Neurobiology of Aging 84 (2019) 217–224

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Effects of L-DOPA on quadripulse magnetic stimulation–induced long-term potentiation in older adults

Nobuyuki Tanaka [1], Ryosuke Tsutsumi, Yuichiro Shirota, Takahiro Shimizu [2], Shinya Ohminami, Yasuo Terao [3], Yoshikazu Ugawa [4], Shoji Tsuji, Ritsuko Hanajima*,[2]

Department of Neurology, University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan

ARTICLE INFO

Article history:
Received 20 October 2018
Received in revised form 3 July 2019
Accepted 6 August 2019
Available online 9 August 2019

Keywords:
Aging
Plasticity
Dopamine
Primary motor cortex
TMS
Human

ABSTRACT

Reduced cortical plasticity has been previously reported in older adult as compared with young adults. However, the effects of dopamine on this plasticity reduction remain unknown. Here, we assessed the effects of high-dose (200 mg) and medium-dose (100 mg) L-3,4-dihydroxyphenylalanine (L-DOPA) intake on the long-term potentiation (LTP)-like effect induced by quadripulse magnetic stimulation (QPS) in older adults (aged ~65 years). The subjects were 32 (200 mg) and 20 (100 mg) healthy older adult volunteers. This study was designed as a double-blind, crossover and placebo-controlled trial on one dose of L-dopa. Two hours after taking L-DOPA or placebo-drug, QPS was applied over the motor cortex. Motor evoked potentials were recorded to evaluate the motor cortical excitability changes. We found that both doses of L-DOPA enhanced LTP after QPS in older adults as one group. We classified subjects into QPS responders and QPS nonresponders. Both L-DOPA doses produced significant LTP enhancement in QPS nonresponders, whereas either of doses did not produce significant LTP enhancement in QPS responders. Collectively, our findings suggest that the neural plasticity reductions observed in older adults could be partly improved by dopamine.

1. Introduction

Synaptic plasticity plays critical roles in memory and learning. Animal experiments have suggested that reductions in plasticity occur with age (Dumitriu et al., 2010; Gutchess, 2014). Research in humans has likewise suggested that reductions in cortical plasticity may relate with cognitive decline in the aged brain (Gutchess, 2014). Recently, several noninvasive brain stimulation techniques, such as paired associative stimulation (PAS) (Stefan et al., 2002) or transcranial direct current stimulation (tDCS) (Nitsche and Paulus, 2000), have been shown to induce cortical plasticity in the human brain. In older adults, the long-term potentiation (LTP)-like effects induced by PAS or tDCS were diminished or induced in a delayed manner (Fathi et al., 2010; Fujiyama et al., 2014). Another unique type of patterned repetitive transcranial magnetic stimulation (TMS) developed by our laboratory, referred to as "quadripulse magnetic stimulation" (QPS), has also been found to induce LTP-like effects (Hamada et al., 2008). Interestingly, the LTP-like effects induced by QPS are generally smaller in older adults than in younger adults, and the number of "QPS responders," that is, people in whom QPS induced the expected amount of LTP, was lower in older than in younger individuals (Hanajima et al., 2017). However, the effects of dopamine on noninvasive brain stimulation–induced LTP have not been studied in older adults.

Dopamine is known to play important roles in neural plasticity induction, which contributes to memory, learning and other cognitive functions, and motor and sensory functions (Knecht et al., 2004; Spencer et al., 2000). Dopamine deficiency causes impairments in plasticity induction in Parkinson's disease (Calabresi et al., 2007). Animal studies have suggested that dopamine-mediated input to D1/D5 receptors facilitates LTP induction and that the activation of both D1 and D2 receptors induces long-term depotentiation (LTD) (Bailey et al., 2000; Jay et al., 1996; Otani et al., 1998). In parkinsonian animal models, LTP/LTD induction was impaired, but LTP/LTD was restored by dopamine replacement (Calabresi et al., 1992; Kerr and Wickens, 2001). In humans, the effects of dopamine on LTP induction at the primary motor cortex (M1) have been

* Corresponding author at: Division of Neurology, Department of Brain and Neurosciences, Faculty of Medicine, Tottori University, 36-1 Nishicho, Yonago, Tottori 683-8504, Japan. Tel.: +81-589-38-6757; fax: +81-589-38-6759.
E-mail address: hanajima-tky@umin.ac.jp (R. Hanajima).
[1] Department of Neurology, National Shimoshizu Hospital, 934-5 Shikawarari, Yotsukaido, Chiba 284-0003, Japan.
[2] Division of Neurology, Department of Brain and Neurosciences, Faculty of Medicine, Tottori University, 36-1 Nishicho, Yonago 683-8504, Japan.
[3] Department of Cell Physiology, Kyorin University, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan.
[4] Department of Neuro-Regeneration, Fukushima Medical University, 1 Hikarigaoka, Fukushima, Fukushima 960-1295, Japan.

0197-4580/$ – see front matter © 2019 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.neurobiolaging.2019.08.005

studied using PAS or tDCS. However, the results of previous studies evaluating the effects of L-3,4-dihydroxyphenylalanine (L-DOPA) on LTP induction are inconsistent. For instance, Kuo et al. (2008) reported that 100-mg L-DOPA intake enhanced the LTP-like effects after PAS. By contrast, Thirugnanasambandam et al. (2011) reported that 100 mg of L-DOPA had no effects on PAS-induced LTP for up to 120 minutes after the intervention but did enhance it the following day, whereas 25 mg or 200 mg of L-DOPA inverted the PAS-induced LTP to LTD. Other studies demonstrated that the anodal tDCS-induced LTP was inverted to LTD by 100-mg L-DOPA intake and was not affected by the intake of 25 mg or 200 mg of L-DOPA (Kuo et al., 2008; Monte-Silva et al., 2010). A prior study from our laboratory also reported that the QPS-induced LTP/LTD-like effects were enhanced by 100 mg of L-DOPA/carbidopa in healthy young volunteers (Enomoto et al., 2015). However, the effects of L-DOPA on the QPS-induced LTP/LTD in older adults remain to be studied, and high-dose L-DOPA effects have also not been studied. Therefore, the aim of the present study was to evaluate the effects of dopamine on neural plasticity induced by QPS in healthy older adults and to investigate differences in these effects between high- (200 mg) and medium-dose (100 mg) L-DOPA/carbidopa.

2. Subjects and methods

2.1. Ethical approval

This study was performed according to the Declaration of Helsinki; the procedures were approved by the institutional review board of the University of Tokyo (No. P2012004, UMIN000008508). All subjects provided written informed consent to participate in this study.

2.2. Participants

We conducted 2 experiments on different participant groups (no subject participated in both experiments). Participants were 32 subjects in the 200-mg L-DOPA intake study (experiment 1) and 20 different subjects in the 100-mg L-DOPA intake study (experiment 2). We asked a temporary employment agency to recruit participants who were around 65 years of age. The exclusion criteria were in line with the appropriate safety guidelines (Rossi et al., 2009; Wassermann et al., 1996) and identified people with contraindications to L-DOPA/carbidopa; gastric or duodenal ulcers; internal metal such as a cardiac pace maker, drug delivery pump, or deep brain stimulator; severe cardiac diseases; hepatic diseases; renal diseases; pulmonary diseases; ileus; bronchial asthma; uncontrolled diabetic mellitus; chronic open angle glaucoma; cerebral stroke; brain injury; brain tumor; epilepsy; psychiatric disorders; and/or pregnant women, those contemplating pregnancy, and people whom the investigators considered inappropriate for participation. None of the participants had any history of neurological or psychiatric diseases or seizure episodes. Participants were not taking any medications for neurological or psychiatric diseases before and during the experiments or caffeine on the experimental day. For experiment 1, the agency identified 3610 people aged 63–67 years as candidates for our experiments, and of these, 99 applied to take part in our experiments. Five were excluded based on the exclusion criteria, and the other 94 applicants were considered candidates for selection. Thirty-four individuals were randomly selected from these 94 applicants. One person withdrew after our explanation of the experiments, and one withdrew after participation because she developed nausea and vomiting after taking the L-DOPA/carbidopa (200 mg). Thirty-two participates (18 men and 14 women; mean ± standard deviation age: 64.9 ± 1.7 years) finally completed the 200-mg L-DOPA intake study (Fig. 1A). We analyzed the data from these 32 participants. For experiment 2, 2111 people were identified as candidates, and of these, 79 applied. Ten were excluded based on the exclusion criteria and the other 69 applicants were considered candidates for selection. Twenty people were randomly selected from these 69 applicants. All of these 20 healthy volunteers (10 men and 10 women; mean age: 65.1 ± 1.3 years) completed the 100-mg L-DOPA intake study (Fig. 1B).

In the present analyses, we used the normal range of the baseline fluctuation of motor evoked potential (MEP) amplitude determined by the placebo-intervention experiment in our previous article (Hanajima et al., 2017; Nakamura et al., 2016).

2.3. MEP recordings

Participants sat in a comfortable chair during the experiments. Surface electromyograms were recorded from the relaxed right first dorsal interosseous (FDI) muscle using surface electrodes placed with a belly-tendon montage. Signals were filtered with stopband frequency between 100 Hz and 3 kHz using an analog filter (Neuropack mu MEP-9100: Nihon Kohden Co, Ltd, Japan) with which signals attenuated -6 dB/oct at low cut filter and -12 dB/oct at high cut filter. All data were stored in the computer for later offline analyses (TMS Bistim Tester; Medical Try System, Japan). We delivered TMS over the left motor "hot spot" (M1) for the right FDI with a figure-eight-shaped magnetic coil (7-cm external diameter at each wing; The Magstim Co, Ltd, UK) connected to a magnetic stimulator (Magstim 200; The Magstim Co, Ltd). The coil was oriented to induce currents in the brain in the posterolateral to anteromedial direction. To find the motor hot spot in each subject, we changed the stimulation site in 1-cm steps anteroposteriorly and mediolaterally, starting at a point 4 cm lateral to the vertex, and the location at which the largest MEPs were evoked was defined as the hot spot. We marked the hot spot and the location of the edge of the coil on the head with a felt pen for repositioning the coil, and kept the location of the coil by hand during MEP recordings. At this point, we measured the active motor threshold (AMT) and resting motor threshold (RMT). The AMT was defined as the intensity required to elicit at least 100-μV MEPs from the FDI in half of the single TMS trials when the subjects maintained 10% maximal voluntary contraction, and the RMT was defined as the intensity required to elicit at least 50-μV MEPs in half of the trials in the relaxed muscle.

2.4. QPS for LTP induction

We delivered QPS through a special combining module (The Magstim Co, Ltd) connected to 4 monophasic stimulators (Magstim 200 square; The Magstim Co, Ltd). Similar to our previous paper (Hamada et al., 2008), the QPS consisted of bursts of 4 monophasic subthreshold TMS pulses (90% of the AMT) repeated every 5 seconds for 30 minutes (360 bursts). We used an interstimulus interval of 5 ms (QPS-5), which is the best interval for LTP induction (Hamada et al., 2008). We administered QPS over the left M1 with a figure-eight-shaped coil held by hand.

2.5. Experimental procedures

Each experiment (one L-DOPA dose) of this study had a double-blind, crossover and placebo-controlled design. The details of the experimental procedures are illustrated in Fig. 2. For both experiment 1 and 2, each participant visited our hospital twice at an interval separated by at least 1 week.

In experiment 1, participants took 200 mg of L-DOPA (with 50 mg of carbidopa) or placebo before QPS at each visit. In experiment 2, participants took 100 mg of L-DOPA (with 25 mg of carbidopa) or

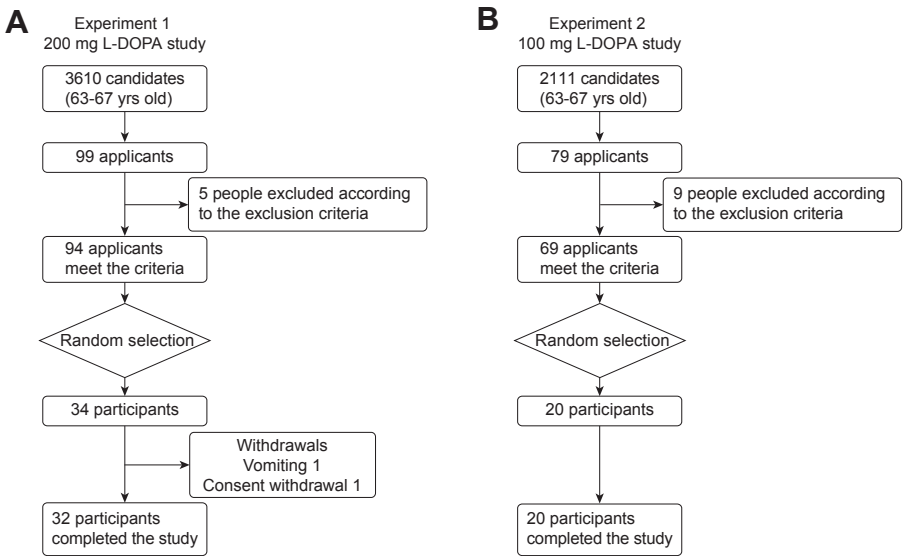

Fig. 1. Subject recruitment procedures. (A). For experiment 1, 34 people were selected. Two individuals withdrew, and 32 participants completed the experiment. (B). For experiment 2, 20 people were selected and all 20 completed the study.

placebo before QPS at each visit. The active drug or lactate (for placebo) was put in a capsule of identical form and size and masked by one fixed experimenter. All participants and all other experimenters were blinded to the experimental condition (placebo or L-DOPA). The assignment of the capsules was determined by 2 other fixed experimenters who did not perform the TMS study. Two hours after capsule intake, AMT and RMT were measured. We set the single-pulse TMS intensity to elicit baseline MEPs at an amplitude of approximately 0.5 mV in the relaxed condition and recorded 20 MEPs as the baseline. After QPS-5, we obtained 20 MEPs at time points 5, 10, 15, 20, and 25 minutes, using the same intensity as that used during the baseline recordings. MEPs were elicited by a single-pulse TMS with a figure-eight-shaped coil held by hand over M1.

2.6. Data analysis

A two-factor repeated measures analysis of variance (ANOVA) was used to statistically analyze the RMT, AMT, amplitude of baseline MEPs, and single-pulse TMS intensity, with the 2 independent variables CONDITION (placebo and L-DOPA) and DOSE (200 mg and 100 mg: experiment 1 and 2) (see Table 1). After we separated participants into "QPS responders" and "QPS non-responders" (see the following), differences in these parameters were analyzed with a three-factor repeated measures ANOVA and the 3 independent variables CONDITION (placebo and L-DOPA), DOSE (200 mg and 100 mg: experiment 1 and 2), and RESPONDER (QPS responders and QPS nonresponders).

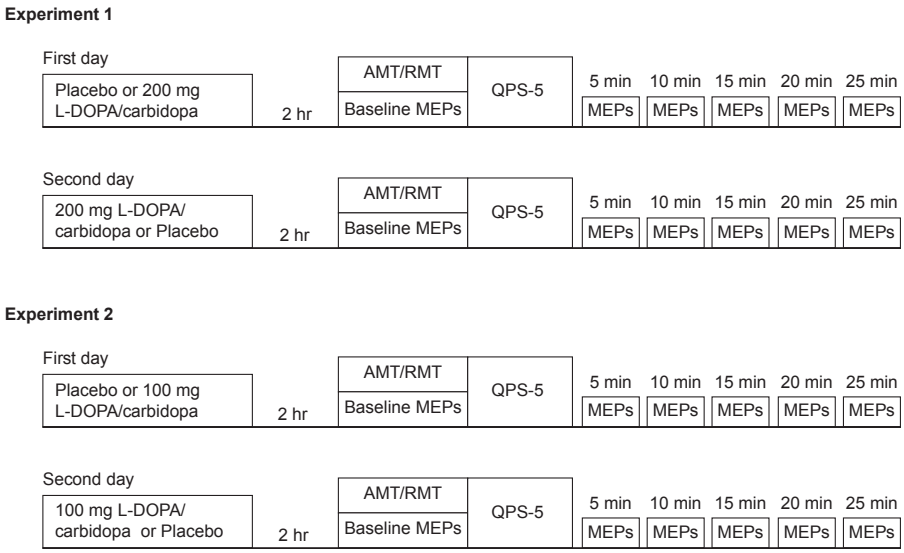

Fig. 2. Experimental procedures. The present study used a double-blind, crossover, and placebo-controlled design for each experiment of 100 mg or 200 mg L-DOPA intake. Experiment 1: On the first day, each participant took a capsule containing 200 mg of L-DOPA/carbidopa or a placebo-capsule, which were randomly allocated. Two hours later, we recorded the active motor threshold (AMT), resting motor threshold (RMT), and 20 baseline motor evoked potentials (MEPs) from the right first dorsal interosseous muscle (FDI). Immediately after the baseline recordings, quadripulse magnetic stimulation with interstimulus intervals of 5 ms (QPS-5) was administered over the left motor cortex for 30 minutes. Subsequently, 20 MEPs were recorded at time points of 5, 10, 15, 20, and 25 minutes after QPS. On the second day, which was at least 1 week after the first visit, the participants took the other capsule. All other procedures were the same as those performed at the first visit. Experiment 2: the participants took 100 mg of L-DOPA/carbidopa or placebo-drug. The experimental procedures were the same as those performed in experiment 1.

Table 1
Physiological parameters before QPS intervention
RMT, AMT, baseline MEP amplitude, and single-pulse TMS intensity to be used to induce MEP in all session in each group

A.

	L-DOPA200 (N = 32)		L-DOPA100 (N = 20)		Two-factor ANOVA		
	Placebo	L-DOPA	Placebo	L-DOPA	CONDITION	DOSE	DOSE × CONDITION
AMT (%)	30.1 ± 5.6	31 ± 5.3	32.1 ± 5.1	33.1 ± 5.9	$p = 0.77$	$p = 0.21$	$p = 0.67$
RMT (%)	51.1 ± 11.8	49.5 ± 9.5	52.9 ± 10.3	53.2 ± 10.0	$p = 0.51$	$p = 0.12$	$p = 0.77$
AMP (mV)	0.48 ± 0.17	0.48 ± 0.12	0.5 ± 0.06	0.51 ± 0.07	$p = 0.96$	$p = 0.25$	$p = 0.83$
TMS intensity (%)	60.8 ± 19.4	62.9 ± 17.4	65.6 ± 17.9	62.7 ± 15.5	$p = 0.91$	$p = 0.53$	$p = 0.45$

B.

	L-DOPA200 (N = 32)				L-DOPA100 (N = 20)						
	Significant responders (N = 19)		Nonresponders (N = 13)		Significant responders (N = 8)		Non-responders (N = 12)		Three factor ANOVA		
	Placebo	L-DOPA	Placebo	L-DOPA	Placebo	L-DOPA	Placebo	L-DOPA	CONDITION	DOSE	RESPONDER
AMT (%)	29.9 ± 5.9	30.5 ± 5.6	31.7 ± 5.1	31.8 ± 5.0	32.9 ± 5.3	34.3 ± 6.7	31.5 ± 5.1	32.3 ± 5.4	$p = 0.59$	$p = 0.36$	$p = 0.89$
RMT (%)	50.3 ± 12.9	47.6 ± 8.4	52.2 ± 10.4	52.2 ± 10.7	54.9 ± 9.5	56.1 ± 11.6	51.6 ± 11.1	51.3 ± 12.3	$p = 0.054$	$p = 0.26$	$p = 0.97$
AMP (mV)	0.46 ± 0.18	0.46 ± 0.14	0.51 ± 0.15	0.5 ± 0.1	0.5 ± 0.06	0.54 ± 0.08	0.51 ± 0.06	0.49 ± 0.06	$p = 0.94$	$p = 0.39$	$p = 0.69$
TMS intensity (%)	58.6 ± 21.0	60.6 ± 17.9	64.1 ± 17.1	66.2 ± 16.7	69.4 ± 16.4	67.5 ± 16.2	63.1 ± 19.1	59.5 ± 15	$p = 0.82$	$p = 0.62$	$p = 0.87$

Key: AMP, amplitude of MEP; AMT, active motor threshold; L-DOPA, L-3,4-dihydroxyphenylalanine; RMT, resting motor threshold; TMS, transcranial magnetic stimulation; MEP, motor evoked potential.

We used the mean amplitude of the MEPs to 20 single TMS pulses at each time point (5, 10, 15, 20, and 25 minutes) to evaluate the excitability changes after QPS-5. We calculated the MEP size ratio, defined as the mean MEP amplitude at each time point divided by the mean baseline MEP amplitude. The time course of LTP induction was illustrated by plotting the mean MEP size ratio against the time after QPS. A three-factor repeated measures ANOVA was used to determine the effects of L-DOPA on LTP; the dependent variable was the MEP size ratio, and the 3 independent variables were CONDITION (placebo and L-DOPA), TIME (5, 10, 15, 20, and 25 minutes after QPS), and DOSE (200 mg and 100 mg: experiment 1 and 2).

We classified all subjects into 2 groups (QPS responders and QPS nonresponders) in a manner similar to that used in previous articles (Hanajima et al., 2017; Nakamura et al., 2016). For each participant, we calculated the average MEP size ratio as the grand average of the size ratios from 5 to 25 minutes (the average MEP ratio 5–25 minutes: average MEP ratio [5–25 minutes]). We defined QPS responders as the participants whose average MEP ratio (5–25 minutes) was >1.24, whereas QPS nonresponders were defined as those whose average MEP ratio (5–25 minutes) was ≤1.24, based on the normal range in our previous article under the sham-intervention condition (mean ± 2 standard deviations: 0.76–1.24) (Nakamura et al., 2016). In the present study, QPS nonresponders include the categories labeled “no significant responder to QPS” and “opposite responders to QPS” in the previous article. We first analyzed the effects of L-DOPA on LTP using a three-factor repeated measures ANOVA in each experiment (with 100 mg or 200 mg). The dependent variable was the MEP size ratio, and the 3 independent variables were CONDITION (placebo and L-DOPA), TIME (5, 10, 15, 20, and 25 minutes after QPS), and RESPONDER (QPS responders and QPS nonresponders). As post hoc analyses, we analyzed the effects of L-DOPA on LTP in QPS responders and QPS nonresponders separately (see results), using a two-factor repeated measures ANOVA. The dependent variable was the MEP size ratio, and the 2 independent variables were CONDITION (placebo and L-DOPA) and TIME (5, 10, 15, 20, and 25 minutes after QPS).

All statistical analyses were performed using IBM SPSS version 17.0 for Windows (IBM Japan, Ltd, Tokyo, Japan). The level of statistical significance was set at $p \leq 0.05$ in all analyses.

3. Results

In experiment 1, one participant vomited after taking the L-DOPA/carbidopa (200 mg) and withdrew from the study. The other 32 participants completed the experiment. In those 32 subjects, 3 participants felt slight nausea after taking the L-DOPA/carbidopa (200 mg), and 2 after placebo intake. However, without any intervention, the symptoms improved spontaneously. In experiment 2, all 20 participants completed the experiment; although one participant felt light, transient nausea after taking the placebo, this participant finished the experiment completely. The QPS procedures and TMS stimulation provoked no adverse effects in any of the participants.

The RMT, AMT, baseline MEP amplitude, and single-pulse TMS intensity in the follow-up session did not differ significantly among the 200-mg L-DOPA, 100-mg L-DOPA, and placebo group (two-factor ANOVA; AMT: CONDITION: $F[1, 100] = 0.09$, $p = 0.77$; DOSE: $F[1, 100] = 1.57$, $p = 0.21$; CONDITION × DOSE: $F[1, 100] = 0.19$, $p = 0.67$; RMT: CONDITION: $F[1, 100] = 0.44$, $p = 0.51$; DOSE: $F[1, 100] = 2.50$, $p = 0.12$; CONDITION × DOSE: $F[1, 100] = 0.09$, $p = 0.77$; amplitude: CONDITION: $F[1, 100] = 0.003$, $p = 0.96$; DOSE: $F[1, 100] = 1.32$, $p = 0.25$; CONDITION × DOSE: $F[1, 100] = 0.05$, $p = 0.83$; TMS intensity: CONDITION: $F[1, 100] = 0.01$, $p = 0.91$; DOSE: $F[1, 100] = 0.41$, $p = 0.53$ CONDITION × DOSE: $F[1, 100] = 0.48$, $p = 0.49$) (Table 1A). Furthermore, when we separated participants into “QPS responders” and “QPS nonresponders”, none of these parameters differed significantly, neither for CONDITION nor for DOSE or RESPONDER [three-factor repeated measures ANOVA: AMT: CONDITION: $F[1, 48] = 0.301$, $p = 0.59$; DOSE: $F[1, 48] = 0.86$, $p = 0.36$; RESPONDER: $F[1, 48] = 0.018$, $p = 0.89$; RMT: CONDITION: $F[1, 48] = 3.91$, $p = 0.054$; DOSE: $F[1, 48] = 1.28$, $p = 0.26$; RESPONDER: $F[1, 48] = 0.002$, $p = 0.97$; amplitude: CONDITION: $F[1, 48] = 0.006$, $p = 0.94$; DOSE: $F[1, 48] = 0.76$, $p = 0.39$; RESPONDER: $F[1, 48] = 0.161$, $p = 0.69$; TMS intensity: CONDITION: $F[1, 48] = 0.054$, $p = 0.82$; DOSE: $F[1, 48] = 0.24$, $p = 0.62$; RESPONDER: $F[1, 48] = 0.03$, $p = 0.87$] (Table 1B).

We found that L-DOPA intake significantly enlarged the MEP size ratio after QPS-5 compared with placebo intake. Representative data from a single subject is shown in Fig. 3. In this single QPS nonresponder, MEP amplitude after QPS did not change comparing with baseline MEP with placebo intake (before QPS) (average MEP waveforms in Fig. 3A and each MEP amplitude plots as time course

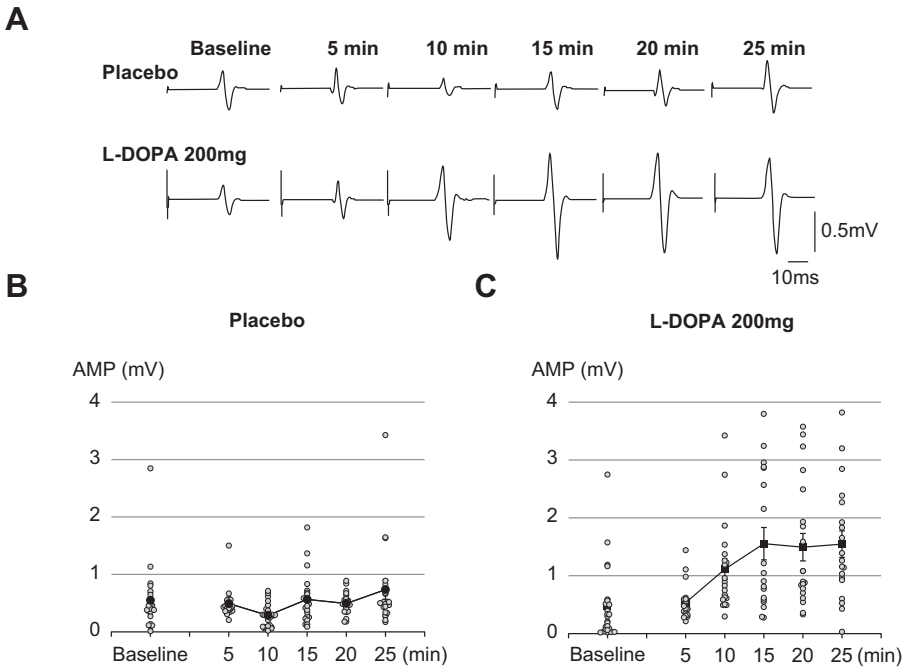

Fig. 3. Representative results in a single nonresponder in experiment 1. (A). Waveforms of average MEPs at each time point (pre-QPS and at time points of 5, 10, 15, 20, 25 minutes after QPS) in the placebo condition (upper rows) and in the L-DOPA/carbidopa 200-mg intake condition (lower rows). The amplitudes of MEPs after QPS were not significantly larger than baseline MEPs in the placebo condition (nonresponders). In the L-DOPA condition, MEP amplitudes were larger than baseline MEPs. (B). Amplitude of each MEP for 20 trials at each time point in the same single participant in the placebo condition. Gray dots indicate MEP amplitudes of single trials and black dots indicate the average amplitude. One-factor ANOVA revealed that TIME had no effect on MEP amplitude in this participant [$F(5, 117) = 2.16$, $p = 0.06$]. (C). Amplitude of each MEP in the L-DOPA 200-mg intake condition. TIME had significant effects on MEP amplitudes [one-factor ANOVA $F(5, 116) = 7.16$, $p < 0.0001$]. Post hoc analyses revealed that MEPs at 15 minutes, 20 minutes, and 25 minutes were significantly larger than baseline MEPs (Bonferroni analysis, $p < 0.05$).

in Fig. 3B). With intake L-DOPA 200 mg, MEP amplitude was enlarged after QPS (Fig. 3A and C)

Fig. 4 shows time course of the average MEP amplitude of all participants in experiment 1 (A, C, and E) and experiment 2 (B, D, and F). A three-factor repeated measures ANOVA revealed that L-DOPA intake had significant effects on MEP ratio (CONDITION: $F[1, 100] = 10.5$, $p = 0.02$; TIME: $F[4, 400] = 0.70$, $p = 0.59$) (Fig. 4E and F). However, the dose of L-DOPA did not have any significant effects on the LTP-like effects after QPS (DOSE: $F[1, 100] = 0.42$, $p = 0.52$). No significant interactions were found between any of the factors.

Based on the average MEP ratio (5–25 minutes) in the placebo-drug intake experiment, 19 and 8 participants were classified as QPS responders in experiments 1 and 2, respectively (Fig. 5A). The grand average MEP ratio (5–25 minutes) in the placebo experiment from all participants was 1.31 ± 0.11 in experiment 1 and 1.14 ± 0.08 in experiment 2. The rate of QPS responders in the placebo-drug condition was 19/32 (59.4%) in experiment 1 and 8/20 (40%) in experiment 2. The rate of QPS responders when taking L-DOPA was 24/32 (75%) in experiment 1 and 8/12 (66.7%) in experiment 2 (Fig. 5A). The conversion rate from the QPS nonresponders to QPS responders by L-DOPA was 10/13 (76.9%) in experiment 1 and 8/12 (53.3%) in experiment 2. The reverse conversion rate from the QPS responders to QPS nonresponders by L-DOPA was 4/19 (21.0%) in experiment 1 and 2/8 (25.0%) in experiment 2.

We first analyzed the effects of CONDITION, TIME, and RESPONDERS in each experiment. In experiment 1 (with L-DOPA 200 mg), a three-factor repeated measures ANOVA showed significant effects of CONDITION and RESPONDER (CONDITION: $F[1, 60] = 8.67$, $p = 0.005$; RESPONDER $F[1, 60] = 10.9$, $p = 0.002$) but not TIME ($F[4, 240] = 1.19$, $p = 0.32$) and there are no significant interactions (CONDITION × RESPONDER $F[1, 60] = 2.78$, $p = 0.1$). In experiment 2 (with L-DOPA 100 mg), a three-factor repeated measures ANOVA revealed the same results (CONDITION: $F[1, 36] = 7.38$, $p = 0.01$; RESPONDER $F[1, 36] = 8.20$, $p = 0.007$, TIME ($F[4, 144] = 0.572$, $p = 0.68$) and there are no interactions (CONDITION × RESPONDER $F[1, 36] = 0.26$, $p = 0.61$). Because the factor RESPONDER significantly affected the MEP size ratio, we analyzed the data of QPS responders and those of QPS nonresponders independently as post hoc analyses. In 19 QPS responders in experiment 1, neither CONDITION nor TIME had significant effects on MEP ratio (two-factor repeated measures ANOVA; CONDITION: $F[1, 36] = 0.81$, $p = 0.37$; TIME: $F[4, 144] = 0.41$, $p = 0.80$), and no interaction was found between those factors (CONDITION × TIME: $F[4144] = 0.86$, $p = 0.49$) (Fig. 5B). In 8 QPS responders in experiment 2, there were no significant effects of CONDITION or TIME on MEP ratio (CONDITION: $F[1, 14] = 1.55$, $p = 0.23$; TIME: $F[4, 56] = 0.24$, $p = 0.92$) and no interaction (CONDITION × TIME: $F[4, 56] = 0.44$, $p = 0.78$) (Fig. 5C). By contrast, in 13 QPS nonresponders in experiment 1, L-DOPA/carbidopa significantly enlarged the MEP size ratio (CONDITION: $F[1, 24] = 13.9$, $p = 0.01$), whereas TIME had no effect (TIME: $F[4, 96] = 0.92$, $p = 0.46$), and no interaction was found (CONDITION × DOSE: $F[4, 96] = 0.78$, $p = 0.54$) (Fig. 5D). In 12 QPS nonresponders in experiment 2, L-DOPA/carbidopa also showed significant effects on the MEP size ratio (CONDITION: $F[1, 22] = 9.00$, $p = 0.007$) but no effects of TIME (TIME: $F[4, 88] = 0.61$, $p = 0.65$) and no interaction (CONDITION × DOSE: $F[4, 88] = 0.33$, $p = 0.86$) were observed (Fig. 5E).

4. Discussion

The present study shows that both investigated doses of L-DOPA (100 mg and 200 mg) similarly enhanced the LTP-like effects after QPS-5 in M1 in healthy older adults as a whole (~65 year old). Especially, in QPS nonresponders, the L-DOPA intake enhanced plasticity considerably.

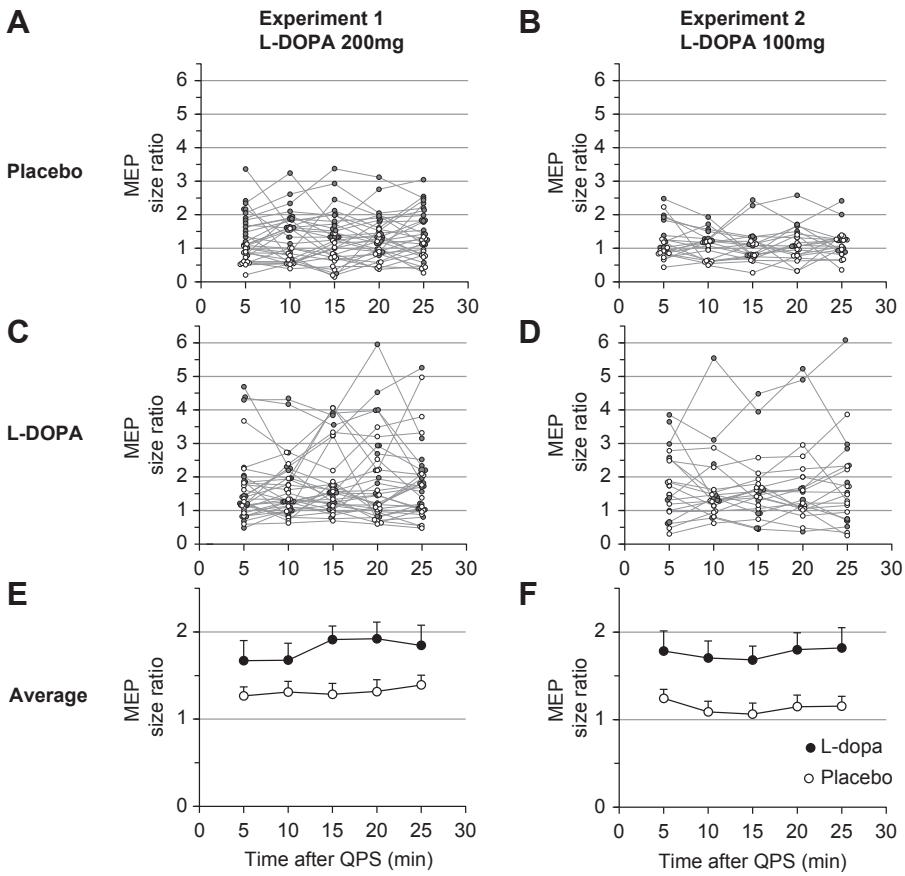

Fig. 4. (A, C). Time courses of the MEP size ratio after QPS for all participants after the intake of placebo (A) and L-DOPA 200 mg (C) in experiment 1. (B, D). Time courses of the MEP size ratio after QPS for all participants after the intake of placebo (B) and L-DOPA 100 mg (D) in experiment 2. (E, F). Time courses of the motor evoked potential (MEP) mean size ratio after quadripulse magnetic stimulation with interstimulus intervals of 5 ms (QPS-5). The grand averaged MEP size ratio (mean ± standard error) of all participants plotted against time (min) after QPS-5. Dots show the (E) 200-mg L-DOPA intake and (F) 100-mg L-DOPA intake conditions and circles represent the placebo-drug condition. The ratios were significantly larger in the L-DOPA intake group than in the placebo intake group. The three-factor repeated measures ANOVA showed a significant effect of CONDITION (placebo or L-DOPA) on the MEP size ratio but did not show any effects of DOSE or TIME; no interaction was identified between DOSE and CONDITION.

4.1. Effects of L-DOPA on plasticity

Our results demonstrating enhancement of LTP by L-DOPA/carbidopa intake are consistent with previous animal experiments showing dopaminergic enhancement of LTP (Jay et al., 1996; Otani et al., 1998). Similar enhancement of LTP was induced by 100 mg of L-DOPA after PAS (Kuo et al., 2008) and 100 mg of L-DOPA after QPS (Enomoto et al., 2015) in young healthy individuals. The enhancement of PAS-induced LTP after dopamine intake has been suggested to depend mainly on D1 group receptor activation (Nitsche et al., 2009). In our previous study, we reported that D2 agonists did not affect the QPS-5–induced LTP, which indicated that the QPS-5–induced LTP was also mediated by D1 receptor activation (Enomoto et al., 2015).

By contrast, the dopaminergic effects on LTP after tDCS were differently affected by L-DOPA. Namely, 100 mg dopamine abolished the tDCS-induced LTP (Kuo et al., 2008) or inverted the LTP to LTD (Monte-Silva et al., 2010). LTP after tDCS was abolished by sulpiride (D2 antagonist) (i.e., relative increase of D1 activity), which suggests that D2 receptor activities could contribute to the tDCS-induced LTP (Nitsche et al., 2006). However, a combination of 100 mg L-DOPA and sulpiride did not affect the tDCS-induced LTP (Nitsche et al., 2009), suggesting that mutual influences between D1 and D2 activities on LTP after tDCS might be complicated. Kuo et al. (2008) concluded that dopamine enhances only "focally induced synaptic plasticity". Dopamine enhanced the PAS-induced plasticity on the specific subgroup synapses for somatosensory and motor cortical neuronal connections, but tDCS induced plasticity on diffuse, nonspecific subgroups of cortical synapses. QPS probably induces homotopic synaptic plasticity of the primary motor cortex using monophasic TMS (a kind of focal synaptic plasticity). That suggests that D1 and D2 receptor influences on LTP were simple in the case of QPS-induced LTP and it is similar to that on PAS-induced LTP.

The effects of dopamine on QPS-induced LTP in older adults shown here are compatible with the abovementioned findings of dopamine enhancement of LTP.

4.2. Dose-dependency of the effects of L-DOPA

The effects of L-DOPA on QPS-induced LTP did not show dose-dependency in our study. Nonlinear dose-dependency of the effects of L-DOPA was revealed previously when the PAS or tDCS plasticity induction method was used (Monte-Silva et al., 2010; Thirugnanasambandam et al., 2011). A nonlinear dose-dependency of dopamine effect with an inverted-U–shaped curve was first observed in studies evaluating the effects of dopamine on cognitive functions (Granon et al., 2000; Williams et al., 1995). This dose-dependent effect identified with animal data was suggested to be due to presynaptic D2 receptor activation by low-dose dopamine (Schmitz et al., 2003) or to the disinhibition of N-methyl-D-aspartate (NMDA) receptor activity under high-dose dopamine (Misonou et al., 2004; Seamans and Yang, 2004). However, another article (Kolomiets et al., 2009) reported that neither NMDA receptor activity nor D1-mediated increases in neural excitability showed an

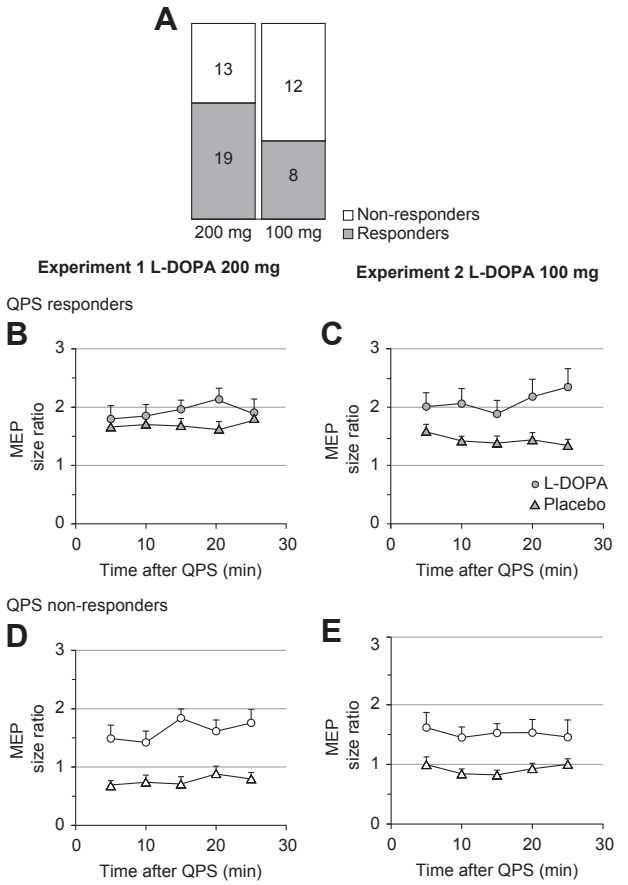

Fig. 5. QPS responders and QPS nonresponders. (A). Split bar graphs show the rates of significant responders (average MEP size ratio >1.24; gray) and nonresponders (average MEP size ratio ≤1.24; white). (B, C). The time courses show the average motor evoked potential (MEP) ratio after QPS in significant responders in experiment 1 (B) and experiment 2 (C). Neither CONDITION nor TIME had significant effect on MEP ratio in experiment 1 in responders (two-factor repeated measures ANOVA; CONDITION: $F[1, 36] = 0.81$, $p = 0.37$; TIME: $F[4, 144] = 0.41$, $p = 0.80$). In experiment 2 of QPS responders, neither CONDITION nor TIME had no effects on the MEP ratio (CONDITION: $F[1, 14] = 1.55$, $p = 0.23$; TIME: $F[4, 56] = 0.24$, $p = 0.92$). (D, E). The time courses show the average motor evoked potential (MEP) ratio after QPS in non-responders. In nonresponders, in both experiments, CONDITION significantly influenced the MEP ratio (experiment 1: CONDITION: $F[1, 24] = 13.9$, $p = 0.01$, TIME: $F[4, 96] = 0.92$, $p = 0.46$; experiment 2: CONDITION: $F[1, 22] = 9.00$, $p = 0.007$, TIME: $F[4, 88] = 0.61$, $p = 0.65$). It indicates that both doses of L-DOPA increased the average MEP ratio more compared with the placebo-drug intake condition.

inverted-U curve. Thus, the mechanisms underlying the inverted-U curve dose-dependency remain unclear (Otani et al., 1998). The complicated effects of dopamine on PAS- and tDCS-induced LTP have been hypothesized to be related to the superimposition of presynaptic D2 receptor activation and the disinhibition of NMDA receptor activity. In the present experiments using doses of 100 mg and 200 mg, the effects of L-DOPA on QPS-induced LTP were similar in terms of the amount of LTP enhancement. Nevertheless, our data are insufficient for discussing the dose-dependency of the effects of L-DOPA on QPS-induced LTP, because we only used 2 dosages. Higher doses of L-DOPA, such as 300 mg or 400 mg, may show decrements in LTP induction. Another possibility is that the plasticity induced by QPS may not exhibit the inverted-U–shaped dose-dependency. If QPS does not have the inverted-U–shaped curve, its lack of dose-dependency may be explained by the notion that QPS-induced homotopic plasticity is mostly influenced by D1 receptor activation.

4.3. Effects of L-DOPA in QPS nonresponders

We targeted older adults who were approximately 65 years old. In our previous article (Hanajima et al., 2017), the LTP induced by QPS-5 in older adults (the grand average MEP size ratio between 0 minutes to 25 minutes was 1.25 ± 0.07) was smaller than that in younger individuals (1.60 ± 0.08), and the rate of QPS responders was lower in older adults (47%) than in younger adults (76%). In the present article, the amount of LTP and the rate of QPS responders in the placebo-drug intake group were compatible with those of old subjects reported in our previous article and smaller than those of younger individuals in our previous article. The present amount of LTP induction under 100 mg L-DOPA intake was also smaller than that in younger individuals in our previous report (Enomoto et al., 2015). These all may show an age dependency of LTP induced by QPS-5.

The present separate analyses of QPS responders and nonresponders revealed that L-DOPA intake produced significant LTP enhancement in QPS nonresponders but not in QPS responders. With L-DOPA intakes, the rate of QPS responder increased; that is, some nonresponders were converted to QPS responder (76.9% of nonresponders in experiment 1 and 53.3% in experiment 2).

The fact that we studied healthy older adults without any neurological disorders, such as Parkinson's disease or dementia, suggests that normal aging likely reduces plasticity. Our data showed that dopamine replacement may improve the age-related LTP attenuation. It is known that D1 receptors in the cortex decrease with age and that the cortical dopaminergic neurotransmission reductions occur in parallel with the cognitive decline in older adults (de Keyser et al., 1990). This age-related reduction in cortical dopamine may partly explain the high rate of QPS nonresponders in older adults and/or the age-related declines in plasticity, memory, and high-order cognitive functions in older adults. As such, dopamine replacement could have the potential to restore some part of the age-related, high-order cognitive decline. Another interesting possibility is that some of the functionally normal older adults that participated in the present study may have had preclinical Parkinson's disease or Alzheimer's disease. This indicates that QPS-induced plasticity may be a preclinical biomarker of some disorders. This issue, however, should be examined further in the future.

It should also be mentioned that in the present study, L-DOPA did not significantly influence LTP in QPS responders. However, the small number of QPS responders studied here likely explains the lack of statistical significance. A ceiling effect of dopamine may be another explanation. Moreover, some QPS responders (21% in experiment 1 and 25.0% in experiment 2) were converted to nonresponder by L-DOPA intake. This may indicate that L-DOPA effects are weak and sometimes variable. It may also be partly explained by an intertrial variability of QPS effects in some subjects whose baseline average MEP ration (5–25 minutes) was just over the normal range. Nevertheless, the overall L-DOPA effect found here suggests that L-DOPA enhances the plasticity induced by QPS-5.

Here, we used only MEPs as a marker of excitability changes. An interesting future project could therefore be to study QPS effects on cognitive/behavioral performance; especially focusing on the difference between responders and nonresponders might be challenging. For example, investigating whether QPS responders also show changes in cognitive performance may give us some information about the mechanism underlying the response to QPS.

4.4. Limitations

One critical limitation of this study is that we only investigated LTP-like effects. To clarify the dopaminergic effects on neural plasticity in older adults, it will be important to also study LTD-like effects; this is one of our future projects. The second limitation is the number of participants, which was too small to perform independent analyses of subgroups. The third limitation is that we did not include young people in the present investigation. The fourth limitation is that we did not study reproducibility of "significant responder" and "nonresponder" classification in each participant. We can therefore not firmly conclude that our effects show age-dependency. The fifth limitation is that this study does not have a full crossover design, and future investigations should therefore compare effects of both doses in the same participants in a complete crossover design.

5. Conclusions

In this investigation, we show that dopamine improves the QPS-induced LTP in healthy older adults as a whole, and especially in QPS nonresponders. Our findings support the hypothesis that age-related neural plasticity reductions may be partly explained by age-related dopamine decreases in older adults. Furthermore, our results indicate that these LTP reductions in older adults could serve as a biomarker for age-related dopamine decreases.

Disclosure

RH received research support from Sumitomo Dainippon Pharma Co, Ltd, and GlaxoSmithKline KK.

Acknowledgements

The authors are very thankful to Dr. Shun-ichi Matsuda and Dr. Shin-ichi Tokusige for allocating the participants, to Dr. Satomi Inomata-Terada for performing the drug blinding, and to Dr. Ryoji Yanagihara for making the drug-blinding adjustments.

This work was supported in part by a research grant from Catecholamine and the Neurological Disorders Symposium (RH), research funds from the Magnetic Health Science Foundation (RH, YT) and by research grants from Grant-in-Aid for Scientific Research (KAKENHI) from Japan Society for the Promotion of Science [grant numbers 26461308 (RH), 17K09809 (RH), 25293206 (YU), 22390181 (YU), 16H05322 (YU), 16K09709 (YT), 16H01497 (YT), and 22590954 (YT)] and Grant-in-Aid for Scientific Research on Innovative Areas (Non-linear Neuro-oscillology: Towards Integrative Understanding of Human Nature [15H05881 (RH, YU)]) from The Ministry of Education, Culture, Sports, Science and Technology. The study was also supported by Grants for Research on Rare and Intractable Diseases: Clinical Research for Establishment of Evidence-Based Guideline for Hereditary Dystonias and Huntington Disease) (RH) and a Grant-in-Aid for the Research Committee for Ataxic Diseases (RH, YU) from the Ministry of Health, Labor and Welfare, Japan.

References

Bailey, C.H., Giustetto, M., Huang, Y.Y., Hawkins, R.D., Kandel, E.R., 2000. Is heterosynaptic modulation essential for stabilizing Hebbian plasticity and memory? Nat. Rev. Neurosci. 1, 11–20.

Calabresi, P., Maj, R., Pisani, A., Mercuri, N.B., Bernardi, G., 1992. Long-term synaptic depression in the striatum: physiological and pharmacological characterization. J. Neurosci. 12, 4224–4233.

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

Dumitriu, D., Hao, J., Hara, Y., Kaufmann, J., Janssen, W.G., Lou, W., Rapp, P.R., Morrison, J.H., 2010. Selective changes in thin spine density and morphology in monkey prefrontal cortex correlate with aging-related cognitive impairment. J. Neurosci. 30, 7507–7515.

Enomoto, H., Terao, Y., Kadowaki, S., Nakamura, K., Moriya, A., Nakatani-Enomoto, S., Kobayashi, S., Yoshihara, A., Hanajima, R., Ugawa, Y., 2015. Effects of L-DOPA and pramipexole on plasticity induced by QPS in human motor cortex. J. Neural Transm. (Vienna) 122, 1253–1261.

Fathi, D., Ueki, Y., Mima, T., Koganemaru, S., Nagamine, T., Tawfik, A., Fukuyama, H., 2010. Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. Clin. Neurophysiol. 121, 90–93.

Fujiyama, H., Hyde, J., Hinder, M.R., Kim, S.J., McCormack, G.H., Vickers, J.C., Summers, J.J., 2014. Delayed plastic responses to anodal tDCS in older adults. Front Aging Neurosci. 6, 115.

Granon, S., Passetti, F., Thomas, K.L., Dalley, J.W., Everitt, B.J., Robbins, T.W., 2000. Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. J. Neurosci. 20, 1208–1215.

Gutchess, A., 2014. Plasticity of the aging brain: new directions in cognitive neuroscience. Science 346, 579–582.

Hamada, M., Terao, Y., Hanajima, R., Shirota, Y., Nakatani-Enomoto, S., Furubayashi, T., Matsumoto, H., Ugawa, Y., 2008. Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. J. Physiol. 586, 3927–3947.

Hanajima, R., Tanaka, N., Tsutsumi, R., Enomoto, H., Abe, M., Nakamura, K., Hamada, M., Shimizu, T., Terao, Y., Ugawa, Y., 2017. The effect of age on the homotopic motor cortical long-term potentiation-like effect induced by quadripulse stimulation. Exp. Brain Res. 235, 2103–2108.

Jay, T.M., Burette, F., Laroche, S., 1996. Plasticity of the hippocampal prefrontal cortex synapses. J. Physiol. (Paris) 90, 361–366.

Kerr, J.N., Wickens, J.R., 2001. Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro. J. Neurophysiol. 85, 117–124.

de Keyser, J., De Backer, J.P., Vauquelin, G., Ebinger, G., 1990. The effect of aging on the D1 dopamine receptors in human frontal cortex. Brain Res. 528, 308–310.

Knecht, S., Breitenstein, C., Bushuven, S., Wailke, S., Kamping, S., Flöel, A., Zwitserlood, P., Ringelstein, E.B., 2004. Levodopa: faster and better word learning in normal humans. Ann. Neurol. 56, 20–26.

Kolomiets, B., Marzo, A., Caboche, J., Vanhoutte, P., Otani, S., 2009. Background dopamine concentration dependently facilitates long-term potentiation in rat prefrontal cortex through postsynaptic activation of extracellular signal-regulated kinases. Cereb. Cortex 19, 2708–2718.

Kuo, M.F., Paulus, W., Nitsche, M.A., 2008. Boosting focally-induced brain plasticity by dopamine. Cereb. Cortex 18, 648–651.

Misonou, H., Mohapatra, D.P., Park, E.W., Leung, V., Zhen, D., Misonou, K., Anderson, A.E., Trimmer, J.S., 2004. Regulation of ion channel localization and phosphorylation by neuronal activity. Nat. Neurosci. 7, 711–718.

Monte-Silva, K., Liebetanz, D., Grundey, J., Paulus, W., Nitsche, M.A., 2010. Dosage-dependent non-linear effect of L-DOPA on human motor cortex plasticity. J. Physiol. 588, 3415–3424.

Nakamura, K., Groiss, S.J., Hamada, M., Enomoto, H., Kadowaki, S., Abe, M., Murakami, T., Wiratman, W., Chang, F., Kobayashi, S., Hanajima, R., Terao, Y., Ugawa, Y., 2016. Variability in response to quadripulse stimulation of the motor cortex. Brain Stimul. 9, 859–866.

Nitsche, M.A., Lampe, C., Antal, A., Liebetanz, D., Lang, N., Tergau, F., Paulus, W., 2006. Dopaminergic modulation of long-lasting direct current-induced cortical excitability changes in the human motor cortex. Eur. J. Neurosci. 23, 1651–1657.

Nitsche, M.A., Kuo, M.F., Grosch, J., Bergner, C., Monte-Silva, K., Paulus, W., 2009. D1-receptor impact on neuroplasticity in humans. J. Neurosci. 29, 2648–2653.

Nitsche, M.A., Paulus, W., 2000. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J. Physiol. 527, 633–639.

Otani, S., Blond, O., Desce, J.M., Crépel, F., 1998. Dopamine facilitates long-term depression of glutamatergic transmission in rat prefrontal cortex. Neuroscience 85, 669–676.

Rossi, S., Hallett, M., Rossini, P.M., Pascual-Leone, A., 2009. Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin. Neurophysiol. 120, 2008–2039.

Schmitz, Y., Benoit-Marand, M., Gonon, F., Sulzer, D., 2003. Presynaptic regulation of dopaminergic neurotransmission. J. Neurochem. 87, 273–289.

Seamans, J.K., Yang, C.R., 2004. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog. Neurobiol. 74, 1–58.

Spencer, J.P., Murphy, K.P., 2000. Bi-directional changes in synaptic plasticity induced at corticostriatal synapses in vitro. Exp. Brain Res. 135, 497–503.

Stefan, K., Kunesch, E., Cohen, L.G., Benecke, R., Classen, J., 2000. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123, 572–584.

Thirugnanasambandam, N., Grundey, J., Paulus, W., Nitsche, M.A., 2011. Dose-dependent nonlinear effect of L-DOPA on paired associative stimulation-induced neuroplasticity in humans. J. Neurosci. 31, 5294–5299.

Wassermann, E.M., 1998. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the safety of repetitive transcranial magnetic stimulation, June 5-7, 1996. Electroencephalogr. Clin. Neurophysiol. 108, 1–16.

Williams, G.V., Goldman-Rakic, P.S., 1995. Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 376, 572–575.