Cohen et al. 2011). Similar phenomena were also observed in our PD rats (Fig. 4A), although we did not analyze them quantitatively. Further analysis revealed a high correlation between the level of functional dopaminergic cell in the substantia nigra and iTBS-induced changes in MEP amplitudes. Moreover, there was the significant negative correlation between the rotational behavior and the percentage change of MEP size. These results confirm that the amount of motor plasticity decreases as the disease progresses and the dopamine level decreases. However, we cannot fully exclude the possibility that decreased motor plasticity in PD rats reflects secondary phenomena adopted in the motor system to compensate for motor impairment due to dopamine depletion.

At first glance, our results may conflict with a previous report showing severely impaired plasticity in early and treatment-naïve PD patients who were at averaged 3.4 years of their PD symptoms (Kishore et al. 2012). However, in consideration of the dopamine cell loss, which is ~62% in our early PD rats and is ~70–80% when PD patients are first diagnosed (Dauer and Przedborski 2003; Davie 2008), it is reasonable to see severely impaired plasticity in early PD patients and such results may in contrast support the current findings.

Although it has been reported that reduced plasticity could be restored by levodopa in PD patients (Huang et al. 2011; Kawashima et al. 2013), there has been no direct evidence of the correlation between rTMS-induced plasticity and the

pathological change of the substantia nigra in PD. The present study for the first time provides such evidence and shows a strong correlation between the amount of plasticity tested by TMS and the functional dopaminergic neuron in the substantia nigra.

In the past few years, the therapeutic potential of rTMS has been shown on PD patients (Randhawa et al. 2013; Shirota et al. 2013). Few studies tried to answer the mechanism of the therapeutic effect of rTMS in 6-OHDA PD rats. Four weeks of daily rTMS could improve the motor deficits and have neuroprotective effect to dopamine neurons (Yang et al. 2010; Lee et al. 2013). A single session of cortical iTBS increased striatal excitability and restored corticostriatal LTD (Ghiglieri et al. 2012). So far, such issue has been mainly approached in the behavioral response or in histology or physiology analysis such that the sacrifice of animals is commonly required. Although we did not focus on this issue in the current study, we have demonstrated another option to assess the physiological condition in the PD rat model without sacrificing the animal.

Dopamine has long been known as a crucial chemical in the regulation of plasticity in the brain (Law-Tho et al. 1995; Calabresi et al. 2000). There is a growing evidence suggesting that the dopamine deficit alters *N*-methyl-D-aspartate (NMDA) receptors to impair striatal and hippocampal plasticity in PD rats (Costa et al. 2012; Picconi et al. 2012). Rebalancing the composition of NMDA receptors may restore plasticity and motor function (Paille et al. 2010). NMDA receptors are known to be

crucial for rTMS to produce motor plasticity in humans (Huang et al. 2007, 2008) or in vitro (Vlachos et al. 2012). Hence, it is reasonable to speculate that the modulation of NMDA receptors by dopamine deficiency involves the impaired rTMS-induced plasticity in PD.

Our study shows that motor plasticity is strongly influenced by the degree of dopaminergic cell loss. However, it cannot be fully excluded that the impairment of motor plasticity in PD rats could reflect the secondary reorganization of motor cortex or the compensation of motor function with the progression of PD. This issue may be worth to be considered for further investigation. Also, for the translational application, in addition to test dopamine depletion in PD, it would be needed to understand the relationship between the changes of motor plasticity and other symptom-related indices such as the motor abilities when planning to test or modulate motor plasticity for therapeutic purposes.

Conclusions

In conclusion, we have set up an animal model for testing rTMS-induced motor plasticity in vivo in rats. Our results confirm that the TMS rat model mimics the situation in humans and might be used for translating TMS studies from humans to rats and vice versa. However, a slightly modified protocol in the stimulus intensity and the coil position and anesthesia were required for rat stimulation. Such technical limitations may cause some discrepancies between the model and human studies and should be aware in data interpretation and further applications. With the help of this model, we showed reduced rTMS-induced motor plasticity, which strongly correlated with the depletion of dopaminergic neuron in the substantia nigra and the severity of PD symptoms, in PD rats. To our knowledge, this is the first evidence that motor plasticity tested by TMS relies on the amount of dopamine in the substantia nigra. The newly developed TMS rat model would be useful for studying on the mechanism of rTMS-induced plasticity and for developing therapeutic strategies of rTMS for PD and other disorders.

Supplementary Material

Supplementary material can be found at: <http://www.cercor.oxfordjournals.org/>.

Funding

This study was funded by National Health Research Institutes (NHRI-EX103-10139EI, NHRI-EX100-9913EC), National Science Council (NSC100-2314-B-182A-001-MY2, NSC101-2632-B-038-001-MY3, NSC102-2321-B-038-003), and Chang Gung Memorial Hospital (CMRPG381281 and BMRP844) of Taiwan.

References

Aydin-Abidin S, Trippe J, Funke K, Eysel UT, Benali A. 2008. High- and low-frequency repetitive transcranial magnetic stimulation differentially activates c-Fos and zif268 protein expression in the rat brain. *Exp Brain Res*. 188:249–261.

Bagnato S, Agostino R, Modugno N, Quartarone A, Berardelli A. 2006. Plasticity of the motor cortex in Parkinson's disease patients on and off therapy. *Mov Disord*. 21:639–645.

Benali A, Trippe J, Weiler E, Mix A, Petrasch-Parwez E, Girzalsky W, Eysel UT, Erdmann R, Funke K. 2011. Theta-burst transcranial magnetic stimulation alters cortical inhibition. *J Neurosci*. 31:1193–1203.

Benninger DH, Berman BD, Houdayer E, Pal N, Luckenbaugh DA, Schneider L, Miranda S, Hallett M. 2011. Intermittent theta-burst transcranial magnetic stimulation for treatment of Parkinson disease. *Neurology*. 76:601–609.

Berlim MT, Van den Eynde F, Daskalakis ZJ. 2013. High-frequency repetitive transcranial magnetic stimulation accelerates and enhances the clinical response to antidepressants in major depression: a meta-analysis of randomized, double-blind, and sham-controlled trials. *J Clin Psychiatry*. 74:e122–e129.

Bologna M, Conte A, Suppa A, Berardelli A. 2012. Motor cortex plasticity in Parkinson's disease: advances and controversies. *Clin Neurophysiol*. 123:640–641.

Bowenkamp KE, David D, Lapchak PL, Henry MA, Granholm AC, Hoffer BJ, Mahalik TJ. 1996. 6-hydroxydopamine induces the loss of the dopaminergic phenotype in substantia nigra neurons of the rat. A possible mechanism for restoration of the nigrostriatal circuit mediated by glial cell line-derived neurotrophic factor. *Exp Brain Res*. 111:1–7.

Bruckner S, Kiefer M, Kammer T. 2013. Comparing the after-effects of continuous theta burst stimulation and conventional 1 Hz rTMS on semantic processing. *Neuroscience*. 233:64–71.

Calabresi P, Gubellini P, Centonze D, Picconi B, Bernardi G, Chergui K, Svenningsson P, Fienberg AA, Greengard P. 2000. Dopamine and cAMP-regulated phosphoprotein 32 kDa controls both striatal long-term depression and long-term potentiation, opposing forms of synaptic plasticity. *J Neurosci*. 20:8443–8451.

Calabresi P, Picconi B, Tozzi A, Di Filippo M. 2007. Dopamine-mediated regulation of corticostriatal synaptic plasticity. *Trends Neurosci*. 30:211–219.

Centonze D, Gubellini P, Picconi B, Calabresi P, Giacomini P, Bernardi G. 1999. Unilateral dopamine denervation blocks corticostriatal LTP. *J Neurophysiol*. 82:3575–3579.

Cohen AD, Zigmond MJ, Smith AD. 2011. Effects of intra-striatal GDNF on the response of dopamine neurons to 6-hydroxydopamine: time course of protection and neurorestoration. *Brain Res*. 1370:80–88.

Costa C, Sgobio C, Siliquini S, Tozzi A, Tantucci M, Ghiglieri V, Di Filippo M, Pendolino V, de Iure A, Marti M et al. 2012. Mechanisms underlying the impairment of hippocampal long-term potentiation and memory in experimental Parkinson's disease. *Brain*. 135:1884–1899.

Dauer W, Przedborski S. 2003. Parkinson's disease: mechanisms and models. *Neuron*. 39:889–909.

Davie CA. 2008. A review of Parkinson's disease. *Br Med Bull*. 86:109–127.

Degardin A, Devos D, Defebvre L, Destee A, Plomhause L, Derambure P, Devanne H. 2012. Effect of intermittent theta-burst stimulation on akinesia and sensorimotor integration in patients with Parkinson's disease. *Eur J Neurosci*. 36:2669–2678.

Doeltgen SH, Ridding MC. 2011. Low-intensity, short-interval theta burst stimulation modulates excitatory but not inhibitory motor networks. *Clin Neurophysiol*. 122:1411–1416.

Dragasevic N, Potrebic A, Damjanovic A, Stefanova E, Kostic VS. 2002. Therapeutic efficacy of bilateral prefrontal slow repetitive transcranial magnetic stimulation in depressed patients with Parkinson's disease: an open study. *Mov Disord*. 17:528–532.

Eggers C, Fink GR, Nowak DA. 2010. Theta burst stimulation over the primary motor cortex does not induce cortical plasticity in Parkinson's disease. *J Neurol*. 257:1669–1674.

Elahi B, Chen R. 2009. Effect of transcranial magnetic stimulation on Parkinson motor function—systematic review of controlled clinical trials. *Mov Disord*. 24:357–363.

Froc DJ, Chapman CA, Trepel C, Racine RJ. 2000. Long-term depression and depotentiation in the sensorimotor cortex of the freely moving rat. *J Neurosci*. 20:438–445.

Funke K, Benali A. 2011. Modulation of cortical inhibition by rTMS—findings obtained from animal models. *J Physiol*. 589:4423–4435.

Gersner R, Kravetz E, Feil J, Pell G, Zangen A. 2011. Long-term effects of repetitive transcranial magnetic stimulation on markers for neuroplasticity: differential outcomes in anesthetized and awake animals. *J Neurosci*. 31:7521–7526.

- Ghiglieri V, Pendolino V, Sgobio C, Bagetta V, Picconi B, Calabresi P. 2012. Theta-burst stimulation and striatal plasticity in experimental parkinsonism. *Exp Neurol*. 236:395–398.
- Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC. 2013. The role of interneuron networks in driving human motor cortical plasticity. *Cereb Cortex*. 23:1593–1605.
- Hess G, Aizenman CD, Donoghue JP. 1996. Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex. *J Neurophysiol*. 75:1765–1778.
- Hoppenrath K, Funke K. 2013. Time-course of changes in neuronal activity markers following iTBS-TMS of the rat neocortex. *Neurosci Lett*. 536:19–23.
- Hsieh TH, Chen JJ, Chen LH, Chiang PT, Lee HY. 2011. Time-course gait analysis of hemiparkinsonian rats following 6-hydroxydopamine lesion. *Behav Brain Res*. 222:1–9.
- Huang YZ, Chen RS, Rothwell JC, Wen HY. 2007. The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin Neurophysiol*. 118:1028–1032.
- Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. 2005. Theta burst stimulation of the human motor cortex. *Neuron*. 45:201–206.
- Huang YZ, Lu CS, Rothwell JC, Lo CC, Chuang WL, Weng YH, Lai SC, Chen RS. 2012. Modulation of the disturbed motor network in dystonia by multisession suppression of premotor cortex. *PLoS One*. 7: e47574.
- Huang YZ, Rothwell JC, Edwards MJ, Chen RS. 2008. Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. *Cereb Cortex*. 18:563–570.
- Huang YZ, Rothwell JC, Lu CS, Chuang WL, Chen RS. 2011. Abnormal bidirectional plasticity-like effects in Parkinson's disease. *Brain*. 134:2312–2320.
- Huang YZ, Rothwell JC, Lu CS, Wang J, Chen RS. 2010. Restoration of motor inhibition through an abnormal premotor-motor connection in dystonia. *Mov Disord*. 25:696–703.
- Iglesias-Bregna D, Hanak S, Ji Z, Petty M, Liu L, Zhang D, McMonagle-Strucko K. 2013. Effects of prophylactic and therapeutic teriflunomide in transcranial magnetic stimulation-induced motor-evoked potentials in the dark agouti rat model of experimental autoimmune encephalomyelitis. *J Pharmacol Exp Ther*. 347:203–211.
- Jeon BS, Jackson-Lewis V, Burke RE. 1995. 6-Hydroxydopamine lesion of the rat substantia nigra: time course and morphology of cell death. *Neurodegeneration*. 4:131–137.
- Kawashima S, Ueki Y, Mima T, Fukuyama H, Ojika K, Matsukawa N. 2013. Differences in dopaminergic modulation to motor cortical plasticity between Parkinson's disease and multiple system atrophy. *PLoS One*. 8:e62515.
- Kishore A, Joseph T, Velayudhan B, Popa T, Meunier S. 2012. Early, severe and bilateral loss of LTP and LTD-like plasticity in motor cortex (M1) in de novo Parkinson's disease. *Clin Neurophysiol*. 123:822–828.
- Koch G, Brusa L, Caltagirone C, Peppe A, Oliveri M, Stanzione P, Centonze D. 2005. rTMS of supplementary motor area modulates therapy-induced dyskinesias in Parkinson disease. *Neurology*. 65:623–625.
- Larson J, Lynch G. 1986. Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. *Science*. 232:985–988.
- Law-Tho D, Desce JM, Crepel F. 1995. Dopamine favours the emergence of long-term depression versus long-term potentiation in slices of rat prefrontal cortex. *Neurosci Lett*. 188:125–128.
- Lee JY, Kim SH, Ko AR, Lee JS, Yu JH, Seo JH, Cho BP, Cho SR. 2013. Therapeutic effects of repetitive transcranial magnetic stimulation in an animal model of Parkinson's disease. *Brain Res*. 1537:290–302.
- Lefaucheur JP, Drouot X, Von Raison F, Menard-Lefaucheur I, Cesaro P, Nguyen JP. 2004. Improvement of motor performance and modulation of cortical excitability by repetitive transcranial magnetic stimulation of the motor cortex in Parkinson's disease. *Clin Neurophysiol*. 115:2530–2541.
- Magnuson DS, Trinder TC, Zhang YP, Burke D, Morassutti DJ, Shields CB. 1999. Comparing deficits following excitotoxic and contusion injuries in the thoracic and lumbar spinal cord of the adult rat. *Exp Neurol*. 156:191–204.
- McAllister SM, Rothwell JC, Ridding MC. 2009. Selective modulation of intracortical inhibition by low-intensity theta burst stimulation. *Clin Neurophysiol*. 120:820–826.
- Morgante F, Espay AJ, Gunraj C, Lang AE, Chen R. 2006. Motor cortex plasticity in Parkinson's disease and levodopa-induced dyskinesias. *Brain*. 129:1059–1069.
- Muller-Dahlhaus JF, Orekhov Y, Liu Y, Ziemann U. 2008. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. *Exp Brain Res*. 187:467–475.
- Nielsen JB, Perez MA, Oudega M, Enriquez-Denton M, Aimonetti JM. 2007. Evaluation of transcranial magnetic stimulation for investigating transmission in descending motor tracts in the rat. *Eur J Neurosci*. 25:805–814.
- Paille V, Picconi B, Bagetta V, Ghiglieri V, Sgobio C, Di Filippo M, Viscomi MT, Giampa C, Fusco FR, Gardoni F et al. 2010. Distinct levels of dopamine denervation differentially alter striatal synaptic plasticity and NMDA receptor subunit composition. *J Neurosci*. 30:14182–14193.
- Paxinos G, Watson C. 2005. *The rat brain in stereotaxic coordinates*. Amsterdam: Elsevier Academic Press.
- Picconi B, Centonze D, Hakansson K, Bernardi G, Greengard P, Fisone G, Cenci MA, Calabresi P. 2003. Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. *Nat Neurosci*. 6:501–506.
- Picconi B, Piccoli G, Calabresi P. 2012. Synaptic dysfunction in Parkinson's disease. *Adv Exp Med Biol*. 970:553–572.
- Prescott IA, Dostrovsky JO, Moro E, Hodaie M, Lozano AM, Hutchison WD. 2009. Levodopa enhances synaptic plasticity in the substantia nigra pars reticulata of Parkinson's disease patients. *Brain*. 132:309–318.
- Randhawa BK, Farley BG, Boyd LA. 2013. Repetitive transcranial magnetic stimulation improves handwriting in Parkinson's disease. *Parkinsons Dis*. 2013:751925.
- Rotenberg A, Muller PA, Vahabzadeh-Hagh AM, Navarro X, Lopez-Vales R, Pascual-Leone A, Jensen F. 2010. Lateralization of forelimb motor evoked potentials by transcranial magnetic stimulation in rats. *Clin Neurophysiol*. 121:104–108.
- Shirota Y, Ohtsu H, Hamada M, Enomoto H, Ugawa Y. 2013. Supplementary motor area stimulation for Parkinson disease: a randomized controlled study. *Neurology*. 80:1400–1405.
- Suppa A, Marsili L, Belvisi D, Conte A, Iezzi E, Modugno N, Fabbri G, Berardelli A. 2011. Lack of LTP-like plasticity in primary motor cortex in Parkinson's disease. *Exp Neurol*. 227:296–301.
- Trepel C, Racine RJ. 1998. Long-term potentiation in the neocortex of the adult, freely moving rat. *Cereb Cortex*. 8:719–729.
- Truong L, Allbutt H, Kassiou M, Henderson JM. 2006. Developing a preclinical model of Parkinson's disease: a study of behaviour in rats with graded 6-OHDA lesions. *Behav Brain Res*. 169:1–9.
- Ueki Y, Mima T, Kotb MA, Sawada H, Saiki H, Ikeda A, Begum T, Reza F, Nagamine T, Fukuyama H. 2006. Altered plasticity of the human motor cortex in Parkinson's disease. *Ann Neurol*. 59:60–71.
- Vahabzadeh-Hagh AM, Muller PA, Pascual-Leone A, Jensen FE, Rotenberg A. 2011. Measures of cortical inhibition by paired-pulse transcranial magnetic stimulation in anesthetized rats. *J Neurophysiol*. 105:615–624.
- Vlachos A, Muller-Dahlhaus F, Roskopp J, Lenz M, Ziemann U, Deller T. 2012. Repetitive magnetic stimulation induces functional and structural plasticity of excitatory postsynapses in mouse organotypic hippocampal slice cultures. *J Neurosci*. 32:17514–17523.
- Volz LJ, Benali A, Mix A, Neubacher U, Funke K. 2013. Dose-dependence of changes in cortical protein expression induced with repeated transcranial magnetic theta-burst stimulation in the rat. *Brain Stimul*. 6:598–606.
- Yang X, Song L, Liu Z. 2010. The effect of repetitive transcranial magnetic stimulation on a model rat of Parkinson's disease. *Neuroreport*. 21:268–272.
- Yoon MC, Shin MS, Kim TS, Kim BK, Ko IG, Sung YH, Kim SE, Lee HH, Kim YP, Kim CJ. 2007. Treadmill exercise suppresses nigrostriatal dopaminergic neuronal loss in 6-hydroxydopamine-induced Parkinson's rats. *Neurosci Lett*. 423:12–17.
- Zamir O, Gunraj C, Ni Z, Mazzella F, Chen R. 2012. Effects of theta burst stimulation on motor cortex excitability in Parkinson's disease. *Clin Neurophysiol*. 123:815–821.